Straight Walking and Steering of Gait Neuronal Correlates in the Young Healthy Human Brain: an [^{18}F]-FDG-PET Study

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August 2016

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

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ABSTRACT

Steering of gait is an essential element of goal-directed locomotion involving a high degree of sensorimotor integration, which places a significant load on the central nervous system. Previous functional neuroimaging studies in subjects imagining walking and stance have found common activations involving the sensorimotor and premotor cortices, basal ganglia, midline cerebellar regions and occipital visual areas (Malouin, et al., 2003; Jahn et al., 2004). Additionally, when imagining walking around obstacles the inferior parietal regions and precuneus were recruited. However, these results are limited in understanding cerebral activations during gait modifications, as such protocols do not involve actual walking and cannot account for sensorimotor integration. Therefore, the aim of this Masters study was to use [^{18}F]-fluorodeoxy-glucose (FDG) Positron Emission Tomography (PET) to quantify regional cerebral glucose metabolism (rCGM) during steering of gait and straight walking. It was predicted that cortical regions such as premotor, posterior-parietal cortices and midline cerebellar area would be actively recruited during steering of gait, as more automatic-lower-ordered regions would be involved in straight walking.

Seven young healthy subjects (*Mean age* = 25yrs) were injected with 150 MBq of [^{18}F]-FDG and began 1 of the 3 motor tasks for 40 minutes (uptake time of tracer is weighted to the first 30 minutes) immediately after injection: 1) steering of gait (SG) involving multiple, successive right and left turns, 2) straight walking (SW) and 3) upright standing (US). The 40-minute image acquisition started ~6 minutes after task completion, therefore images were obtained well before the end of the 2-hour half-life of the tracer. US and SW served as reference

tasks to isolate activations for SW (i.e., SW-US) and SG (i.e. SG-SW), respectively. Regions showing significant increases and decreases in rCGM were thresholded at $p < 0.05$.

Steering of gait showed predominant activation of the posterior parietal areas, including the SPL, precuneus, parahippocampal gyrus and midline cerebellar regions during complex walking. As SW recruited the paracentral lobule, occipital lobe, midline and left cerebellum. These findings display region-specific activations for SG and SW which relate to the functional responsibility of such cerebral cortices during human locomotion.

This study is the first detailed functional neuroimaging study comparing straight walking and steering of gait in the healthy human brain during these locomotor tasks. The findings propose region specific neural areas which supervise steering of gait and straight walking. Furthermore, this understanding of sensorimotor integration in young healthy humans is crucial in understanding the compensatory mechanisms of normal aging and developing rehabilitation therapies in pathological populations afflicted with gait disturbances.

RÉSUMÉ

Le contrôle de la trajectoire de marche est un élément essentiel de la locomotion qui implique un important degré d'intégration sensorimotrice, sollicitant grandement le système nerveux central. Des études en neuroimagerie fonctionnelle ont démontré qu'imaginer la marche ou la station debout active des régions du cortex sensorimoteur et prémoteur, des noyaux gris centraux, des régions centrales cérébelleuses et des aires visuelles occipitales (Malouin et al. 2003., ;Jahn et al., 2004). De plus, en imaginant la marche autour d'obstacles, les régions pariétales et précuneus sont recrutées. Cependant, ces résultats limitent notre compréhension des activations cérébrales lors de modifications de la marche car ces protocoles ne s'appliquent pas à la marche réelle et par conséquent, ne peuvent expliquer la fonction sensorimotrice car elle n'est recrutée que lors de mouvements réels. Le but de cette étude de maîtrise était donc d'utiliser la tomographie par émission de positrons (PET) avec le marqueur [^{18}F]-fluorodeoxy-glucose (FDG) pour quantifier le métabolisme du glucose cérébral régional (rCGM) pendant la redirection de la marche et lors la marche en ligne droite. Il était postulé que les régions d'ordre supérieur (le cortex moteur primaire, le cortex prémoteur, les aires corticales pariétales et la zone cérébelleuse centrale) seraient recrutées activement pendant le pilotage de la marche. Les régions plus automatiques d'ordre inférieur (le cortex sensorimoteur et les zones occipitales visuelles) seraient impliquées dans la marche en ligne droite.

Sept jeunes individus (*Age moyen* = 25ans) en santé ont effectué l'une des trois tâches motrices pendant 40 minutes (temps d'absorption du traceur est de 30 minutes) immédiatement après avoir reçu l'injection de 150 MBq du marqueur [^{18}F]-FDG: 1) la marche avec virages vers la droite et la gauche (i.e., le contrôle de la trajectoire de marche) 2) la marche en ligne droite et

3) la station debout. L'acquisition des images a débuté ~ 6 minutes après la fin de chacune des tâches motrices. Les images ont donc été obtenues bien avant la fin de la demi-vie du traceur (environ 2 heures). Les images collectées lors des tâches de référence ont été soustraites des images obtenues lors des tâches d'intérêt. Donc, dans le but d'obtenir des régions recrutées uniquement pendant le contrôle de la trajectoire de marche, les images de la marche en ligne droite ont été soustraites. De même, afin d'obtenir les images de la marche en ligne droite, les images lors de la station debout ont été soustraites. Les régions ayant un seuil à p <0,05 sont considérée comme ayant des augmentations et des diminutions significatives en rCGM.

Durant la marche en ligne droite, les résultats démontrent une augmentation d'activation des régions sensorimotrices centrales (M1, S1, PMC, SMA), des aires visuelles occipitales et de la région centrale du cervelet. Durant le contrôle de la trajectoire de marche, les résultats ont permis de voir une augmentation de l'activation des régions pariétales supérieures, des régions prémotrice et primaire, des régions visuelles, des régions du lobe occipital et des régions antérieures du cervelet.

Ceci est la première étude détaillée en neuroimagerie fonctionnelle comparant la marche en ligne droite et le contrôle de la trajectoire de marche dans le cerveau humain sain. Les résultats proposent la présence de régions cérébrales distinctes qui supervisent le contrôle de la trajectoire de marche et la marche en ligne droite. De plus, ces résultats chez les jeunes individus en bonne santé sont essentiels à la compréhension des mécanismes de compensation du vieillissement normal ainsi que pour le développement de thérapies de réadaptation dans les populations pathologiques qui ont des troubles de marche.

ACKNOWLEDGMENTS

This thesis would not have been possible without the contribution and support of several individuals. I would like to begin by thank the members of my advisory committee, Dr. Mallar Chakravarty and Dr. Jean-Paul Soucy, for their expertise, advice, guidance and feedback. I would also like to thank all members of the human brain control of locomotion lab during this thesis, including Dorelle Hinton, Kelly Perlman, Rhéa Coriaty and Rehka Raveendrakumar. With special thanks to Ilse Frias her invaluable moral support and assistance with data collection. I would also like to thank the PET and MRI technicians and support staff at the Montreal Neurological Institute for their professional assistance during data collections. I would express gratitude to my parents and family for their continuous support and belief in my academic aspirations throughout this degree. Lastly, I would like to thank my supervisor, Dr. Caroline Paquette, for her unwavering support, encouragement, critical feedback and mentoring during this thesis. I am very grateful for the opportunity she provided me with and I am very happy with the experience in neuroimaging she provided me with as it has developed my passion and curiosity in the field. This has been a continuous learning process and would not have been possible without each of these persons support, encouragement and expertise. I am forever grateful for the experience they were a part of.

This thesis was funded through studentships from the Canadian Institutes of Health Research, Department of Kinesiology and Physical Education, and GREAT travel award in 2015 and 2016.

CONTRIBUTION OF AUTHORS

Faryn Starrs, the candidate, was responsible for the adjustment of the study design, recruitment and screening of subjects, setting up the protocol and the data collection, analyzing all data and wrote the manuscript presented in Chapter 3. Caroline Paquette developed the initial design for this study and received funding for this project. They revised the manuscript and figures for publication. Jean-Paul and Paul Gravel assisted in developing a customized script for used in pre-processing of the data. Ilse Frias assisted in data collections and in developing the pre-processing methodology. Caroline Paquette supervised all aspects of the study, conceptual framework, developing the experimental protocol, data analysis procedures and correcting the manuscript and figures.

CHAPTER 1. INTRODUCTION

1.1 Rationale

Turning while walking is an essential element of goal-directed locomotion as it allows one to steer around and avoid obstacles, and steer around obstacles as they move through the environment (Lamontagne, Paquette et al. 2007). Therefore, steering of gait involves a high degree of sensorimotor integration. This includes various sensory inputs (e.g. vestibular, vision, proprioception) (Lamontagne and Fung 2009), along with fine coordination of movements and motor commands to maintain posture and body reorientation towards a desired direction (Lamontagne, Paquette et al. 2007). Additionally, we make about 1000 turns per day while walking (Mancini, El-Gohary et al. 2015), which equates to approximately 20% of steps taken during our lifespan are during turning while walking (Sedgman, Goldie et al. 1994).

Directional changes and steering of gait are complex motor tasks which place a significant burden on the central nervous system, especially with normal aging and those afflicted with neurological diseases or disorders such as Parkinson's disease (Orendurff, Segal et al. 2006, Lamontagne and Fung 2009). It is estimated that one third to one half of older adults over the age of 65 falls at least once per year (Hornbrook, Stevens et al. 1994, Hausdorff, Rios et al. 2001). Hyndman et al. (2002) observed an increased risk of falls during turning while walking, rather than standing or straight walking in community-dwelling individuals post-stroke. In light of the aforementioned risk of injury or death from falling, it is surprising that the majority of research pertaining to the study of human gait has been focused on straight walking (Orendurff, Segal et al. 2006). Moreover, it is important to understand the interaction between neural areas responsible for such gait modulations and control of locomotion in the young

healthy brain to identify the mechanisms and patterns of activations during steering of gait. Such findings will influence the development of rehabilitation therapies for aging individuals and clinical populations with gait disturbances.

1.2 Objectives

Therefore, the objectives of this study were to, 1) Identify neuronal networks activated during straight walking and steering of gait in young healthy humans; 2) Determine the effect of locomotor task (straight walking or steering) on the activation of neuronal network and the walking speed, and 3) Determine if there is any relationship with the regions recruited during steering of gait and straight walking, walking speed and error trajectories of the corresponding walking condition.

1.3 Hypotheses

1. More executive cortical structures will be actively recruited with increasing complexity of the walking task.
2. Increased activation in the posterior parietal regions and the cerebellar vermis during steering of gait
3. Increased activation during straight walking in the primary motor cortices, visual cortical regions of the occipital lobe and the cerebellum.
4. Straight walking and steering of gait networks will be controlled by distinct neural networks.

CHAPTER 2. LITERATURE REVIEW

2.1 Control of Locomotion in Animals

Most knowledge about the control of locomotion in humans stems from studies of the intact cat or in cats with lesions of the neuraxis at lower (spinal; i.e. spinal cord transection, decerebrate; i.e. pre- or post- mammillary lesions) or higher (decorticate; i.e. a transection through the caudal end of the diencephalon) level (Jahn et al., 2008). It is well known that cats with lesions of the spinal cord are able to generate locomotor patterns below the spinal cord lesion (Grillner and Wallen 1985, Whelan 1996). Such locomotor-like movements of the hind paw can be modulated to enable the cat to walk within a slow range of treadmill velocities (Whelan 1996). These studies uncovered the existence of a central rhythm-generating network located within the spinal cord, termed central pattern generators (CPGs) (Grillner and Zangger 1975). Grillner (Grillner, McClellan et al. 1981, Grillner 2006) showed that the coordination (synergistic alternating movement of lower limbs) and rhythmic motor outputs of locomotion are produced via the CPGs (segmentally organized interneurons at the spinal cord level). CPGs are regulated by descending input from supraspinal locomotor regions located in the brainstem and cerebellum (Jahn et al., 2008).

