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The Effect of Movement Rate and Complexity on Functional Magnetic Resonance Signal Change During Pedaling

**Jay P. Mehta, Matthew D. Verber, Jon A. Wieser,
Brian D. Schmit, and Sheila M. Schindler-Ivens**

We used functional magnetic resonance imaging (fMRI) to record human brain activity during slow (30 RPM), fast (60 RPM), passive (30 RPM), and variable rate pedaling. Ten healthy adults participated. After identifying regions of interest, the intensity and volume of brain activation in each region was calculated and compared across conditions ($p < .05$). Results showed that the primary sensory and motor cortices (S1, M1), supplementary motor area (SMA), and cerebellum (Cb) were active during pedaling. The intensity of activity in these areas increased with increasing pedaling rate and complexity. The Cb was the only brain region that showed significantly lower activity during passive as compared with active pedaling. We conclude that M1, S1, SMA, and Cb have a role in modifying continuous, bilateral, multijoint lower extremity movements. Much of this brain activity may be driven by sensory signals from the moving limbs.

Keywords: locomotion, fMRI, supraspinal, cycling

Many studies have used functional imaging technologies such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) to examine the effects of movement rate and complexity on human brain activity (Blinkenberg et al., 1996; Jancke et al., 1998; Jancke, Specht, Mirzazade, & Peters, 1999; Jenkins, Passingham, & Brooks, 1997; Khushu et al., 2001; Rao et al., 1996; Sadato et al., 1996; Sadato et al., 1997; Wexler et al., 1997). These studies, which have typically involved finger tapping, have shown that the intensity and volume of brain activity vary with task demands and that these activation changes are specific to particular brain regions. In comparison with the upper extremity, less is known about the way in which brain activity changes across different lower extremity movement tasks, particularly tasks that involve continuous, reciprocal, multijoint movements of both legs, which is the focus of this paper.

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Observations from the upper limb suggest that the intensity and volume of activity in the primary sensory and motor cortices (S1 and M1) and in the cerebellum (Cb) is sensitive to movement rate (Blinkenberg et al., 1996; Jancke et al., 1999; Jenkins et al., 1997; Khushu et al., 2001; Rao et al., 1996; Sadato et al., 1996; Sadato et al., 1997; Wexler et al., 1997). Several studies have shown that activation intensity in S1 and M1 increases linearly with movement rate at finger tapping frequencies up to 5 Hz (Blinkenberg et al., 1996; Rao et al., 1996; Sadato et al., 1997; Wexler et al., 1997). The volume of activation in these cortical regions also increases with increasing movement rates between 1 and 2 Hz, but movement rates beyond 2 Hz produce no further increases in activation volume as measured by functional imaging (Khushu et al., 2001; Rao et al., 1996; Sadato et al., 1997). Similar effects, albeit not without exception, have also been observed in the Cb during tapping and copying geometrical shapes (Jancke et al., 1999; Jenkins et al., 1997; Lewis et al., 2003; Sadato et al., 1996).

In contrast to M1, S1, and Cb, the effect of upper extremity movement rate is less robust in the premotor and supplemental motor areas (PMA and SMA). Instead, these structures may be sensitive to changes in task complexity. At least two studies have shown no change in activity in the PMA and SMA with tapping rates between 0.5 and 4 Hz (Blinkenberg et al., 1996; Wexler et al., 1997). Sadato and colleagues showed a "reverse rate effect" in the SMA whereby activity in this structure decreased with increasing tapping rate (Sadato et al., 1996). One of the few studies that have shown a direct relationship between movement rate and activity in the SMA and PMA used a joystick task in which volunteers made ballistic movements in response to a tone and were asked to freely select the direction of movement (Jenkins et al., 1997). Because of the decision making involved in this "free selection" task, it may have been a more sophisticated movement paradigm than tapping, and the observed rate effect may have reflected an interaction between movement rate and task complexity. This conclusion is supported by the observations of Wexler et al. who compared brain activation patterns during repetitive movements of the same finger and sequential movements of different fingers (Wexler et al., 1997). They found that the contralateral PMA and SMA showed a significant increase in activation volume during sequential as compared with repetitive tapping.

Observations from the upper limb provide a framework for understanding how brain activity changes across different lower limb movement tasks. However, it remains unclear whether upper and lower limb movements are similarly controlled with respect to rate and complexity because few studies have examined this issue during locomotor-like tasks of the lower limbs. Moreover, differences in the characteristics of arm and leg movements suggest that supraspinal control may also be different. Unlike finger tapping and other hand movements that have been examined to date, functionally important lower limb movements such as walking are characterized by bilateral, continuous, multijoint movements. These types of movements are strongly influenced by pattern generating circuits in the spinal cord (Duysens & Van de Crommert, 1998) and by sensory signals from muscle and joint afferents (McCrea, 2001; Van de Crommert, Mulder, & Duysens, 1998). Hence, the same supraspinal control strategies that modulate the rate and complexity of upper limb movement may not be used in the lower limb. Alternatively, control strategies that govern changes in movement rate and complexity may not be influenced by the end effectors (i.e., upper versus lower limb), the nature of the task (i.e., discrete versus continuous movement), or the number of limbs and/or joints involved. If

the latter explanation is correct, then the upper and lower limbs may use the same supraspinal control strategies to modulate movement rate and complexity.

