Journal of Neuroscience Methods 204 (2012) 306–317

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Clinical Neuroscience

A non-human primate model of bipedal locomotion under restrained condition allowing gait studies and single unit brain recordings

L. Goetz[a,b,c], B. Piallat[a,b,c], Y. Thibaudier[a,b,c], O. Montigon[a,b,c,d], O. David[a,b,c,e], S. Chabardès[a,b,c,f,*]

[a] *Grenoble Institut des Neurosciences, Chemin Fortuné Ferrini, BP 170, 38042 Grenoble, France*
[b] *Université Joseph Fourier, Fonctions cérébrales et neuromodulation, Grenoble, France*
[c] *INSERM, U836, Grenoble Institut des Neurosciences, Grenoble, France*
[d] *Plate forme IRM Préclinique, Grenoble, France*
[e] *Clinique de Neuroradiologie et Unité IRM, Pôle Imagerie, Centre Hospitalier Universitaire, Grenoble, France*
[f] *Clinique de Neurochirurgie, Pôle Tête et Cou, Centre Hospitalier Universitaire, Grenoble, France*

ARTICLE INFO

Article history:
Received 16 June 2011
Received in revised form
23 September 2011
Accepted 9 November 2011

Keywords:
Non-human primate
Locomotion
Gait disorders
Experimental model
Kinematic
Micro electrode recording
Parkinsons disease

ABSTRACT

For decades, several animal models of locomotion have allowed a better understanding of the basic physiological mechanisms of gait. However, unlike most of the mammals, the Order Primates is characterized by fundamental changes in locomotor behaviour. In particular, some primates use a specific pattern of locomotion and are able to naturally walk bipedally due possibly to a specific supra-spinal control of locomotion. These features must be taken into account when one considers to study the intrinsic properties of human gait. Thus, an experimental model of bipedal locomotion allowing precise and reproducible analysis of gait in non-human primate is still lacking. This study describes a non-human primate model of bipedal locomotion under restrained condition. We undertook a kinematic and biomechanic study in three *Macaca fascicularis* trained to walk bipedally on a treadmill. One of the primate was evaluated in complete head fixation. Gait visual analysis and electromyographic recordings provided pertinent description of the gait pattern. Step frequencies, step lengths, cycle and stance phase durations were correlated with Froude number (dimensionless velocity), whereas swing phase durations remained non-correlated. Gait patterns observed in our model were similar to those obtained in freely bipedal *Macaca fuscata* and to a lesser extend to Humans. Gait pattern was not modified by head fixation thereby allowing us to perform precise and repetitive micro electrode recordings of deep cerebral structures. Thus, the present model could provide a pertinent pre-clinical tool to study gait parameters and their neuronal control but also could be helpful to validate new therapeutics interventions.

1. Introduction

For decades, animal models of locomotion, developed in the lamprey, cat, and rodent, have allowed a better understanding of the basic physiological mechanisms of gait (Grillner, 2003; Clarac, 2008; McCrea and Rybak, 2008). However, unlike most of the mammals, the Order Primates (including Humans) is characterized by fundamental changes in locomotor behaviour. Nevertheless, a primate model of bipedal locomotion under restrained condition which allows for reproducible gait analysis for electrophysiological studies and/or pre-clinical treatment assessments, is still lacking. In particular, such a primate model, phylogenetically close to human, would allow a better understanding of pathophysiological mechanisms underlying gait disorders that may occur in neurodegenerative diseases, movement disorders and after spinal cord trauma.

Locomotion in primates has been extensively studied for decades and is a key element to understand the origins and evolution of human bipedalism (Schmitt, 2003; Hirasaki and Ogihara, 2006). The Order Primates is characterized by diverse locomotor behaviours depending on the substrate used (terrestrial, arboreal). Most of the primates commonly use quadripedalism to move in their environment with a great variety of displacement, using models such as walker, leaper, arm swinger, vertical clinger as detailed by D. Schmitt in a recent review on primate neuroethology (Platt and Ghazanfar, 2010).

Furthermore, primates, that belong to Catarrhini parvorder (including old world primates, apes and humans) are characterized by locomotor behaviour that fundamentally differs from all the other mammals regarding the pattern of locomotion and its neuronal control: (i) primate locomotor control is roughly thought to rely on a supra-spinal control that includes cerebral cortex, basal ganglia and brainstem nuclei. Furthermore, the existence of

* Corresponding author at: Université Joseph Fourier, Fonctions cérébrales et neuromodulation, Grenoble, France. Tel.: +33 4 76 76 54 72
E-mail address: SChabardes@chu-grenoble.fr (S. Chabardès).