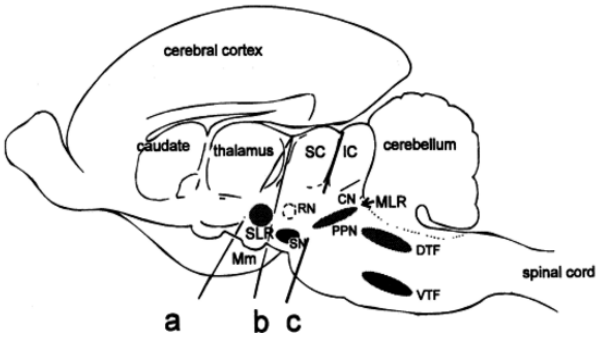


Figure 1. Mid-sagittal section showing different levels of transections (Whelan 1996).

Grillner and Wallen (1985) postulated that decorticate cats would maintain goal-directed locomotion (i.e. voluntary adaptation of gait patterns). This was based on previous studies in

decerebrate and decorticate cats which implied that the central nervous system produces; 1. synergistic movement to promote forward propulsion, 2. sufficient control over equilibrium during complex movements (i.e. locomotion) and 3. locomotor adaptation is based on the specie, the environment, visuomotor demands, and compensatory adjustments for perturbations (Grillner and Wallen 1985). It was found that decorticate cats (i.e. post mammillary preparation- transection rostral to the superior colliculi and rostroventrally to the caudal point of mammillary bodies- see Figure 1; label c) were able to initiate locomotion via electrical stimulation of MLR. However, the movements produced were not goal directed suggesting control of movement synergy was impaired (Grillner, McClellan et al. 1981) and higher stimulation induced trotting like movements (Shik and Orlovsky 1976). This suggests that locomotor synergy is able to be regulated by a phasic sensory signal to control spinal motor circuits and that higher order regions (such as basal ganglia, lateral hypothalamic areas and more frontal brain regions) are crucial for goal-directed locomotion (Grillner and Wallen 1985). Therefore, the midbrain and lower parts of the central nervous system produce synergistic patterns of locomotor movements, as more rostra1 structures are necessary for initiation of locomotion (Shik and Orlovsky 1976).

The ability of lesioned cats and other species to initiate locomotion while mounted and fixed on a treadmill implies the presence of a sensory feedback control mechanism. Forssberg et al. (1980), suggested that this sensory feedback mechanism is likely integrated as part of the central control system of locomotion and important during rapid changes in acceleration, deceleration and during turning. Based on animal studies over the last century, the cerebellum has been understood as playing a crucial role in the coordination of locomotion. It functions as a feedback mechanism to compensate for perturbations and precise locomotor movements. Armstrong (1978) suggested that the vermis of the cerebellum integrates multisensory

information and it was speculated that the vermis of the cat's cerebellum sends efferent projections to the deep fastigial nuclei (Chambers and Sprague 1955). Any lesion or infarct to the fastigial nucleus of the cerebellum can result in gait disturbances (i.e. locomotor ataxia) (Yu and Eidelberg 1983). It was that the fastigial nucleus is involved in the coordination of axial muscles during postural modifications (i.e. upper trunk and neck movements) and coordination of locomotion (Armstrong 1986). Orlovsky (1970) suggested that the cerebellum does not directly elicit locomotion, rather it is more important in continuous locomotor limb coordination (Armstrong 1978, Andersson, Forssberg et al. 1981). Arshavsky (1983) also found that spinal feedback systems do produce compensatory mechanisms for precise locomotor movements (e.g. placement of foot on the ground) and perturbations, but this feedback is reliant on the projection from the cerebellum to the spinal cord being intact. This is because the cerebellum receives specific feedback concerning the movements of each joint and efference copies from the CPGs via spinocerebellar pathways (Arshavsky YuI 1983).

Based on these results it can be assumed that two events co-occur when the cat initiates locomotion; stepping automatisms of limbs are activated and an interaction between these automatisms is elicited (Shik and Orlovsky 1976). Such an interaction between automatisms results in a significant reduction in the degrees of freedom which is the primary basis for motor coordination (Bernstein 1967). Thus, gait and posture are controlled by automated spinal motor programs (i.e. CPGs) initiated and regulated by supraspinal networks (Grillner and Wallen 1985).

The above studies of cats with various lesions have been vital in building a basic framework in understanding the more automatic mechanisms and structures involved in the control of human locomotion. However, human locomotion differs from quadruped mammal

locomotion, due to the fact that an erect posture along with bipedal locomotion are fundamental characteristics of human locomotion (Jahn et al., 2008). The transition to bipedal locomotion in humans is considered to be a critical evolutionary characteristic of humans because of the elimination of forelimbs involved in walking and more importantly the ability to use our hands for other tasks while walking (Harcourt-Smith and Aiello 2004).

2.2 Control of Locomotion in Humans

Much of our knowledge regarding mechanisms and structures involved in the control of human locomotion has been acquired from traditional neuroimaging techniques. Traditional functional magnetic resonance imaging (fMRI) studies which assess the blood oxygen level-dependent (BOLD) signal alterations in subjects while laying down and fixed in the scanner have been used to assess the neural networks involved in human locomotion. Various studies using this technique have demonstrated the presence of a supraspinal locomotor network including the frontal cortex, basal ganglia, brainstem tegmentum (specifically, the mesencephalic locomotor region-MLR), and the cerebellum (Jahn et al., 2008; Jahn and Zwergal 2010). A study by Jahn et al., (2008) provided strong evidence that hierarchically organized supraspinal locomotor regions which were electrophysiologically defined in cat studies, were preserved in the evolutionary transformation from quadrupedal to bipedal locomotion. The following is a simplification of findings from this study, which supports this evolutionary preservation. Subjects were trained to imagine four different conditions while in the scanner (eyes open and eyes closed): lying (rest condition), standing, walking, and running in 20-second intervals. This study found that control of locomotion involves frontal and parahippocampal areas, which send projections to the basal ganglia (BG) and to gait initiation centers of the dorsal brainstem. Then the midbrain tegmentum

receives input from the cerebellar vermal and paravermal cortices by way of the fastigial nuclei and cerebellar locomotor region (CLR). Jahn et al., (2008) defined the subthalamic (SLR) and MLR to elicit locomotion when disinhibited from tonic BG input and the CLR receiving rhythmic input from the vermis and paravermal cortex to control for speed of gait. Then the CLR projections converge with descending MLR projections in the pontine medullary reticular formation (PMRF), where locomotor commands are conveyed to CPGs within the spinal cord. These findings suggest several similarities between human and animal supraspinal control of locomotion (see Figure 2). Such results propose that adjustments of the straight walking pattern (i.e. increasing cadence) may be achieved through modulation of neural drive from the posterior midbrain (i.e. MLR), altering the more automatic pattern generators for walking (i.e.CPGs) (Grillner and Zangger 1975). Moreover, the oxygenation of hemoglobin using Near-infrared spectroscopy (NIRS) was used to observe cortical activations during imaginary gait which showed that the sensorimotor and supplementary motor cortices are integrated in human locomotion (Mayai et al., 2001). While these studies have been crucial in developing a basic framework of cortical networks involved in human locomotion, concerns among researchers exist due to low resolution and the absence of sensory feedback as the participants are in the scanner imagining the locomotion, thus there is no physical locomotor task being performed.

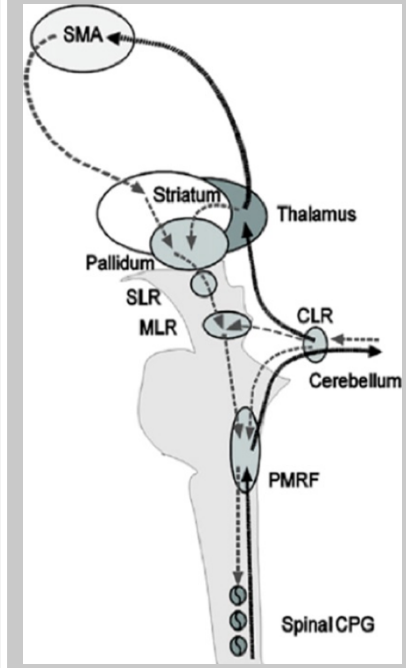


Figure 2. Schematic of the proposed "planning" locomotor network (la Fougere, Zwergal et al. 2010)

Furthermore, it is also important to understand the mechanisms of human locomotion at a cortical and subcortical level. In contrast to previously discussed techniques, the use of Transcranial magnetic Stimulation (TMS) combined with EMG has been used to measure the motor-evoked potential of the lower and upper-limb during an locomotion. Using this technique, Peterson et al., (2001) found that activation of M1 is directly involved in continuously activating motorneurons within the lower-leg muscles (i.e. Tibialis anterior) during human locomotion. Additionally, Barthelemy and Nielsen (2010) findings suggest that the corticospinal tract (CST) is integrated in activating proximal arm musculature during human locomotion. Such findings extend to the idea of concurrent coupling at the M1 and CST of both arm and leg movements during locomotion (Barthelemy and Nielsen, 2010).

Locomotion in humans has been defined as a dynamic sensorimotor task requiring a complex interaction between CPGs and hierarchically organized supraspinal locomotion centers in the brainstem tegmentum, cerebellum, frontal cortex, & basal ganglia (la Fougere, Zwergal et al. 2010). Real locomotion using [^{18}F]-fluorodesoxyglucose ([^{18}F]-FDG) PET was directly compared to mental imagery of locomotion with functional magnetic resonance imaging (fMRI) (la Fougere, Zwergal et al. 2010). This locomotion paradigm required subjects to walk for 10 minutes and then receive an injection of [^{18}F]-FDG and continued walking straight for another 10 minutes. The first 30 minutes following injection accounts for the tracer uptake time, thus the tracer will be metabolically trapped in areas of increased glucose metabolism during the real walking task. Immediately after this walking task, PET images were acquired which is well within the 109.8 minute half-life of the tracer (i.e. time during which tracer concentration is sufficient to be imaged). For the fMRI condition subjects were trained in a uniform visual

environment for various conditions; lying (rest condition), standing, walking, and running in 20 second sequences over 20 minutes. After training, subjects were told to imagine these conditions while supine in the scanner and functional MR images were acquired. In contrast to Figure 2, during imaginary locomotion with fMRI, Figure 3 displays the execution of real (straight walking) locomotion ([^{18}F]-FDG-PET paradigm) which appears to go from the primary motor cortex areas, integrates sensory input from visual cortical areas of the occipital lobe in order to produce rhythmic outputs of locomotion at the level of the spinal cord (la Fougere, Zwergal et al. 2010), thereby bypassing the basal ganglia loop. As illustrated in Figure 3, there is a feedback loop from the spinal cord, supraspinal structures, the cerebellum, through the thalamus and to sensorimotor cortical regions. Primary motor cortex (M1) was only activated during real locomotion as BOLD signal increases occur in supplementary motor areas (SMA) and basal ganglia during mental imagery of locomotion (see Figure 2).