The purpose of this study was to elucidate the role of the brain in controlling the rate and complexity of locomotor-like movements of the lower limbs. To this end, we examined brain activity during pedaling using fMRI. Pedaling is not the same as walking, but it involves rhythmic, reciprocal, flexion and extension movements of multiple joints in both legs, and it can be accomplished while lying supine on a scanner bed. We hypothesized that, if the brain employs similar control strategies for upper and lower limb movement, then activity in M1, S1 and Cb would increase with increasing movement rate, while activity in the PMA and SMA would increase with increasing movement complexity. Alternatively, if the brain has a lesser role in controlling locomotor-like movements of the lower extremities, as compared with the upper extremities, then brain activation would not be modulated with movement rate and complexity. To our knowledge this is the first report describing task-related changes in brain activation, as measured by fMRI, during continuous, rhythmic, reciprocal, multijoint movements involving both legs. Portions of this work have been reported previously in abstract form (Schindler-Ivens, Mehta, Schmit, Verber, & Wieser, 2008).

Material and Methods

Instrumentation and Data Recording

The pedaling device used for this study has been described previously (Mehta, Verber, Wieser, Schmit, & Schindler-Ivens, 2009). In brief, it was a direct drive apparatus fabricated from nonmetallic materials that could be positioned on an MR scanner bed and used to pedal forward or backward against a frictional load with rates up to 80 RPM. The device was equipped with a custom designed MR compatible optical encoder (model: TD 5207, Micronor Inc., CA) that was coupled to the crank shaft and used to measure pedaling velocity to ± 100 RPM and crank position to a resolution 1.8° . Signals from the encoder were output via a fiber optic cable to a controller unit (model: MR 310, Micronor Inc., CA) located outside the scanner room. The controller unit converted the optical signals to electrical signals and produced analog outputs corresponding to position and velocity. Position and velocity data were sampled at 2000 Hz using a laptop computer, a 16 bit analog to digital converter, and data acquisition software (micro 1401 mk II and Spike, Cambridge Electronic Designs, UK). These data were used to compute mean pedaling velocity across subjects and conditions.

A 3.0T GE short bore MR scanner (General Electric Healthcare, Milwaukee, WI) and a GE single channel transmit/receive split head coil assembly (GE model 2376114) were used for all experiments. Audacity (open source software) and Presentation (Neurobehavioral Systems, CA) software were used to deliver audio output to the subjects via MR compatible earphones (model SRM 212, Stax, Japan).

fMRI images were obtained using a gradient echo, echo planar imaging (EPI) pulse sequence (36 contiguous slices in the sagittal plane, 4 mm slice thickness, echo time (TE) = 25 ms, interscan period (TR) = 2 s, flip angle = 77° , field of view (FOV) = 24 cm, and 64×64 matrix). The resolution of the images was $3.75 \times 3.75 \times 4$ mm. Approximately half way through the pedaling protocol, 148 high resolution spoiled GRASS (gradient-recalled at steady state) anatomical images

were collected with TE = 3.9 ms, TR = 9.5 ms, flip angle = 12°, matrix of 256 × 244, and slice thickness of 1 mm.

Subject Selection and Preparation

Ten healthy individuals (6 males, mean age of 31 years, range: 21–53) with no elite training in pedaling volunteered to participate. Each subject gave written informed consent according to the Declaration of Helsinki and institutional guidelines at Marquette University and the Medical College of Wisconsin.

Before participating, subjects underwent at least three fMRI safety screenings and were excluded if they were claustrophobic, pregnant, or had any implants or foreign bodies incompatible with fMRI. Subjects were also excluded if they had a history of neurological impairments or physical conditions contraindicated to pedaling. Each subject participated in a familiarization session outside the MR environment where we explained the experimental procedures and taught them to pedal at 30 and 60 RPM, cued by a metronome. They also practiced relaxing their lower extremities while the experimenter pedaled their legs in the device. During this session we also explained the importance of remaining still during fMRI and encouraged subjects to keep their head and trunk stationary while pedaling. Subjects did not practice the variable rate pedaling task.

Experimental Set-Up

During fMRI scanning, subjects lay supine on the scanner bed with their feet secured in a pair of sandals that were fastened to the pedals as shown in Figure 1. Subjects' buttocks rested on the base of the pedaling device. The position of the pedaling

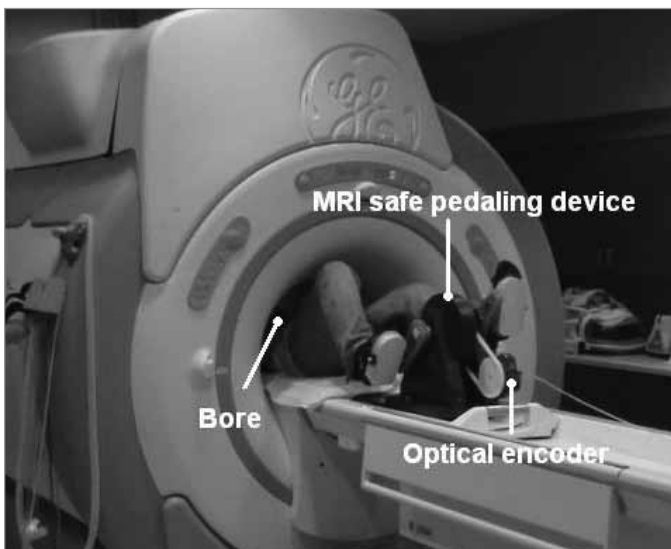


Figure 1 — Experimental setup with subject positioned inside the scanner bore and ready to pedal.