Real steady-state locomotor commands (i.e. Figure 3) were shown to be conveyed via a direct pathway (the "executive" network) from M1, as imagined locomotion commands (i.e. Figure 2) are conveyed via an indirect pathway (the "planning" network) from the supplementary motor areas (SMA) and basal ganglia loop. In both paradigms of this study a consistent locomotor network was found consisting of primary motor, premotor and multisensory cortices, parahippocampal gyri and midline cerebellum activations. While both real and imagined locomotion in humans are able to depict the supraspinal locomotor networks, findings

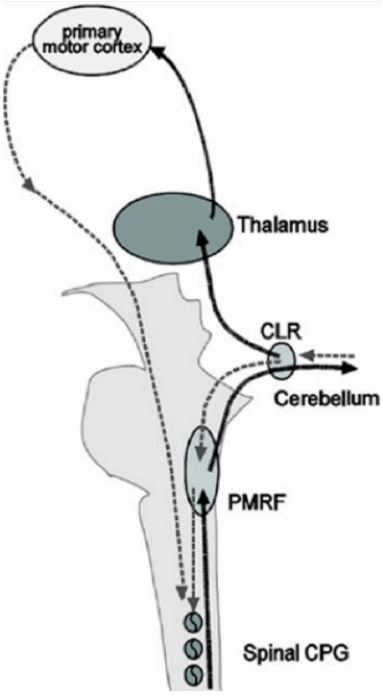
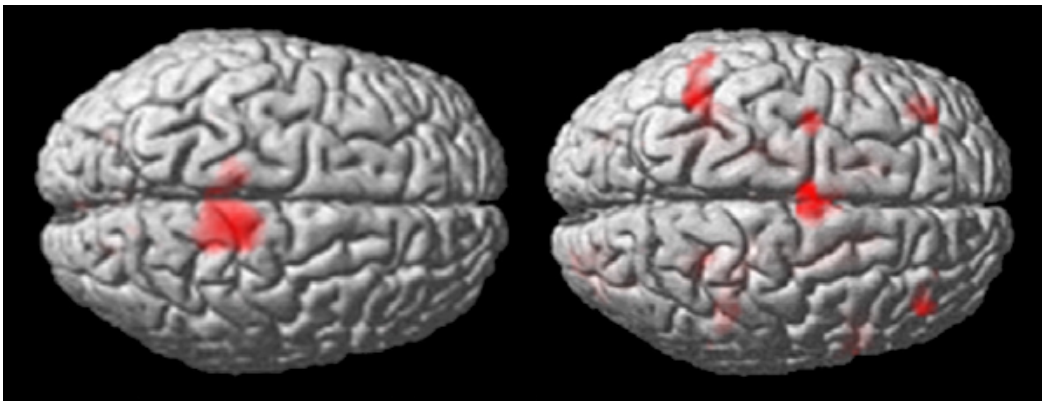


Figure 3. Schematic of the proposed "executive" locomotor network(la Fougere, Zwergal et al. 2010).

from this study suggest that using [^{18}F]-FDG-PET is more accurate and precise in quantifying areas of whole-brain activation during real locomotion when compared to fMRI as the task of imagining locomotion appears to recruit regions involved in visualizing the motor task as opposed to actually carrying out the motor commands (see Figure 4) (la Fougere, Zwergal et al. 2010).



Real Locomotor Activations: [^{18}F]-FDG-PET **Mental Imagery Locomotor Activations: fMRI**

Figure 4. During real locomotion (left) the M1 (pre- and post-central gyri) are active, as compared to activations of the SMC (superior and medial frontal gyri) and basal ganglia (caudate nucleus and putamen) in the mental imagery of locomotion condition (right) (la Fougere, Zwergal et al. 2010).

2.3 Adapting Patterns for Goal-Directed Locomotion

Single-Photon Emission Tomography (SPECT) has been used to examine pattern of changes in regional cerebral blood flow (rCBF) during over-ground walking which found

activation in the thalamus, supplementary motor area (SMA), sensorimotor (SMC), cingulate, prefrontal, anterior cerebellar, and posterior parietal cortices. Another PET study using $H_2{}^{15}O$ to measure cerebral blood flow while imagining locomotor tasks including: gait initiation, walking, walking with obstacles and standing (Malouin et al., 2003). They found activation of the similar cortical regions as in the aforementioned SPECT study, they also found activation of the pre-SMA, parahippocampal, and precentral gyrus during imaginary locomotion, including the standing condition. They also reported activation in the BG when imagining gait initiation, suggesting the BG is important in more automatic mechanisms of locomotion. Interestingly, when walking was subtracted from the obstacle condition, a network was found including the bilateral precuneus, left SMA, right inferior parietal cortex, and left parahippocampal area. These findings infer that when more complex locomotor adjustments are required, our gait relies greatly on motor and visuospatial sources at the cortical level (Malouin et al., 2003).

2.4 Direct Approach to Studying Human Locomotor Networks

Presently, the only technique capable of quantifying full brain activation during a whole body task is [^{18}F]-FDG-PET as it allows to measure regional cerebral metabolic rates of glucose. PET is a form of nuclear medicine imaging technique, which detects gamma rays emitted by a radioactive tracer and then constructs 3D functional images of various physiological processes in the brain (Hamel 2015). PET detects radioactive decay emitted by a positron-emitting radionuclide (i.e. radioactive tracer), which is injected into the body, binds to its target tissue and is absorbed by brain cells like normal glucose. [^{18}F] is the radioactive tracer (fluorine) as it replaces two hydroxyl groups (-OH) of normal glucose and once [^{18}F]-FDG has been absorbed

by the cells, phosphorylation prevents it from being released again. Thus, the accumulation of [^{18}F] -FDG during the task then reflects regional glucose metabolism during the uptake period (FDG uptake: ~ 30 min), which is the time for the injected dose to be absorbed by active regions and cells in the brain. Immediately after injection, subjects can perform a task and all circulating [^{18}F]-FDG will be trapped in highly metabolically active regions during the task. The task should last no longer than 50 minutes so that images can be acquired long before the 109.8-minute half-life of the tracer is reached (i.e. time during which tracer concentration is sufficient to be imaged). This trapping of [^{18}F]-FDG in regions of interest therefore reflects cerebral glucose metabolism during the task.

In order to address the limitations in transferring results from imaginary locomotor tasks to real steady-state locomotion, Paquette & Soucy (2014) used [^{18}F]-FDG-PET to quantify regional cerebral glucose metabolism (rCGM) during both a straight walking and steering of gait task performed by post-stroke and aged-matched control subjects. The straight walking task activations were subtracted from activations during the steering task to obtain the steering activated networks in both groups. They observed a consistent and reproducible steering network (See Figure 5) in healthy controls, recruiting mostly the intraparietal sulcus region, sensorimotor cortex and cerebellar midline. Subjects post-stroke however, showed an asymmetrical pattern of activation in sensorimotor areas and superior parietal lobule where the affected hemisphere showed no increased activation. Differences between groups were also observed in the cerebellum where there was increased activation in the vermis for controls. However, mildly impaired subjects showed increased activation of the affected hemisphere but more severely

affected subjects showed activation of the non-affected hemisphere. These findings imply that changes in whole-brain activations can in fact be quantified using [^{18}F]-FDG-PET during steering of gait.

2.5 Potential Hierarchical Concept of Locomotion

Current concepts regarding the control of locomotion in humans propose that volitional

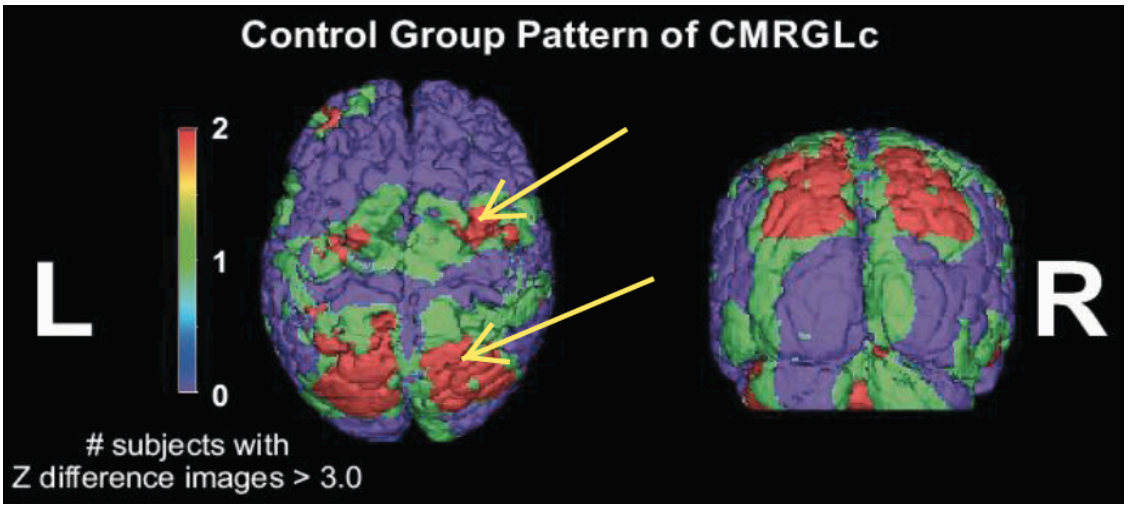


Figure 5. 3D rendering of a consistent activation of the steering network in healthy middle-aged control subjects (Paquette and Soucy 2014).

locomotion (i.e. steering of gait) and more automatic locomotor tasks (i.e. straight walking) may be controlled by different hierarchical neuronal networks (Drew and Marigold 2015). Locomotion is an integral process which is critical in achieving and adapting a safe walking pattern in complex situations such as: changing direction to avoid an obstacle, turning or rounding corners, walking straight, and accelerating to catch a bus. Findings presented from la

Fougere et al., (2010), Jahn et al., (2004) and Paquette et al., (2011) suggests that "volitional" goals originate from supplementary motor areas of the cortex which then alter the more automatic brainstem structures (i.e. CPGs) by way of the basal ganglia loop which then produce rhythmic outputs of locomotion at the level of the spinal cord.

2.6 The Importance of Postural Control during Functional Locomotion

While aforementioned neuroimaging studies have been crucial in developing an understanding of cortical networks involved in human locomotion, they fail to account for differences between postural control mechanisms required to achieve functional locomotion and regions involved in straight walking. The postural control system is required to achieve independent and functional locomotion, however posture is maintained by different and higher level neuronal structures. Multisensory inputs such as vestibular, visual and somatosensory inputs all influence postural control to maintain balance and orientation based on external modifications (Kandel 2012). Modifications to gait, such as turning are potentially destabilizing and therefore must be compensated with postural adjustments to provide support during such voluntary movements (Drew, Prentice et al. 2004). Corticoreticular projection pathways connecting cortical motor areas (i.e. primary motor cortex) via locomotor regions with reticulospinal neurons (RSNs) located within the PMRF (Drew, Prentice et al. 2004), strongly suggests that the PMRF of the cat likely plays a role in integrating higher level postural responses during movement and adapting gait pattern to the external environment (Mori 1987, Mori, Matsuyama et al. 1992). It was later demonstrated using electromyography (EMG) that cortical neurons projecting to the PMRF increased in phasic discharge when making adjustments

during the swing phase of the gait pattern in cats, therefore such sensory inputs modulate CPGs at the spinal cord level (Kably and Drew 1998, Kably and Drew 1998).

While cats with spinal transections are capable of initiating locomotor patterns, normal postural responses such as active balance are not present (Kandel 2012). A cat with such a lesion likely sustains minimal stability to maintain standing given the quadrupedal stance, recruitment of the hind limbs (tonic contraction of flexors and extensors- antigravity muscle tone), and compensatory mechanisms of the forelimbs to promote postural control mechanisms (Kandel 2012). Such results that show locomotor patterns are preserved but absent control of balance suggests that higher brain regions are involved in postural control. Cortical projections of locomotion which have been discussed may form a basis of feedforward commands involved in postural responses involved in voluntary movement such as altering gait (Drew, Prentice et al. 2004). Jahn et al., (2004) had healthy subjects imagine standing, walking and running during fMRI. Results showed activation occurring predominantly in the basal ganglia and thalamus during standing. Additionally, in a similar fMRI study during imagined stance, there was activations of the prefrontal cortex, SMA, middle temporal gyri and thalamus (Jahn et al., 2008). Activations of these areas is directly linked to each structures functionalities and outputs. The thalamus is crucial in maintaining upright posture, which is evidenced from patients with lesions to the posterolateral thalamus (i.e., thalamic astasia) as these individuals show body tilt and falls (Jahn et al., 2004). Higher order structures such as the basal ganglia is important for quickly modifying posture in response to the changing surroundings to ensure that postural responses are appropriate (Kandel 2012). Cerebral cortex structures such as the SMA and temporoparietal cortex have been implicated in optimizing postural control as a part of motor planning which explains recruitment of these areas during imaginary standing. SMA has also been suggested to

be a part of anticipatory postural adjustments of voluntary movements such as changes to gait pattern and the temporoparietal cortex integrates multisensory inputs which may be involved in perception of upright posture (Kandel 2012) .

Therefore, balance and locomotion seem to be controlled by distinct regions within the CNS. Nevertheless, results of functional imaging studies investigating neural activations during human locomotion often fail to account for postural control mechanisms required to achieve functional locomotion. A postural control condition is important to include in such paradigms as higher order regions such as the thalamus, BG, SMA and temporoparietal cortices are implicated in optimizing and maintaining posture as part of motor planning and gait modifications.

Overall, much of the prior research examining human locomotor networks has predominately been focused on imaginary stance and locomotion (Malouin et al., 2003; Jahn et al., 2004; Jahn et al., 2008; la Fougere et al., 2010). While these previous studies have advanced locomotor models by better understanding the planning of sequential lower-limb movements, imaginary standing and locomotion does not allow to examine cortical regions involved in the motor task itself. Moreover, a select number of studies have used [^{18}F]-FDG-PET to investigate straight walking, however postural control and balance mechanisms were not removed, thus regions involved in the maintenance of balance and postural responses are present which does not allow to make conclusion about cerebral networks exclusive to the locomotor task (la Fougere et al., 2010). To date, no previous neuroimaging studies have investigated real time, steady-state straight walking and complex locomotion involving turning. Therefore, the purpose of this study was to [^{18}F]-FDG-PET to quantify rCGM during straight ahead walking and steering of gait in the young-healthy human brain.

CHAPTER 3. RESEARCH ARTICLE

Complex and straight walking involve different brain regions as measured with [^{18}F]-FDG PET imaging

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Sources of funding: This project was supported by grants from the National Sciences and Engineering Research Council of Canada, Canada Foundation for Innovation, Centre for Interdisciplinary Research in Rehabilitation of Greater Montreal and McGill University to CP. FS was supported by a Canadian Institutes of Health Research (CIHR) Fredrick Banting and Charles Best Canada Graduate Scholarship and KPE McGill Graduate Excellence Award.

Running Head: Cerebral activations of complex and straight walking

Abstract

Functional neuroimaging studies in subjects imagining walking and stance have found common activations involving the sensorimotor and premotor cortices, basal ganglia, midline cerebellar regions and occipital visual areas (Jahn et al., 2004; Malouin et al., 2003). Additionally, imagining walking around obstacles (Malouin et al., 2003) recruited inferior parietal and precuneus regions. However, such protocols do not involve actual walking and cannot account for the high degree of sensorimotor integration involved in goal-directed locomotion. The aim of this study was to use [^{18}F]-fluorodeoxy-glucose (FDG) Positron Emission Tomography (PET) to quantify regional cerebral glucose metabolism (rCGM) during steering of gait (SG) and straight walking (SW). It was predicted that more executive regions and midline cerebellum would be recruited during SG, as more automatic-lower-order regions and visual occipital areas would be involved in SW. Seven young healthy subjects were injected with 150 MBq of [^{18}F]-FDG and began 1 of the 3 motor tasks for 40 minutes immediately after injection: 1) SG, 2) SW and 3) upright standing (US). Metabolic trapping of [^{18}F]-FDG in the brain was completed by the time subjects finished the task (within the tracer uptake time of approximately 30 minutes), subjects were scanned for 40-minutes immediately after task completion to quantify changes in rCGM during the task (Zwergal et al., 2016). Reference PET images were subtracted from the intervention PET images to obtain regions recruited only during SW (SW- US) and SG (SG – SW). Regions showing significant increases and decreases in rCGM were thresholded at $p < 0.05$. Steering of gait showed predominant activation of the posterior parietal areas, including the SPL, precuneus, parahippocampal gyrus and midline cerebellar regions during complex walking. As SW recruited the paracentral lobule, occipital

lobe, midline and left cerebellum. These findings display region-specific activations for SG and SW which relate to the functional responsibility of such cerebral cortices during human locomotion. This is the first detailed functional neuroimaging study to propose distinctive cerebral regions subserving SW and SG which is crucial in understanding compensatory mechanisms of aging and developing rehabilitation therapies for those afflicted with gait disturbances.

Keywords: Behavior, Brain Activation, Glucose Metabolism, Straight Walking, Steering of Gait

Introduction

Turning while walking is an essential element of goal-directed locomotion involving a high degree of sensorimotor integration, allowing one to avoid, or steer around obstacles while walking (Lamontagne et al., 2007). Directional changes and steering of gait are complex motor tasks which place a significant burden on the central nervous system, especially with normal aging and those afflicted with various types of neurological diseases or disorders (Lamontagne and Fung, 2009; Orendurff et al., 2006). We make about 1,000 turns per day while walking (Mancini et al, 2016), representing approximately 20% of all steps taken during our lifespan (Sedgman et al., 1994). Therefore, it is important to understand the interaction between neural networks responsible for such gait modulations and those involved in the control of straight walking in the healthy brain in order to develop rehabilitation therapies for aging individuals and clinical populations.

Much of our knowledge regarding the control of locomotion in humans stems from cat studies which were paramount in identifying supraspinal structures responsible for conveying motor commands to central pattern generators (CPGs) at the level of the spinal cord to initiate and terminate rhythmic locomotion (Grillner, 2006; Jahn et al., 2008). Additionally, several animal studies investigating the modulation of locomotion during obstacle avoidance, found the primary motor cortex (M1) provides input to alter limb trajectory and paw placement during goal-directed locomotion (Armstrong, 1986; Drew et al., 2004). A study by Jahn et al., (2008) provided strong evidence that hierarchically organized supraspinal locomotor regions, which were electrophysiologically defined in cats, were preserved in the evolutionary transformation from quadrupedal to bipedal locomotion. Despite such findings suggesting several similarities between quadruped and human locomotion, other researchers have proposed that the activation

of lower order supraspinal structures in humans are more reliant upon descending input from higher order cortical and subcortical areas (Malouin et al., 2003). Therefore, it is also important to understand these mechanisms of human locomotion at a cortical and subcortical level. Previously, transcranial magnetic Stimulation (TMS) has been combined with EMG to measure the motor-evoked potential of the lower and upper-limb during an locomotion. Using this technique, Peterson et al., (2001) found that activation of M1 is directly involved in continuously activating motorneurons within the lower-leg muscles (i.e. Tibialis anterior) during human locomotion. Additionally, Barthelemy and Nielsen (2010) findings suggest that the corticospinal tract (CST) is integrated in activating proximal arm musculature during human locomotion. Such findings extend to the idea of concurrent coupling at the M1 and CST of both arm and leg movements during locomotion (Barthelemy and Nielsen, 2010). The oxygenation of hemoglobin using Near-infrared spectroscopy (NIRS) was used to observe cortical activations during imaginary gait which showed that the sensorimotor and supplementary motor cortices are integrated in human locomotion (Mayai et al., 2001).

More recently, functional neuroimaging techniques have been used to explore neural networks involved in human locomotion. Current concepts regarding the control of locomotion in humans propose that volitional locomotion (i.e. steering of gait) and more automatic locomotor tasks (i.e. straight walking) may be controlled by differential neuronal networks (Drew and Marigold, 2015; Malouin et al., 2003). Findings from functional imaging studies propose that “volitional” mobility goals originate from more frontal (SMA, SMC, premotor) and M1 cortical areas which alter the more “automatic” brainstem structures by way of the basal ganglia loop , sensory input from visual cortical areas of the occipital lobe and cerebellar midline

in order to produce rhythmic outputs of locomotion at the level of the spinal cord (Jahn et al., 2008; la Fougere et al., 2010; Peterson et al., 2001; Barthelemy and Nielsen, 2010; Mayai et al., 2001). While these studies have been crucial in developing a basic framework of cortical networks involved in human locomotion, they are limited by the absence of sensory feedback as the participants are imagining locomotion and not actually performing a physical locomotor task. Furthermore, these studies fail to account for differences between postural control mechanisms required to achieve functional locomotion and regions involved in straight walking. Since regions recruited during straight walking were examined in the present study, a postural control condition was included given that higher order regions such as the thalamus, BG, SMA and temporoparietal cortices are implicated in optimizing and maintaining posture as part of motor planning (Kandel, 2012).

Therefore, the aim of this study was to identify regions involved in steering of gait and straight walking by measuring regional cerebral glucose metabolism (rCGM) during steering of gait and straight walking using FDG-PET in young healthy humans. A secondary, explorative aim was to analyze whether or not there was any relationship between walking speed and rCGM during both SW and SG. It was hypothesized that more executive sensorimotor cortical structures will be actively recruited with increasing complexity of the walking task. Specifically, we predicted there would be increased activation in the posterior parietal regions and the cerebellar vermis during steering of gait. As for straight walking, we expected there would be increased activation in the primary motor cortices, visual cortical regions of the occipital lobe and the cerebellar vermis.

Materials and Methods

Subjects

Seven young healthy subjects (4 females, mean age: 25 ± 3 years) participated in this study. Exclusion criteria were metal implants and cardiac pacemakers (precluding MRI), diabetes mellitus (tracer injection requiring fasting) and pre-existing neurological or orthopedic disorders which could affect balance or mobility. All subjects were right-handed as assessed with the Edinburgh Handedness Inventory (Oldfield, 1971). The study was approved by the McGill Faculty of Medicine Institutional Review Board (study number A11-M109-12B). All subjects gave their informed consent in accordance to the Board's regulations for human subjects' studies and the Helsinki Declaration prior to their participation in the study.

Protocol

The subjects were tested on three separate days on three different motor tasks (one task per visit, at least 48h apart): 1) steering of gait (SG), 2) straight walking (SW), and 3) upright standing (US), performed in random order. Four walking lanes (34m X 1.5m) were delineated by small yellow and red disc cones (5.08 cm high and 19.05 cm diameter, 5 cones X 30 cones creating 4 walking lanes as illustrated in Figure 1A-B). For the SG task, subjects had to steer around the gray cones that were positioned pseudo-randomly to ensure an irregular pattern of walking so that the path remained unpredictable to participants (Fig. 1A), thus requiring subjects to constantly adjust gait trajectory. For SW, the subjects were instructed to walk straight up and down the lanes (Fig. 1B). The same cone setup was used for all participants so that the walking trajectories were identical. Upon arrival, subjects were given uniform instructions regarding the motor task they were to perform and were then required to practice a minimum of one walking

lane for the selected task, until they demonstrated a full understanding of the task (i.e., performing the task correctly without input from the experimenter). The US required subjects to stand in the middle of the room with the cones set-up in the same order (same visual aspect of the room), hands at their side. Subjects were filmed with a camcorder to calculate the average speed (m/s) for each task obtained by dividing the distance measured with a measuring wheel a posteriori (m) by the time (s) to perform the task. Trajectory errors as measured by the number of times participants took an incorrect path were calculated from video recordings.