device was adjusted until subjects were able to pedal comfortably and their legs did not touch the scanner. Each subject wore MR compatible ear phones through which audio cues were delivered and an additional set of headphones to protect against scanner noise. The subject's head was placed in the head coil and adjusted to achieve symmetry in all 6 planes of movement (superior-inferior, left-right, anterior-posterior, roll, pitch, and yaw). To minimize head movement, the subject's head rested in a beaded vacuum pillow that enveloped the entire head (except the face) and created a firm, comfortable, "brace" around the head. A chin strap was used to prevent inferior-superior head movement. The subject's torso was stabilized with a wide Velcro strap to minimize trunk movement. Additional padding under the buttocks and shoulders was provided for comfort. Subjects grasped an emergency squeeze ball that could be used at any time to signal a problem. Participants were monitored for safety and comfort and were able to communicate via intercom with the scanner technician.

Experimental Protocol

Participants performed four different tasks, slow (30 RPM), fast (60 RPM), passive (30 RPM), and variable rate pedaling (mean rate: 45 RPM) while scanning to obtain functional images of the brain. Tasks were presented in a block design consisting of 3 runs of each pedaling condition. Each run lasted 4 min. In a single run, subjects pedaled for 30 s, then rested for 30 s. This sequence was repeated 4 times. During the slow, fast, and variable conditions, subjects actively pedaled the bicycle at the rate indicated by the metronome. During passive pedaling, both the subject and an experimenter listened to the metronome cues. Subjects were instructed to remain completely relaxed while the experimenter pedaled the bicycle. During the variable task, the pedaling rate varied randomly between 30 and 60 RPM with a mean rate of 45 RPM. In all other tasks, pedaling rate was constant. Throughout the experiment, subjects' pedaling performance was visually monitored through the control room window and by examining the position and velocity data from the optical encoder. We also had access to real time information about head position. If the subject did not perform the task as instructed or if their head moved more than 2 mm, we checked the subject for comfort, repeated the instructions to remain still, and restarted the run.

Data Processing and Statistics

fMRI signal processing was completed using Analysis of Functional NeuroImages (AFNI) software. Raw fMRI data were converted into 3 dimensional (3D) images and were registered to the functional scan obtained closest in time to the anatomical scan. Functional datasets were averaged across all runs within a pedaling condition to minimize the effect of noise. Correlation analysis was performed on the average signal.

Conventional fMRI signal processing techniques for block designs in which the entire BOLD signal is fit with a boxcar function are not appropriate for analyzing pedaling-related brain activation because limb movement causes signal distortion (Mehta et al., 2009). To overcome this confound, we extracted the portion of the signal after movement stopped (i.e., the rest period) and correlated only this portion of the signal with the corresponding portion of the standard model (box car convolved with a gamma function). See Figure 2, where the shaded region denotes

the rest period during which the BOLD signal (blue) was correlated with the model (green). From these data we used standard AFNI functions to calculate the correlation between the model and the data and the percent change in BOLD signal from the baseline. This approach is justified because the onset and termination of BOLD signals are delayed with respect to a given task (Bandettini & Cox, 2000; Blamire et al., 1992). Hence, movement-free BOLD signal should be present when pedaling has stopped. The validity of this approach has been described previously (Mehta et al., 2009).

Group activity maps were obtained as follows. After overlaying the functional data on the anatomical images and identifying standard landmarks, data from each subject were transformed into the standardized coordinate system of Talairach and Tournoux (Talairach & Tournoux, 1988). Functional data were blurred using a 4 mm full width half maximum Gaussian filter. These data were then entered into a t test to identify voxels containing BOLD signal that was significantly different from baseline. A threshold for the t test was selected using a Monte Carlo simulation (AlphaSim) that set an appropriate individual voxel p -value ($p < .000001$) and cluster size threshold to maintain a familywise error rate of $p < .05$. The values for percent change in BOLD signal for each significant voxel were displayed as a group color map superimposed on the Talairach reference brain. The spatial coordinates for the Brodmann areas and cerebellar lobules that were significantly activated were also determined from the Talairach atlas.

Values for volume and intensity of activation were calculated for each subject as follows. We applied the standard Talairach atlas to the group activation maps and identified regions that were commonly activated for all subjects and across all 4 pedaling conditions. The spatial coordinates of commonly active regions, referred to as regions of interest (ROIs), were then obtained from a standard Talairach and Tournoux maximum probability map (TT-MPM) atlas and transformed into each

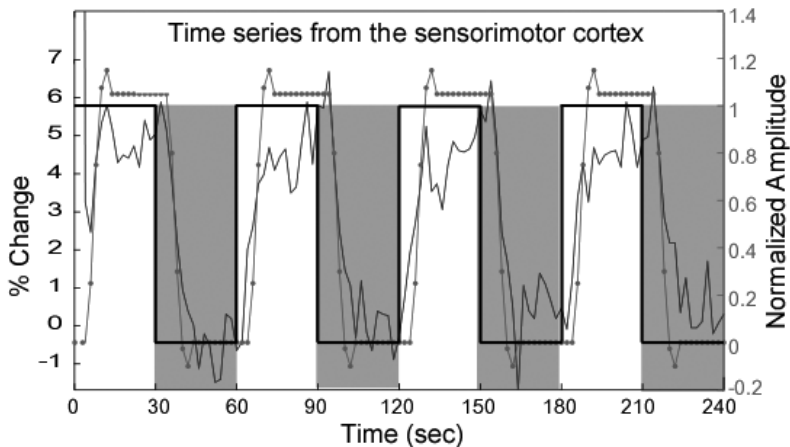


Figure 2 — Time series voxel from a single subject taken from the sensorimotor cortex. Gray shaded region shows portion of the data (blue) fit to the model (green). Black line represents the block design of the pedaling task. The x-axis represents time, and the y-axes represent the amplitude of the BOLD signal (left) and the model (right).

subject's original space. After transformation we visually inspected the position of the ROIs in each individual subject's map and confirmed that the group coordinates represented the same anatomical regions in individual subjects as in the group maps. Individual subject values from each ROI were entered into a *t* test to identify voxels containing BOLD signal that was significantly different from baseline. A threshold for the *t* test was selected using a Monte Carlo simulation to maintain a familywise error rate $p < .05$. Individual voxel *p*-values were 0.0002 for M1/S1, 0.0001 for SMA, and 0.0006 for Cb. Voxels that passed this threshold were included in subsequent analyses.

The number of significantly active voxels in each ROI was counted to obtain a value for activation volume. The mean percent change in BOLD signal from baseline was computed from these volumes. This analysis was performed on each subject for each ROI activated in the group dataset for each pedaling condition. These values, which were used for the group statistics explained below, described the spatial extent and intensity of activation during different conditions and within each ROI.

One way analysis of variance with repeated measures was used to identify differences in activation volume and intensity across pedaling conditions in each ROI. Differences were considered significant at $p < .05$. When significant main effects of condition were identified, Dunnett's post hoc procedure was used to compare the slow condition to every other pedaling condition. Comparisons between slow and variable pedaling and slow and fast pedaling allowed us to examine changes in brain activation associated with changing task demands. The comparison between slow and passive pedaling allowed us to distinguish between brain activation associated with producing movement and activation arising from passive movement of the limbs.

We further examined Brodmann area 6, which contains both the SMA and premotor area (PMA), to understand the extent to which each of these regions was active during pedaling, and we found that nearly all the activity in Brodmann area 6 was in the SMA. This processing was completed as follows. Before applying a threshold for brain activation to the group maps, the full range of *t* values in the combined SMA and PMA region for each pedaling condition was examined. There were two peaks in the *t* values. One peak corresponded to the known anatomical location of the SMA and the other to the PMA (Jenkins et al., 1997; Kandel E.R., Schwartz J.H., Jessell T.M., 1991; Wexler et al., 1997). Manual editing was performed to remove the "valley" between the peaks, allowing separation of SMA and PMA. Thresholds were applied to each pedaling condition as reported above, and the thresholded maps were divided along the boundary established in the previous step. Upon completing this processing, we saw that more than 98% of the significant voxels were in the SMA. Less than 2% were in the PMA.

To extract individual subject voxel counts and intensity of activation values, the separated maps for each of the four pedaling conditions were combined (OR operation). This thresholded group-level, separated SMA/PMA, combined pedaling condition map (in Talairach space) was converted to each subject's original space. To maintain the size of the regions in original space, and because the original space voxels are larger than the Talairach voxels ($3.75 \times 3.75 \times 4$ mm vs. $1 \times 1 \times 1$ mm), only original space voxels that were overlapped 50% or more by Talairach space voxels were included. Because of this step, the small PMA areas were lost for many of the individual subjects. These observations indicate that the results for Brodmann area 6 were driven predominantly by the SMA.

Results

Task Performance

All subjects were able to perform the pedaling tasks as evidenced by group mean (*SD*) pedaling rates of 30.4 (0.4), 45.0 (1.0), 59.7 (0.6), and 29.9 (0.7) RPM for the slow, variable, fast, and passive conditions, respectively. On average, subjects displayed less than 0.5 mm of translational head movement and less than 0.5° of head rotation during all pedaling conditions except the passive condition where the head moved 1.4 (2.0) mm in the inferior direction. There was no significant effect of pedaling condition on head movement ($p > .06$).