A

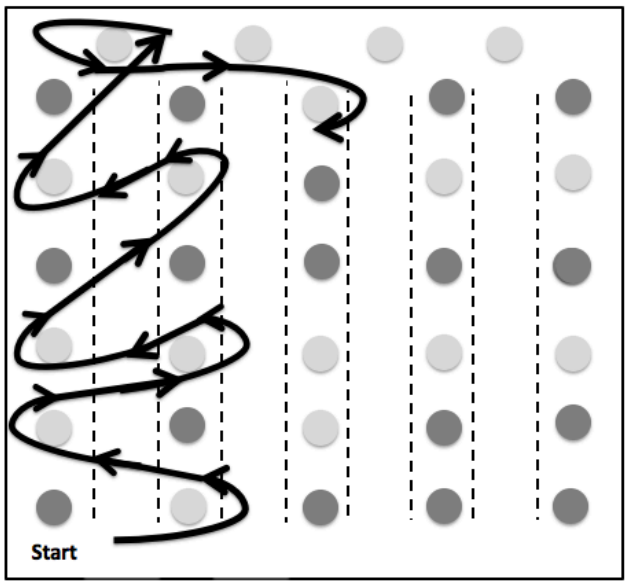


Figure 1A: Zoom in of Steering of Gait "Intervention" task of 6 cones compared to 30 cones. Subjects turn around the light gray cones, with one lap consisting of beginning at the start point and finishing back at the start.

B

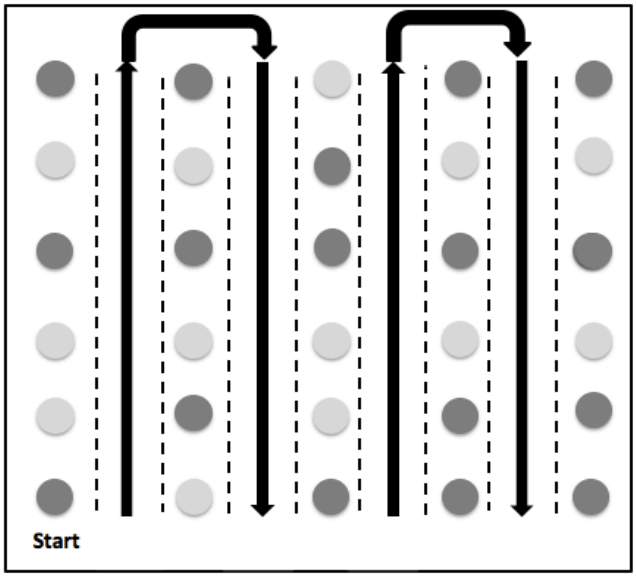


Figure 1B: Zoom in of Straight Walking "Reference" task of 6 cones compared to 30 cones. Subjects walk up and down lanes, with one lap consisting of beginning at the start point and finishing back at the start.

Figure 1. Room setup with walking lanes. Magnified view (only 6 of the 30 cones in length are shown) of the room setup with example trajectory for the (A) Steering of gait and (B) Straight walking tasks. Light gray cones represent the yellow cones at which participants were instructed to turn for the Steering of Gait task.

Before starting the task, subjects were injected with [^{18}F]-fluorodeoxyglucose (FDG) and then immediately began the randomly selected task for 40-minutes non-stop. Subjects were instructed to walk at a comfortable speed. As with previous protocols (Paquette et al., 2011), all subjects wore a safety harness equipped with a handle that a research assistant following the participant could grasp in case of a fall. None of the participants experienced any fall. After task completion, subjects walked to the scanning facilities (average of 4.6 mins [*SD* 0.7]) and PET image acquisition (40-minute scan) began shortly after arrival at the scanning facility (average of 5.2 mins [*SD*1.7]). Additionally, one T1-weighted (T1-W) anatomical MR image for each subject was obtained.

Image Acquisition

On each of the three testing days, subjects were in a fasting state (approximately 6 hours) to allow for optimal cerebral FDG uptake that would not be affected by increased serum glucose levels (Varrone et al., 2009). Subjects were injected with a 150 MBq bolus of [^{18}F]-FDG tracer into an ante-cubital vein immediately before starting the motor task. [^{18}F]-FDG acts as a glucose analog where the 2' hydroxyl group (-OH) of glucose is replaced with fluorine-18 [^{18}F], which serves as the radioactive tracer. Metabolic trapping of [^{18}F]-FDG in the brain was completed by

the time subjects finished the task (within the tracer uptake time of approximately 30 minutes), subjects were scanned for 40-minutes immediately after task completion to quantify changes in rCGM during the task (Zwergal et al., 2016). Six of the subjects' images were obtained with a High-Resolution Research Tomograph (HRRT) PET scanner (CTI/Siemens, Knoxville, Tennessee). The spatial resolution of this scanner is ~2.3 - 3.4 mm full-width-at-half-maximum (FWHM) (Funck et al., 2014). 3D sinograms were produced from the list-mode data acquired over 40 minutes, and reformatted into a series of 4 successive 3D images of 5 minutes each; the use of 4 consecutive static images allowed us to correct for motion artefacts, and then to sum those frames into a single one of 20 minute duration. One subject's PET images were acquired on an ECAT EXACT HR^{+} scanner (Siemens AG, Erlangen, Germany) in 3D mode. With that tomograph, one static 40 minute acquisition was obtained for each condition. For both scanners a 10-minute transmission scan was obtained after the emission scan for attenuation correction.

T1-W images were acquired to co-register PET images to identify regions of increased and decreased glucose metabolism. A 3-T Siemens Trio Tim Scanner (Siemens, Knoxville, TN) located at the Montreal Neurological Institute was used with 3D magnetization rapid gradient echo. These T1 images were acquired with $1mm^3$ voxel sizes (echo time = 2.98ms; repetition time =23ms; flip angle= 9°) consisting of 192 contiguous slices (thickness = 1mm) obtained across the entire brain using an echoplanar imaging sequence (field of view = 256 X $256mm^2$).

PET Image Analysis

Analysis were conducted with the Statistical Parametric Mapping 12 (SPM12, Welcome Trust Centre for Neuroimaging, Institute of Neurology, University College London, London, UK) running within MATLAB 8.5 (MathWorks, Natick, Massachusetts, USA). Reconstructed [^{18}F]-

FDG-PET images for all three tasks were linearly coregistered to their corresponding native T1 anatomical image. T1 MR images were spatially normalized to the Montreal Neurological Institute template the ICBM 152 6th generation linear brain atlas (Mazziotta et al., 2001) using an affine transformation (12 parameters for rigid transformations) (Friston et al., 1995). MRI normalization parameters were then applied to all [^{18}F]-FDG-PET images. Spatially normalized [^{18}F]-FDG-PET images were blurred with a Gaussian filter (FWHM = 12mm) to increase signal-to-noise ratio. All [^{18}F]-FDG-PET images were then normalized to white matter using a mask of the centrum semiovale in MNI space. This mask was used to count-normalize PET images prior to voxel-based statistics (la Fougere et al., 2010). After preprocessing, each subject's reference PET image was subtracted from the intervention PET images to obtain regions of increased or decreased rCGM during SW (SW- US) and SG (SG – SW). Additionally, an exploratory analysis was conducted to examine common or differential patterns of activation during steering of gait based on the subtask being subtracted, therefore US was subtracted from SG. One subject dropped out after completing the SG and SW task, in this case only the SG activations were available since the US task was not performed. To calculate significant increases and decreases in glucose metabolism, group difference images was generated using a one-way repeated measures model in SPM12 for SW (SW - US) and SG (SG – SW and SG - US) and were thresholded at $Z > 2.8$ ($p < 0.005$, uncorrected). Clusters of activation were included in difference images when there was a significant increase or decrease in glucose metabolism and MNI coordinates of peak-activation within clusters were obtained.

Results

Cerebral Activations Vary with Locomotor Subtasks

As shown in Figure 2, SG increases rCGM in several areas of the brain when compared to upright standing (purple panel, top). These regions include the paracentral gyrus, parietal, occipital cortices and cerebellum (cluster size 32,219 voxels, Z-score: 4.63, p=0.000 uncorrected). When isolating complex locomotion by comparing SG with SW, regions of increased rCGM are limited to the parietal cortex and cerebellum (vermis and cerebellar hemispheres). On the other hand, straight walking only (SW-US) increases rCGM of the paracentral gyrus, occipital cortex and cerebellum (mainly vermis). No deactivations were found in any of the contrasts.

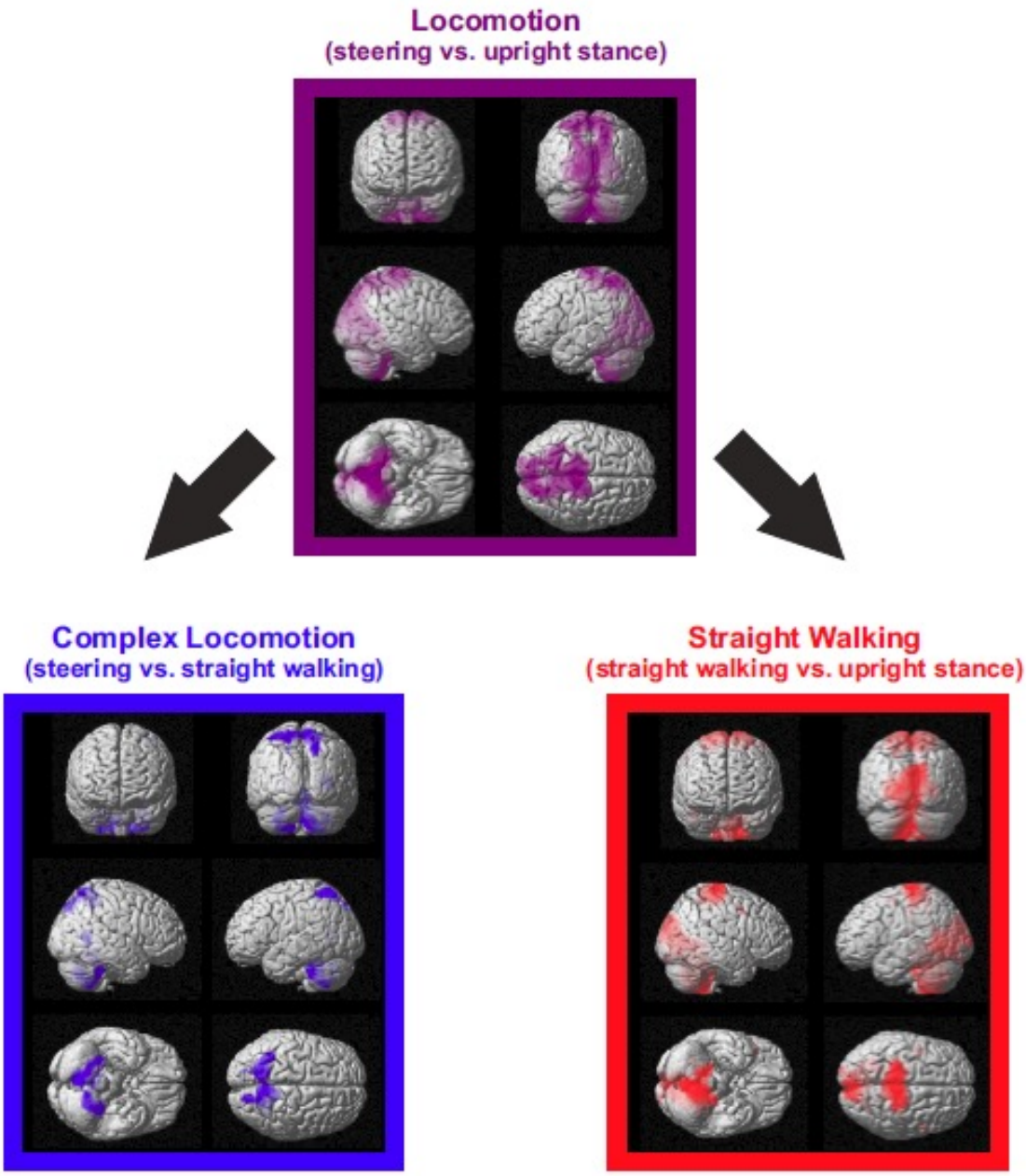
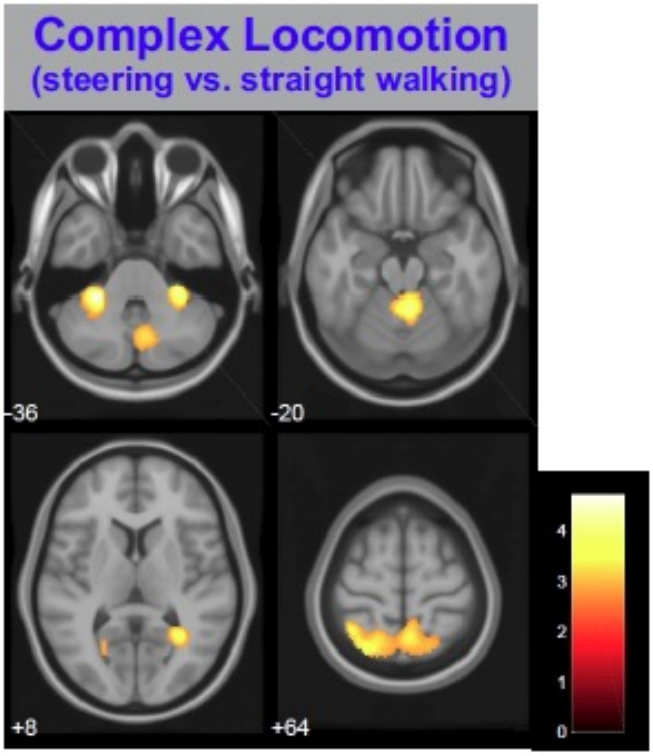


Figure 2. 3D rendering of regions with increased rCGM during locomotion (SG-US; top, purple), complex locomotion only (SG-SW; bottom-left, blue) and straight walking (SW-US; bottom- right, red). Regions activated during walking in top, purple panel differ according to locomotor task (bottom panels).right, red). Regions activated during walking in top, purple panel differ according to locomotor task (bottom panels).



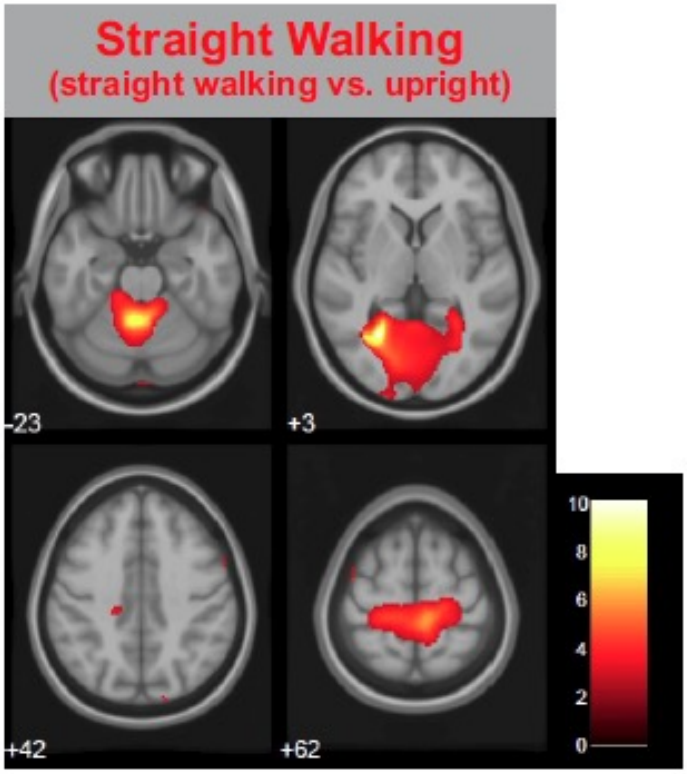


Figure 3. rCGM activations associated with Complex Locomotion (top) by contrasting SG with SW and Straight Walking (bottom) by contrasting SW and US. NOTE: Scale needs to be modified to match between panels.

rCGM during complex walking

Figure 3, top panel, shows the regions with a significant increase in rCGM during complex walking (SG vs. SW). Activations were found bilaterally in the superior parietal lobule (SPL), the right precuneus, parahippocampal gyrus as well as in the bilateral cerebellar hemispheres and vermis (Table 2).

Table 2. [18F]-FDG activation during complex locomotion (SG vs. SW)

	BA	Cluster	Z	T	P	x	y	z
Cerebral Hemispheres								
L Superior Parietal Lobule	40, 7	2122	2.99	3.84	0.001	-40	-52	62
			2.94	3.73	0.002	-26	-64	64
R Superior Pareital Lobule / R Precuneus	7		2.85	3.57	0.002	4	-58	66
R Parahippocampal Gyrus	30	273	3.05	3.95	0.001	32	-56	8
Cerebellum								
L Cerebellum		1131	3.42	4.72	0.000	-28	-40	-36
			2.87	3.61	0.002	-14	-56	-52

Vermis, R Cerebellum		2399	3.23	4.31	0.001	6	-48	-16
			3.22	4.28	0.001	30	-38	-36
			3.16	4.15	0.001	12	-62	-44

R: right; L: left; BA: Brodmann Area; Custer: Cluster size in voxels; Z: Z-score; T: T-value; P: p-value, $p<0.005$ uncorrected; x, y, z: coordinates in MNI space

rCGM During Straight Walking

Figure 2, bottom, and Table 3 show the most prominent activations observed in SW (compared to US). SW-related activations were bilateral in the paracentral lobule (BA 4, 6), the occipital lobe as well as in the vermis and left cerebellum.

Table 3. [18F]-FDG activation during straight walking (straight walking vs. upright stance)

	BA	Cluster	Z	T	P	x	y	z
Cerebral Hemispheres								
L Occipital Lobe, Lingual Gyrus	19	16234	4.97	10.12	0.000	-26	-60	4
			4.81	9.3	0.000	-34	-64	2
R/L Paracentral Lobule	4, 6	4858	4.03	6.32	0.000	8	-32	66
			3.4	4.68	0.000	-18	-30	54

			3.17	4.18	0.001	-24	-26	78
L Precentral Gyrus	6	17	3.08	4	0.001	-44	-4	62
R Precentral Gyrus	6	26	3.04	3.92	0.001	58	8	44
Cerebellum								
Vermis, L Cerebellum			4.58	8.3	0.000	-4	-50	-24

R: right; L: left; BA: Brodmann Area; Custer: Cluster size in voxels; Z: Z-score; T: T-value; P: p-value, $p<0.005$ uncorrected; x, y, z: coordinates in MNI space

Walking Speed and Correlation to rCGM

The average speed was significantly slower in SG compared to SW (SG: 0.7 m/s [*SD* = 0.1] vs. SW: 1.1 m/s [*SD*=0.2], t (6) = 4.223, p = 0.006). As speed was derived from the distance covered by participants, a corresponding reduction in total distance covered was measured (SG: 1,749 m [*SD* = 301] vs. SW: 2537 m [*SD*=388], t (6) = 4.223, p = 0.006).

Discussion

This is the first whole-brain neuroimaging study in young healthy humans to show involvement of the posterior parietal areas, including the SPL, precuneus, parahippocampal gyrus and midline cerebellar regions during complex walking. In contrast, SW predominantly recruited the paracentral lobule, occipital lobe, midline and left cerebellum. These findings display region-specific activations depending on the reference task that steering of gait is contrasted with.

Substantial bilateral activation of posterior parietal regions during SG can be reflected by findings from monkey studies which found that sensorimotor input to frontal motor areas (M1 and PMC) predominantly originate from the posterior parietal cortex (PPC) (Rizzolatti et al., 1998). However, when observing activation of brain regions during human spatial navigation, Zwergal et al., (2016) did not report PPC recruitment which suggests that posterior parietal areas are not linked to human navigation and orientation, but rather in the planning and execution of gait modifications during complex locomotion (Burgess et al., 1999). While activation of the precuneus was not anticipated during SG, recruitment of the precuneus, agrees with findings from a study where straight walking compared to walking with obstacles (Malouin et al., 2003). Previous findings and results of the current study, propose that the precuneus plays a role in integrating visual and somatosensory input while concurrently processing where the cones they need to turn around are within the environment (Maguire et al., 1998). To a lesser degree, the parahippocampal gyrus was also recruited during SG which coincide with the findings where this region was recruited during both the imagination of walking and real steady-state locomotion (Jahn et al., 2004; la Fougere et al., 2010). Parahippocampal gyri activation during complex walking suggests that this area is recuited during visually guided spatial navigation and

topographical memory when landmarks are present withing the environment (Maguire et al., 1998; Malouin et al. 2003; la Fougere et al., 2010). Furthermore, the cerebellum has been found to play a role in reducing walking speed when a slower pace is required in order to process visuospatial information (Jahn et al., 2004). Our findings show activation of the cerebellum and a signifcant decrease in walking speed during SG when compared to SW, thus inferring that walking speed is mediated by decending sensorimotor input from the cerebellum.

Significant bilateral activation of the paracentral gyri (i.e. M1) was seen when US was subtracted from SW. This was anticiapted as primary motor regions have been found to be recruited during real steady-state straight walking using FDG-PET which supports the notion that SW is a more executive task which bypasses the basal ganglia (BG) and sends sensorimotor input directly to CPGs at the level of the spinal cord (la Fougere et al., 2010). Additionally, activation of the M1 during SW align with findings from a previous study which measured MEP of the lower-limb and found constant activation of these muscles during human locomotion (Peterson et al., 2001). Furthermore, based on animal lcoomotor studies it has been thought that visuospatial input from visual areas of the occipital lobe can modulate sensorimotor integration within the M1 (Drew et al., 1988; la Fougere et al., 2010; Zwergal et al. 2010). Activation of occipital areas involved in processing of visual input were recruited during SW suggesting this region is important in processing visuospatial information of the environment during straight ahead walking, which may be due to optic flow as one progresses forwards through an environment (Jong et al., 1994). Similar to SG, the cerebellar vermis was recruited during SW. This alludes to the idea that the midline cerebellum is more important in trunk and balance control, as the cerebellar hemispheres activated during SG are responsible for foot placement during gait modifications (Ilg et al., 2008). In further support, midline cerebellar regions have

also been reported for both imagining and performing real locomotion (Jahn et al., 2008; Jahn et al., 2004; la Fougere et al., 2010).

To further understand brain regions involved in both SW and SG, we subtracted US from SG to reveal neural activations common to both types of gait. Subtracting US from SG revealed various cortical activations including the paracentral gyrus (i.e. M1), parietal and occipital cortices and cerebellum. When these regions of activation are compared with regions activated during SG (SG-SW), recruitment is contained to the parietal cortices and cerebellar (vermis and hemispheres). Therefore, we propose that posterior parietal regions are exclusive to SG, in that this area is involved in integrating sensorimotor input to plan gait modifications (Burgess et al., 1999). However, straight walking (SW-US) showed increased activation of paracentral gyri, occipital cortex and cerebellar vermis. Thus, we can conclude that the paracentral gyri recruitment is important in straight ahead steady state locomotion, however these effects were removed when SW was subtracted from SG. Moreover, activation of the paracentral gyri (specifically the PMC), aligns with findings from monkey studies which found this area to be important in the preparation of sequential movements involving visuomotor integration (Rizzolatti et al., 1998). When US was subtracted from SG, the occipital area showed increased recruitment similar to SW (SW-US) which was removed when SW was subtracted from SG. Interestingly, when looking exclusively at SG, there was increased activation of the parahippocampal gyrus which is known to receive mainly visual input (la Fougere et al., 2010) and therefore, could account for the increased activation of the parahippocampal gyrus only during SG and not during SW only.