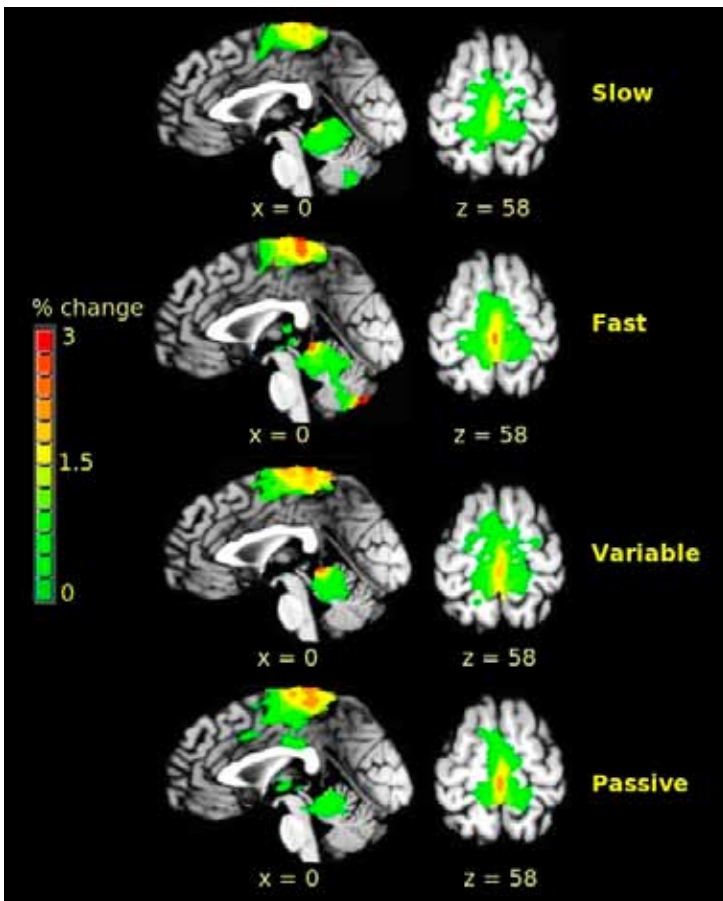


Figure 3 — Functional data (% change values) overlaid on reference brain showing group activity ($N = 10$) for slow, fast, variable, and passive pedaling conditions. Images are snapshots from the sagittal and axial planes, the location of which are represented by x and z coordinates. Color maps indicate percent change in BOLD signal with red being the maximum change from baseline.

Brain Activation During Active Pedaling

All four pedaling conditions produced significant bilateral activation in M1, SI, SMA, and the Cb vermis (lobules IV, V, and VIII). There was no significant activation in PMA. Figure 3 displays active brain regions for the entire group during each condition. Table 1 displays the spatial coordinates and volume of activation for each condition as measured from the group data.

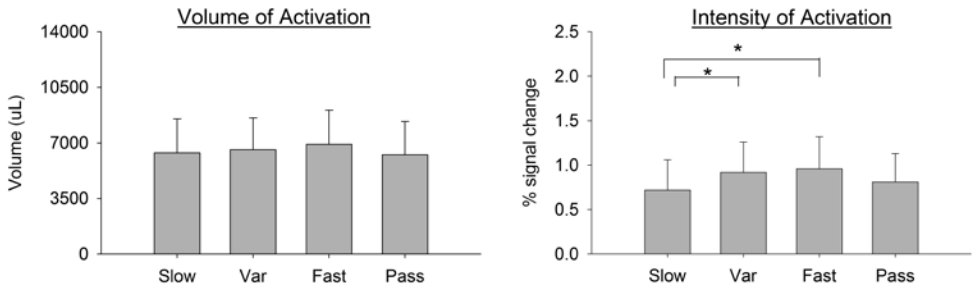
Table 1 Areas of Activation with Coordinates of Center of Mass for Each Pedaling Condition.

| Condition | Region ^a | Coordinates for center of mass | | | Volume (μL) |
|-------------------|--|--------------------------------|----------|----------|-------------|
| | | R-L (mm) | A-P (mm) | I-S (mm) | |
| Slow (30 RPM) | B. Primary sensorimotor cortex (1,2,3,4) | -1.1 | 36.2 | 59.6 | 9299 |
| | B. SMA (6) | -0.7 | 18.5 | 59.8 | 10708 |
| | B. Cerebellar vermis (IV/V) | 1.1 | 44.3 | -9.7 | 6194 |
| | B. Cerebellar vermis (VIII) | 2.5 | 60.0 | -34.3 | 516 |
| Fast (60 RPM) | B. Primary sensorimotor cortex (1,2,3,4) | 0.2 | 37.0 | 58.7 | 10857 |
| | B. SMA (6) | 0.5 | 16.5 | 59.1 | 12762 |
| | B. Cerebellar vermis (IV/V) | 0.8 | 44.9 | -14.9 | 4298 |
| Variable (45 RPM) | B. Primary sensorimotor cortex (1,2,3,4) | 0.0 | 36.6 | 58.5 | 10906 |
| | B. Primary sensorimotor cortex (1,2,3,4) | -53.3 | 17.2 | 30.2 | 201 |
| | B. SMA (6) | -0.4 | 13.8 | 57.5 | 17919 |
| | R. SMA (6) | -31.6 | 11.7 | 47.2 | 185 |
| | B. Cerebellar vermis (IV/V) | -0.9 | 45.7 | -14.3 | 5483 |
| Passive (30 RPM) | B. Primary sensorimotor cortex (1,2,3,4) | -1.0 | 36.1 | 58.6 | 10645 |
| | B. SMA, (6) | -0.6 | 14.3 | 57.6 | 14038 |
| | B. Cerebellar vermis (IV/V) | -3.5 | 27.5 | -13.1 | 2084 |

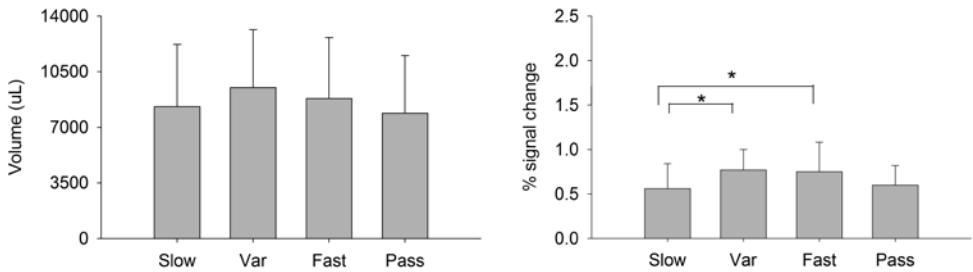
R = Right, L = left, A = anterior, P = posterior, I = inferior, S = superior, B = bilateral, SMA = supplemental motor area. Negative values represent right, inferior, and anterior. ^aBrain regions are described by name, Brodmann area (cortex), and cerebellar lobule (cerebellum).

In comparison with the slow condition, fast and variable pedaling produced a higher intensity of activation in M1, S1, SMA, and Cb ($p \leq .02$) (Figure 4). Increased pedaling rate and variability in pedaling rate had no effect on the volume of activation in any region examined ($p \geq .18$).

PRIMARY SENSORIMOTOR CORTEX



SUPPLEMENTAL MOTOR AREA



CEREBELLUM

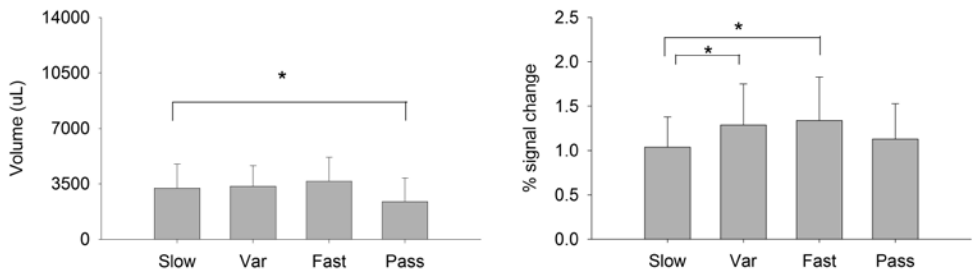


Figure 4 — Graphs represent the intensity (percent signal change) and spatial extent (volume) of activation across pedaling conditions in each region of interest. Asterisks indicate significant differences between conditions.

Effect of Passive Movement

In M1, S1, and SMA, there was no significant difference in the intensity or volume of activation during slow and passive pedaling ($p \geq .23$). In the Cb, the volume of activation was significantly lower during passive as compared with slow pedaling ($p = .045$). The intensity of activity in the Cb was not different during active and passive pedaling ($p = .28$).

Discussion

This study showed that M1, S1, Cb, and SMA were active during pedaling and that the intensity of activity in these areas increased with increasing pedaling rate and complexity. Responses to changing task demands were similar in primary (M1, S1) and secondary (SMA) sensory and motor areas. There was no significant difference in the intensity or volume of brain activity in M1, S1, or SMA during passive and active pedaling. Only the volume of activity in the Cb was lower during passive as compared with active pedaling. These observations demonstrate that cortical and subcortical brain regions are active during continuous, rhythmic, lower extremity movements and that activity in these regions is related to movement rate and complexity. Our data also suggest that this brain activity may be driven by sensory feedback.

Changes in Brain Activity Associated with Increasing Pedaling Rate

Our results agree partially with previous work examining the effects of upper extremity movement rate on brain activity. Consistent with our hypothesis and in accordance with finger tapping studies (Blinkenberg et al., 1996; Jancke et al., 1999; Jenkins et al., 1997; Rao et al., 1996; Sadato et al., 1996; Sadato et al., 1997; Wexler et al., 1997), increased pedaling rate resulted in increased *intensity* of activity in M1, S1, and Cb. However, unlike upper limb tapping studies, increasing movement rate did not increase activation *volume* (Jancke et al., 1999; Khushu et al., 2001; Rao et al., 1996; Sadato et al., 1997). Our findings are also in line with studies involving copying geometric shapes that have shown a direct relationship between movement rate and activation intensity in the medial Cb (Lewis et al., 2003) and with studies demonstrating that activity in M1 can be used to predict hand velocity during tracing (Schwartz, 1993; Schwartz, 1994; Schwartz & Moran, 1999). Moreover, Christensen et al. used PET to show that regional cerebral blood flow in M1 and Cb increased with increasing pedaling rate (Christensen et al., 2000). This group was unable to assess changes in activation volume, per se, because PET cannot make distinctions between intensity and volume changes (Fox, Fox, Raichle, & Burde, 1985).

It is thought that increased BOLD responses detected with fMRI are functionally meaningful and caused by increased synaptic activity and elevated neuronal firing frequencies in the brain (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). Specific to motor tasks, Lutz et al. have suggested that increased BOLD signals are caused by increased neuronal firing rates that are required to generate higher muscle forces to increase movement velocity (Lutz, Koeneke, Wustenberg, & Jancke, 2005; Rao et al., 1996). This idea is supported by single cell recordings in monkeys that have shown that firing rates of M1 neurons were positively correlated with the increased force and velocity that was needed to produce faster finger movements (Humphrey, 1972). Hence, our observations suggest that neurons in M1, S1, and Cb increase their synaptic activity and/or firing frequency to increase pedaling rate. Moreover, the same brain regions that increase their neuronal activity to produce faster finger movements (M1, S1, and Cb) also modulate the rate of continuous, bilateral, multijoint movements of the lower limbs, suggesting shared supraspinal control strategies for the upper and lower limb.