Although previous studies have reported activation of the thalamus and BG for locomotor tasks (Jahn et al., (2004), we did not observe such an increase in rCGM for neither SW nor SG.

This does not mean that these regions are not involved in SW or SG but rather, that the recruitment of these regions is similar and not increased when compared to the respective reference task (i.e., US and SW). Previous work investigating changes in brain activations during locomotion have either used protocols in which participants are imagining locomotion in supine position (Jahn et al., 2008; Jahn et al., 2004) or using FDG-PET imaging with supine as the reference task (la Fougere et al., 2010; Malouin et al., 2003). Therefore, the difference in activation likely arises from the reference task. La Fougère et al., (2010) suggested that PMC-basal ganglia activation could be involved in modulation of gait but it is unlikely in light of our results which would suggest that basal ganglia be more related to postural control and premotor to straight walking. As we know from spinal cord injury research, postural control involves brain regions above the brainstem (i.e. higher level structures) while on a treadmill can promote rhythmic locomotor movements via lower level locomotor regions such as the CLR, MLR or SLR which modulate the spinal cord CPGs (Grillner and Wallen, 1985; Grillner and Zangger, 1975; la Fougere et al., 2010). Postural control and locomotor networks are largely independent and by subtracting activations during active balance control, we can quantify task-specific locomotor activations in the human brain. Unfortunately, due to limitation in radiation exposure, we were unable to add a supine reference task to this protocol to identify balance-related brain regions. In light of the current findings, we would expect that such contrast would show increase in rCGM in basal ganglia and thalamus regions, reflecting postural control specific activity. In fact, Jahn et al., (2004) compared imaginary stance with imaginary locomotion using fMRI and showed predominant activation of the basal ganglia and thalamus during imaginary standing, supporting the thesis that the basal ganglia plays an important role in the control of posture.

With this paradigm, we revealed task-specific neuronal correlates of locomotion. The

strength of our study design was to use reference tasks to isolate SW from postural control and focus specifically on SG. The use of [^{18}F]-FDG PET imaging enabled us to visualize whole brain metabolic activity while performing the task. On the other hand, this imaging technique does add limitations to our walking protocol in that participants need to be engaged in a continuous task for at least 20 minutes. Thus, it is not possible to have participants walk straight, without any turns, for such a long period. Our straight walking paradigm included turn at the end of each lanes. These transitions were still minimal as compared to the amount of straight walking performed. Furthermore, FDG-PET only reveals integrated neuronal activity over a long time period and in large ensemble of cells and does not allow evaluation of mechanisms at a deeper cellular level (Zwergal et al., 2016). Radiation limits number of tasks, we could not test lying down to look at control of basal ganglia and it also limited our number of subjects is relatively small number of subjects requires further investigations to allow for the generalization of these results.

Overall, this study showed the involvement of superior parietal regions during real steering of gait in healthy individuals suggesting the PPC is important in integrating sensorimotor information and planning of gait modifications. Finally, our findings also provide a rationale for the use of [^{18}F]-FDG-PET paradigms in pathological populations with the aim of developing rehabilitation therapies to reduce falls and improve or maintain one's ability to successfully redirect themselves and steer around corners or obstacles.

Acknowledgements

We thank the Cyclotron Unit of the McConnell Brain Imaging Center for their skillful assistance and the McGill Athletics for their help with coordinating the use of the facilities. We would also like to thank our research personnel including Melanie Bañina and Kim Emery for their assistance with data collection.

Author Contribution statement

FS contributed to the study design, recruitment and screening of subjects, data collection, data analysis and wrote the manuscript. CP developed the study design, obtained funding, supervised data collection and analysis and revised the manuscript and figures. JPS revised the manuscript. PG contributed to data analysis. IF assisted in data collection and data analysis.

Disclosure/Conflict of Interest

None.

CHAPTER 4. CONCLUSIONS

This study was the first to use [^{18}F]-FDG-PET to identify cerebral activations during the performance of complex locomotor tasks. Findings of this study revealed differential locomotor networks controlling for SW and SG. SW results confirmed previous imaginary locomotor neuroimaging studies (Jahn et al., 2004, 2008; la Fougere et al., 2009) which postulated a common locomotor network involved in straight ahead walking. Likewise, when US was subtracted from SW, a more direct locomotor network was found including sensorimotor regions, occipital visual areas and the cerebellar midline. Results during SW provide evidence regarding the recruitment of these cerebral areas which are integrated with input from deeper subcortical regions including the thalamus and basal ganglia to optimize postural responses and balance to achieve successful straight ahead walking. Moreover, when SW was subtracted from SG, we saw recruitment of posterior parietal areas (i.e. SPL) involved in visuomotor integration and the planning of gait modifications, precuneus and parahippocampal gyri, and the cerebellar midline. Based on the evidence these findings revealed, we propose a new and novel "volitional" locomotor network involved in controlling SG. This study provides new evidence for distinct locomotor networks involved in functional goal-directed locomotion using [^{18}F]-FDG-PET. While our findings provide a new comprehensive understanding of the cerebral networks involved in gait modifications, however to further understand the integration of volitional and automatic locomotor networks, further neuroimaging studies including protocols involving real performance of locomotor tasks is warranted. Nonetheless, this study has provided findings which can be implemented in formulating rehabilitation studies and therapies (i.e., transcranial magnetic stimulation, and physical and occupational therapy protocols) to reduce falls while turning and improve and/or maintain successful gait modifications in both healthy aging

individuals and those with pathologies involving gait disturbances (i.e., stroke, Parkinson's disease, cerebellar ataxia etc.).

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APPENDIX A: EDINBURGH HANDEDNESS INVENTORY

EDINBURGH HANDEDNESS INVENTORY

(OLDFIELD RC, *NEUROPSYCHOLOGIA* 9:97-113, 1971)

SUBJECT ID CODE: _____

A. Please indicate your preferences in the use of hands in the following activities by putting + in the appropriate column. Where the preference is so strong that you would never try to use the other hand unless absolutely forced to, put ++. If in any case you are really indifferent put + in both columns.

B. Some of the activities require both hands. In these cases the part of the task, or object, for which hand preference is wanted is indicated in brackets.

C. Please try to answer all the questions, and only leave a blank if you have no experience at all of the object or task.

		LEFT	RIGHT
1	Writing		
2	Drawing		
3	Throwing		
4	Scissors		
5	Toothbrush		
6	Knife (without fork)		
7	Spoon		
8	Broom (upper hand)		
9	Striking a match (match)		
10	Opening a box (lid)		

APPENDIX B: MR QUESTIONNAIRE

Subject last name:_____ First name: _____

Date of birth:_____
dd / mm / yy

Sex: F ☐ / M ☐

Previous surgery?	NO	YES	If yes indicate the type
Head			
Heart			
Eyes			
Abdomen			
Extremities			
Spine			

Others:_____

Do you have a:	NO	YES	
Cardiac Pacemaker / Defibrillator			
Cochlear implant or implanted hearing aid			
Implanted insulin pump			***If yes, must be removed for scan***
Coloured contact lenses			***If yes, must be removed for scan***
Transdermal delivery system (e.g. patch)			***If yes, must be removed for scan***
Body piercing			***If yes, must be removed for scan***
IUD			
Foreign metallic objects (e.g. bullets or metal splinters)			
Permanent make-up / tattoos			
Ocular implants or devices			***Specify type*** ▶ _____
Cardiac valve prosthesis			▶ _____
Neurostimulator			▶ _____
Artificial limb or joint			▶ _____
Implanted orthopedic device			▶ _____
Penile implant			▶ _____
Aneurysm Clip			▶ _____
Filter, catheter or stent in a blood vessel			▶ _____
Shunt (programmable)			▶ _____

	NO	YES
Are you pregnant?		
Have you ever been injured by a metallic piece? (e.g. in your eyes)		
Have you ever undergone Magnetic Resonance Imaging?		
If yes when: _____		
Do you suffer from claustrophobia?		

_____ _____
Subject signature Date (dd-mm-yy)

_____ _____
Physician / Researcher signature Date (dd-mm-yy)

APPENDIX C: MNI PET UNIT MEDICAL HISTORY

MONTREAL NEUROLOGICAL INSTITUTE

PET UNIT

MEDICAL HISTORY

LAST NAME : _____ FIRST NAME : _____ PHONE # : _____

ADDRESS : _____ CITY: _____ POSTAL CODE: _____

PROVINCE or COUNTRY of ORIGIN : _____

DATE OF BIRTH (m/d/y) : _____ AGE : _____ OCCUPATION : _____

MEDICARE # : _____

ARE YOU RIGHT OR LEFT HANDED? : _____ WEIGHT: _____ HEIGHT: _____

DO YOU SUFFER FROM ANY OF THE FOLLOWING CONDITIONS?

	YES	NO
CLAUSTROPHOBIA		
NERVOUS CONDITIONS OR ANXIETIES		
EPILEPSY / NEUROLOGICAL DISEASES		
PSYCHOLOGICAL PROBLEMS		
HEAD INJURIES OR TRAUMA		
LOSS OF CONSCIOUSNESS OR FAINTING		
HIGH BLOOD PRESSURE		
LOW BLOOD SUGAR		
BLOOD CLOTTING PROBLEMS		
ANEMIA OR OTHER BLOOD DISORDERS		
EASILY TIRED OR FATIGUED		
DIABETES		
HEART DISEASE		
LIVER DISEASE (HEPATITIS OR OTHER)		
CANCER OR TUMORS		
BREATHING DIFFICULTIES (ASTHMA, BRONCHITIS)		
ALLERGIES TO ANAESTHETICS OR OTHER DRUGS		
ANY X-RAYS OR NUCLEAR MEDICINE TESTS DURING THE PAST YEAR IF YES, WHICH TYPE:		
ARE YOU PREGNANT ?		

ARE THERE ANY OTHER CONDITIONS WE SHOULD BE AWARE OF ? : _____

MEDICINES OR DRUGS CURRENTLY TAKEN : _____

LAST MENSTRUAL PERIOD (females) : _____ TOBACCO USE : _____ ALCOHOL USE : _____

APPENDIX D: CONSENT FORM

INFORMED CONSENT DOCUMENT

Project Title: Neuronal correlates of locomotion: understanding the effects of post-stroke neuroplasticity to develop new add-on therapies

Principal Investigator:

Caroline Paquette, Ph.D., Assistant Professor

Kinesiology and Physical Education, Faculty of Education, McGill University

Dr. Jean-Paul Soucy, M.D., Associate Professor

McConnell-Brain Imaging Centre, Montreal Neurological Institute, McGill University

Sponsoring: Heart and Stroke Foundation

Introduction:

We are asking you to participate in a research project to understand how our brain controls our posture and walking and especially how we control our walking trajectory. Before agreeing to participate in this project, please take the time to read and carefully consider the following information.

This consent form explains the reason of this study, the procedures, disadvantages, advantages, as well as the persons to contact, if necessary.

This consent form may contain words that you do not understand. We invite you to ask any question that you deem useful to the researcher and the other memb ers of the staff assigned to the research project and ask them to explain any word or information that is not clear to you.