We were surprised that increased pedaling rate did not increase activation volume in M1, S1, or Cb because others have shown rate-dependent increases in the activation volume in these structures during finger movements (Jancke et al., 1999; Khushu et al., 2001; Rao et al., 1996; Sadato et al., 1997). Perhaps, unlike the upper limb, the entire network of neurons involved in pedaling was active even at the lowest demand condition. However, this observation must be considered in the context of the reliability and validity of fMRI measurements of brain activity.

In comparison with other quantitative measures of brain activity derived from BOLD fMRI, such as activation intensity, volume is less reliable (Aron, Gluck, & Poldrack, 2006; Kimberley, Birkholz, Hancock, VonBank, & Werth, 2008; Kimberley, Khandekar, & Borich, 2008; Lim, Choo, & Chee, 2007; Loubinoux et al., 2001; Marshall et al., 2004). Hence, our failure to see between-condition differences in activation volume may have been due to measurement variability. Poor reliability of activation volume has been attributed to the "all or none" nature of the calculation (Kimberley, Khandekar et al., 2008). Because a voxel is considered active based on a given threshold, subtle changes in activation can be magnified if a voxel is hovering around threshold. Intensity is not dependent on a threshold, which may make it a more stable measure than volume and more sensitive to changes in neural activity.

It is also possible that activation volume did not change because, in comparison with signal intensity, the volume of the BOLD fMRI signal may be a less valid measure of brain activity. Rao et al. suggested that increased brain activation volume with increased movement rate is not due to a widening of the area of activation, but rather, enhanced signal-to-noise ratio that leads to better detection of voxels on the fringe of the primary activation site (Rao et al., 1996). This conclusion was based on the observation that, during finger tapping, activation volume increased from the nominal to the next fastest condition, but did not increase further with increasing movement rate; whereas, activation intensity increased linearly with increasing tapping rate across all rates examined.

Finally, the pedaling rates used in this study may account for no change in the volume of brain activity. Previous studies showed that activation volume increased most substantially in M1 as finger movement rate increased from 1 to 2 Hz (Rao et al., 1996) and in the Cb as movement rate increased from 1 to 3 Hz (Jancke et al., 1999). In the current study, pedaling rate increased twofold, but never exceeded 1 Hz (i.e., 60 RPM). The slower movement rates used in this study, as compared with finger tapping studies, were selected to minimize head movement. At these relatively slow rates, subjects may not have pedaled fast enough to reveal rate-dependent volume changes.

Brain Activity During Variable Rate Pedaling

Our hypotheses predicted that activity in the PMA and SMA would increase during variable rate pedaling, as compared with the slow condition, but that activity in these structures would not be sensitive to changes in pedaling rate. We also predicted that activity in M1, S1, and Cb would not increase with increasing task complexity. These hypotheses were based on observations from the upper extremity (Blinkenberg et al., 1996; Wexler et al., 1997; Sadato et al., 1996; Jenkins et al., 1997). Consistent with these predictions, the SMA showed an increase in activation

intensity during variable rate pedaling as compared with slow pedaling. However, the intensity of activity in the SMA was similarly sensitive to changes in pedaling rate. Moreover, M1, S1, and Cb showed increases in activation intensity during variable rate pedaling that were similar to those observed during fast pedaling. One possible explanation for these observations is that, during rhythmic, multijoint movements of the legs, movement rate and complexity may not be controlled by different brain regions, like they are in the hand. Instead, all active brain regions may modulate pedaling rate and complexity.

Alternatively, the variable rate condition may not have been sufficiently challenging to reveal complexity-related changes in signal intensity in the SMA. In the upper limb, task complexity has been manipulated by comparing repetitive movements of the same finger to sequential movements of different fingers (Elsinger, Harrington, & Rao, 2006; Wexler et al., 1997). Authors demonstrate that one task is more complex than another by showing increased performance errors, reaction times, and/or movement times. Our subjects were equally capable of producing the desired pedaling rate during all conditions, suggesting that the variable rate task may not have been more difficult than the other conditions. We may have inadvertently examined the effect of three different pedaling rates on brain activity. During the variable condition, pedaling rate varied between 30 and 60 RPM with a mean rate of 45 RPM, which was the midpoint between the slow and fast condition. Therefore, the increase in brain activation during the variable rate condition may be due to rate effects. The observation that there was no additional increase in signal intensity between the variable and fast condition may mean that the effect of pedaling rate saturates somewhere between 45 and 60 RPM.

Another factor that may have influenced our results was the type of cue used to guide pedaling. All tasks were externally cued to ensure that subjects produced the desired pedaling rates. During slow and fast conditions, cues were predictably paced. During the variable condition, cues were unpredictably paced. A common finding is that the SMA is preferentially activated during predictable movements, whether externally cued or self-initiated (Deiber, Honda, Ibanez, Sadato, & Hallett, 1999; Jenkins, Jahanshahi, Jueptner, Passingham, & Brooks, 2000). The PMA is most active during externally cued, unpredictable movements (Elsinger et al., 2006). These findings suggest that the SMA is important for planning and executing actions from memory; while the PMA is important for planning movements that are guided by sensory cues. Hence, we might have expected to see more activity in the SMA during slow and fast pedaling as compared with variable pedaling and more activity in the PMA during variable as compared with fast and slow pedaling. However, the SMA showed more activity during variable and fast pedaling as compared with slow pedaling, which points to a rate-related and not a cue-related trigger. Perhaps the functions ascribed to the PMA and SMA based on discrete movements of the upper limb do not apply to continuous, bilateral movements of the legs.