Reason for the Study:

With this study, we are investigating which areas of the brain are involved in the control of the walking trajectory. We want to understand how the brain reorganizes after stroke to maintain walking capabilities.

We seek your participation in this study as a normal healthy participant or as someone who had a stroke to help us determine which areas of the brain are involved in walking and whether we can use brain stimulation to increase activity in these regions after stroke to help with the recovery of walking.

Procedures:

Your participation in this study will involve 6 visits as described below.

Visit 1 (duration: 1.5 h)

To fill various questionnaires and forms (such as this consent form), schedule upcoming visits and tour the research premises. The goal of this visit is to ensure that you can walk our obstacle course for 30 minutes and that there are no reason why you could not participate in this study.

Visits 2 & 3 (duration: 2 h each)

The goal of those visits is to take images of your brain while you are walking. During one of the visits we will ask you to walk a straight path and to walk around obstacles in the other visit. These visits will be separated by at least 1 week.

We will use 2 different types of imaging techniques:

- PET (Positron Emission Tomography) to be done in both visits 2 and 3.
- MRI (Magnetic Resonance Imaging) to be done one time only in visit 3.

PET Imaging: Upon your arrival, a fine needle-catheter will be inserted into an arm vein for the administration of small amounts of a radioactive substance (18F-FDG,
or fluorodeoxyglucose, which behaves very much like sugar in your body, labeled with a short lived radioactive fluorine isotope (^{18}F) with a physical half-life of 2 hours). Once the catheter is in place, you will start performing the walking task that will last for approximately 30 minutes. After 10 minutes of walking you

will receive the injection of the radioactive substance and continue the walking task for another 20 minutes. The total activity injected from the 2 visits will be of 370 MBq. MBq is short for megabecquerels, a unit used to measure how much radioactivity there is in a sample.
After the walking task is over, we will record images of your brain. A scanning session is tailored to the needs of a specific study and can take up to one hour during which time you will be requested to lie still on the couch in the scanner. All procedures during the PET study will be carried out by a qualified nuclear medicine technician, and supervised by a qualified nuclear medicine physician.

THE FOLLOWING ARE CONTRAINDICATIONS FOR THE PET PROCEDURE:

- Pregnancy or Breastfeeding
- Under 18 years old
- Previous radiation absorbed doses received within the past (12 months) from other experiments that would lead, with inclusion of this study, to an aggregate radiation absorbed dose exceeding 20 mSv (millisievert). The dose of radiation absorbed in the body from this study will be approximately 11.5 mSv.

MRI Imaging: You will be asked to lie on a bed that will be moved into a cylindrical opening where images of your head will be taken during a period of 20 minutes. The cylindrical opening is very narrow and persons with claustrophobia should refrain from participating in the study as they may feel strong discomfort from being in a confined space. The MRI machine will be quite noisy during the scan. To reduce the noise, you will wear headphones. You will be able to communicate with the technician during the procedure.

THE FOLLOWING ARE CONTRAINDICATIONS FOR THE MRI PROCEDURE:

Pacemaker,
Aneurysm Clip,
Heart/Vascular Clip,
Prosthetic Valve,
Metal Prosthesis,
Pregnancy,
Claustrophobia,
Metal fragments in the body,

Transdermal patches (must be removed prior to scanning). There is a concern that the magnetic field from the MRI will affect the drug release from your transdermal patch. You should bring an additional patch to re-apply post scanning. If interruption of the transdermal drug application is not possible for 20 minutes, you cannot take part in this study.

A coronary artery or other stent may also prevent you from partaking in MRI scanning, depending on the type of stent.

Visits 4 & 5 (duration: 1.5 h each)

In these visits we will stimulate your brain using non-invasive brain stimulation (rTMS). After the stimulation you will be asked to walk around obstacles. This walking task will be identical to the ones in visits 2 to 4 but will take place in a smaller room.

Repetitive Transcranial Magnetic Stimulation (rTMS) Procedure: During visits 4 &5, you will receive 20 minutes of rTMS. You will be asked to sit comfortably in a chair and two electrodes will be fixed over your skin over your hand muscles in order to record muscle contractions. A TMS coil will be positioned over different parts of your head and will be used to stimulate your brain. The intensity of stimulation will be increased until a contraction response is recorded by the electrodes and a muscle twitch is seen on the hand. Depending on the stimulation site, you may experience a minimal discomfort caused be slight muscle contraction of the hand muscles. This stimulation procedure will berepeated several times to find the desired intensity and correct location for stimulation. Once the intensity and position have been determined, we will stimulate the brain with one pulse every second continuously or with 10 pluses every second for 5 seconds with 55 second break (the sequence is repeated for 20 minutes). After this stimulation, the electrodes and coil will be removed. You will then perform the walking task for 10 minutes. During the walking task, you will wear a safety harness and assistant will follow you closely to prevent a fall in case you lose your balance.

Potential Risks of Participating in this Study:

Some discomfort may be caused by insertion of the fine needle-catheter into the vein, as well as immobility on the couch. The study you are asked to participate in will involve injection of radioactive material into your body. The specific agent(s), 18F-FDG, to be used is not normally present in your body and will result in a small dose of radiation (expressed in units of millisieverts, mSv) above that which you are inevitably exposed to in daily life (natural radiation in the environment, cosmic rays, etc), or to that you might receive for medical reasons (diagnostic X-rays, radiation therapy). Nationally accepted limits on radiation doses which can be administered for research purposes have been defined (20 mSv/year), and in order to ensure that you do not go above the recommended limit, you must make sure to let us know about any other research protocol you might have been part of that would have involved radiation exposure, as well as to mention the current protocol, if you do take part in it, to any investigator asking you to take part in another protocol in the future.
Most of the radioactivity you will receive will be gone from your body in a matter of hours. The risk which is alluded to when discussing risk associated with radiation exposures of the level seen in PET scanning (specifically in the current study, the radiation exposure is estimated at 11.5 mSv) is that of developing a cancer at some point in the future, which would not have happened if you had not received that radiation dose. Although radiation clearly increases the risk of developing cancer over certain doses, its ability to do so at the levels used in PET imaging has never been observed, is certainly at most very low, and could conceivably not even exist.

During the MRI-study, you will be exposed to a strong magnetic field. No long-term negative side effects have been observed from this type of study. If you have metallic implants you cannot participate in the study, because these implants may become dislocated or may heat up during the measurement due to the strong magnetic field. The MRI is very noisy and you will wear headphones to reduce this effect.

For rTMS, a serious potential risk is the induction of seizure. A seizure is the result a sudden burst of excess activity in the brain. This causes the brain’s messages to become temporarily halted or mixed up. This may affect the body in different ways, and can cause spells of confusion, jerking

movements and even sudden loss of consciousness. For single-pulse TMS, there are no reported cases of seizures in healthy participants; it is considered a safe technique in this group. For rTMS, the technique used in this study, the risk of seizure is a little bit higher. In the past, a total of 8 brief seizures have been reported. Given that the total number of people tested with rTMS is more than 1,000, this means that more than 99% of the participants did not experience a seizure. It is important to point out that seizures occurred in individuals at risk; that is in persons with a history of epilepsy or other neurological disorders. At that time, it was not known that these conditions were incompatible with rTMS. This study will be conducted in accordance with rigorous safety guidelines. These guidelines outline safe stimulation parameters and define who can undergo TMS without risk. Since the establishment of these guidelines, no seizures associated with TMS have been reported. There are no known long-term risks associated with the rTMS procedure. rTMS is accompanied by loud clicking sounds from the stimulator that can exceed 100 dB near the stimulator. While studies conducted in humans found that the clicking sounds produced no measurable hearing loss, you will be provided with earplugs during the rTMS session as a precaution. A potential short-term side effect of rTMS is a tension-type headache localized at the site of the stimulation. It has been estimated that headache can occur in up to 20% of rTMS subjects.

During the walking tasks, a person will always be present to provide any assistance. You may, however, feel tired following the evaluation. You can request to stop the experiment if you are too tired or uncomfortable to participate further in the experiment.

Potential benefits of Participating in this Study:

You will not benefit directly from participating in the study. However, with this study we will determine the role of given brain structures in the control of walking.

Withdrawal from the Study:

Your participation in this study is voluntary. You may refuse to participate or if you agree to participate you may leave the study at any time without affecting future medical care.

Discontinuation of the Study by the Investigator:

At any time during the testing, the investigator has the right to terminate the study for any reason. If this was to occur, the reason(s) will be explained to you.

Compensation:

There are no costs to you for participating in this study other than your time. You will be compensated a total amount of $200 for your time and will be reimbursed your transportation costs.

Research Injury:

Every effort to prevent injury that could result from this study will be taken by the investigator and study personnel. In the event of injury or illness suffered by participating in this study, you will receive appropriate medical care under the Quebec Medicare or private insurance plans.

Confidentiality:

A number of precautions will be taken to guarantee the confidentiality of the information you will provide. Results from this study will be analyzed in group form. Furthermore, in all databases and documentation, participants will be identified by unique identification number only (random values containing no identifier). All personal and identifying information will be kept confidential and under lock and key. Data recorded by computers, will be transferred and kept on computer disk with limited access. Only the members of the research team will have access to the information gathered during the project. This information will be kept for 5 years, after which they will be destroyed. The research data will appear only in the form of a scientific presentation or publication, without your name, or any potentially identifying information being disclosed. Imaging data will be stored in a secure room at the brain imaging centre. The Research Ethics Board may consult the study data to ensure the sound management of this study.

Results of the Research:

Any relevant information regarding the results of the research will be communicated to you, upon your request. Brain images and other test

results, obtained in this study are not routinely scanned for abnormalities. Should there be any incidental findings and should you wish to receive this information, it will be communicated to you and your physician.

For More Information:

The following are the names, addresses and telephone numbers of persons you may contact for questions about the research or any injuries or adverse reactions:

Caroline Paquette
(514) 398-4184 ext. 00890

Department of Kinesiology and Physical Education
475 Pine Avenue West
Montreal, Quebec H2W 1S4

For PET imaging:

Jean-Paul Soucy
(514) 398-1585

Montreal Neurological Institute
3801University St.
Montreal, Quebec H3A 2B4

Contact Information for Subjects:

If you have any questions regarding your rights as a research volunteer and wish to discuss with someone other than the individual(s) conducting the study, you may contact the following impartial third party, who are not associated with the study.

You may contact the Patient Ombudsman at the Montreal Neurological Institute at **(514) 934-1934 ext. 48306**.

Any other kinds of comments or concerns or assistance needed regarding participation as a research subject in the project can be addressed to the Montreal Neurological Hospital Patients' Committee, Room 354, tel. (514) 398-5358.

You will be informed of any major new findings during the course of your participation in this study, which may affect your willingness to continue in the study.

SIGNATURE/CONSENT: I have read (or have had read to me) this consent form. This consent form should only be signed if I did have a chance to ask and receive satisfactory answers to all my questions. I voluntarily consent to participate in this study. I have received a copy of this signed consent form.

I have asked that this consent form be provided in English.

I do not waive my legal rights by signing this form.

Research Participant:

_____ Date: _____
(Signature)

_____ Tel: _____
(Print Name)

Investigator:

_____ Date: _____
(Signature)

_____ Tel: _____
(Print Name)

Person Explaining Consent Form:

_____ Date: _____
(Signature)

_____ Tel: _____
(Print Name)

Incidental Findings:

Test results obtained in this study are not routinely scanned for abnormalities. Should there be any incidental findings do you wish to receive this information?

- ☐ Yes, I would like to receive this information. *If applicable, it will be communicated to you and your physician.*
- ☐ No, I do not wish to receive this information.

_____
Research Participant Initials