Brain Activity During Passive Pedaling

The brain activation observed in this study may have been induced by sensory signals from the moving limbs. Our data suggest that supraspinal structures, particularly the cerebral cortex, do not produce the fundamental pattern of muscle output associated with locomotor-like leg movements. Instead, these structures

may monitor sensory information from the moving limbs and use this information to respond to external perturbations or to maintain, reinforce, or shape the pattern of ongoing locomotor output.

This study showed that activity in M1, S1, and SMA was not significantly different during passive and active pedaling. Comparable brain activation during active and passive movement is a counterintuitive observation, as one might assume that the observed activity represents an internally generated, descending locomotor command. We cannot rule out the possibility that some of the brain activity observed during passive pedaling may have been due to unintended muscle activity. However, our result is not surprising in light of other studies that have shown comparable activity in motor areas of the brain during active and passive movements (Christensen et al., 2000; Radovanovic et al., 2002; Terumitsu, Ikeda, Kwee, & Nakada, 2009; Weiller et al., 1996). Weiller et al. (Weiller et al., 1996) showed that during active and passive elbow movements there was a strong increase in regional cerebral blood flow (rCBF) in the contralateral sensory and motor cortices and in the SMA. Christensen et al. (Christensen et al., 2000) also showed that passive pedaling activated areas of the brain that were similar to and nearly as extensive as active pedaling. Both groups concluded that sensory feedback from the moving limbs may explain a substantial part of cerebral activation, and Christensen et al. suggested that sensory feedback to the cortex may help maintain rhythmic, alternating movements.

Further support for the assertion that the cortical activity during pedaling was caused by sensory input used to modify locomotor output comes from studies demonstrating a transcortical contribution to stretch and cutaneous reflexes elicited during sitting and walking (Christensen, Morita, Petersen, & Nielsen, 1999; Christensen, Andersen, Sinkjaer, & Nielsen, 2001; Nielsen, Petersen, & Fedirchuk, 1997; Petersen, Christensen, Morita, Sinkjaer, & Nielsen, 1998; Zuur, Christensen, Sinkjaer, Gray, & Nielsen, 2009). Zurr et al. showed that the late component of the tibialis anterior (TA) stretch reflex elicited during the swing phase of gait was suppressed after 20 min of repetitive transcranial magnetic stimulation (rTMS), a technique that inhibits motor cortex output (Zuur et al., 2009). The authors concluded that the TA stretch reflex was partially mediated via the motor cortex and suggested that the motor cortex interacts with proprioceptive feedback to deal with the complex demands of walking.

The animal literature also suggests that sensory signals modulate activity of M1 neurons. Cheney and Fetz (Cheney & Fetz, 1984) showed that corticospinal cells in the monkey cortex respond at short latency to stretch of their target muscles. Others have demonstrated that corticospinal neurons in the motor cortex of standing and walking cat are modulated at short latency by afferent input (Karayannidou et al., 2008; Marple-Horvat, Amos, Armstrong, & Criado, 1993). Comparable observations have been made in humans where cutaneous and proprioceptive input enhances motor responses to TMS of M1 during sitting and walking (Christensen et al., 1999; Christensen et al., 2001; Nielsen et al., 1997; Petersen et al., 1998).

The Cb was the only structure observed in this study to demonstrate a lower level of activity during passive as compared with active pedaling. Christensen and colleagues (Christensen et al., 2000) also showed a decrease in activity in the Cb during passive versus active pedaling. However, it must be emphasized that, in both studies, there was a substantial amount of activity in the medial aspect of the Cb

during the passive condition. Again, we cannot completely rule out motor contributions to this observation; however, these data suggest that the Cb may have a role in producing locomotor movement and processing locomotor-related sensory feedback. Mori et al. (Mori, Matsui, Mori, Nakajima, & Matsuyama, 2000) have shown that, in the decerebrate cat, stimulation of the hook bundle of Russell, which is located in the white matter of the medial Cb, produces well-coordinated treadmill locomotion that is similar to that evoked by stimulating the mesencephalic locomotor region. White matter tracts in the hook bundle of Russell originate in the fastigial nucleus of the Cb and project to reticulospinal and vestibulospinal neurons in the brainstem and to areas 4 and 6 of the cortex. Hence, portions of the Cb may initiate and regulate locomotion via cortical and brainstem pathways. Information from nearly all sensory systems converges on the Cb (Brodal, 1978), and it can be activated by tactile and proprioceptive stimuli in the absence of movement (Fox, Raichle, & Thach, 1985; Gao et al., 1996; Naito et al., 2002). Some authors have even suggested that the Cb is involved in sensory discrimination and not movement, *per se* (Gao et al., 1996). Others have reported movement-related Cb activation in the absence of sensory input (Weeks, Gerloff, Honda, Dalakas, & Hallett, 1999). Our data point to both a sensory and motor role of the Cb during pedaling.

Conclusions

In conclusion, this study demonstrated significant activity in M1, S1, SMA, and Cb during pedaling that increased with increasing pedaling rate and complexity, suggesting that these structures have a role in modifying continuous, bilateral, multijoint lower extremity movements. Similar levels of cortical and Cb activity were present during active and passive pedaling, which suggests an important role for sensory signals in producing brain activity during pedaling. Additional studies are needed to further elucidate the relationships among brain activity and pedaling rate, task complexity, and sensory feedback.

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