0165-0270/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2011.11.025

central pattern generators in primates (including humans), located in the spinal cord has still not been demonstrated (Schmitt, 2003; Vilensky and O'Connor, 1998; Vilensky, 1989; Eidelberg et al., 1981; Capaday, 2002; Duysens and de Crommert, 1998; Hultborn and Nielsen, 2007; Guertin, 2009; Mori et al., 2004; MacKay-Lyons, 2002). (ii) A functional differentiation between hind limbs (HL) and fore limbs (FL): the former are thought to act as the locomotor propelling component, supporting most of the body weight while the latter, released from it, could provide stability, guidance during locomotion and the ability to perform precise manipulative or grasping movements presumably due to the requirement of arboreal locomotion on thin branches (Vilensky, 1989; Schmitt, 2003; Kimura et al., 1983). (iii) Primates preferentially use diagonal sequence (DS) footfall pattern during which each HL foot contact is followed by the contralateral FL foot contact (DS = Right HL, Left FL, Left HL, Right FL) as first described by Prost (1965) and Hildebrand (1967). Interestingly, primates can occasionally switch from DS to a lateral sequence (LS) commonly used by most other mammals. This typical DS pattern of locomotion and the ability to switch from DS to LS could reflect the fundamental changes in the neuronal control of locomotion by supra-spinal brain structures (Vilensky, 1989; Schmitt, 2003; Kimura et al., 1983). (iv) Primates ability to use natural bipedal locomotion on short distance has been observed in most primate species (Hirasaki and Ogihara, 2006; Schmitt, 2003; Aerts et al., 2000; Kimura et al., 1983). Of special interest in this study, bipedalism in primate have been studied for more than 50 years since the pioneering study of Elftman and Manter in 1935 who analyzed the distribution of pressure in the foot of a chimpanzee during bipedalism (Elftman and Manter, 1935). Kimura et al. confirmed that voluntary bipedal locomotion could be observed in many non-human primates (NHP) (Kimura et al., 1983). In particular, some zoological observations of *Macaca arctoides* and *Macaca fascicularis* reported that those macaque species could naturally adopt bipedal posture and locomotion in specific situations, such as scanning the environment (Cant, 1988; Hemmi and Menzel, 1995; Yeager, 1996; Sigmon, 1971).

More recently, numerous studies focused on the ability of the *Macaca fuscata* to walk bipedally as it has been displayed for centuries in Asian entertainments. More precisely, several Japanese groups performed biomechanical studies to compare quadrupedal and bipedal locomotion in order to describe the bipedal gait pattern of the *M. fuscata* at different gait velocity (Nakajima et al., 2004; Kimura et al., 1983; Mori et al., 2004). Hirasaki's group compared energetic and kinematic parameters of two groups of bipedal macaques (ordinary *versus* highly trained) and found that in highly trained group, bipedal locomotor features were characterized by an increasing stride length and a reduced step frequency possibly due to the use of the inverted pendulum mechanic (Hirasaki et al., 2004, 2006; Nakatsukasa et al., 2004). All the aforementioned studies underlined that when *M. fuscata* was included in an intensive bipedal training protocol, step kinematic parameters (phases durations, frequency, length, joints angles) showed great similarities to those observed in human bipedalism leading to "the development of human-like gait characteristics" (Hirasaki et al., 2004).

In neuroscience, most of recent studies were conducted on decerebrate animals or on spinal preparations but few on live animals, pointing out the need for a new experimental model of locomotion in NHP. Hence, those studies could not take into account the role of afferent inputs of spinal cord circuitry and supra-spinal structures (Capitanio and Emborg, 2008; Capaday, 2002). Due to the specific nature of the primate locomotion and because of their relative phylogenetic and neuroanatomical closeness to Humans, NHP, particularly macaque monkeys, remain a useful experimental model in neuroscience (Vilensky, 1989; Anonymous, 2008; LeDoux, 2005; Capitanio and Emborg, 2008).

Electrophysiological recordings are useful tools to study neuronal networks involved in brain functions during normal and/or pathological states. Multi-electrode arrays have been recently proposed in freely moving animals (Ludvig et al., 2001; Eliades and Wang, 2008). Nevertheless, successive and precise micro electrode recordings (MER) of deep brain structures still require complete immobility of the animal's head. This limitation points out the need to develop a new experimental set up for locomotion based on the use of a treadmill that would allow head fixation and control of all experimental conditions. Besides, using a treadmill allows exact control of experimental conditions required for gait analysis and optimal reproducibility of the experiments. For both reasons, locomotion on treadmill is very useful to evaluate gait characteristics between subjects and in different pathological conditions.

The aim of this study was to characterize an experimental model of bipedal locomotion in the NHP under restrained condition. To that goal, we conducted a kinematic analysis on three *M. fascicularis*, in two different conditions (head free *versus* head fixed). The data obtained were then compared to data available in the literature obtained in *M. fuscata* (Kimura et al., 1983; Nakajima et al., 2004; Nakatsukasa et al., 2004, 2006) and in human beings (Nilsson et al., 1985; Grillner et al., 1979). In addition, we present preliminary biomechanical data and electrophysiological recordings to demonstrate the potential feasibility and usefulness of our model for gait disorder research.

2. Materials and methods

2.1. Experimental setup

In order to obtain a complete control on the primate's behaviour during a locomotor sequence, we designed and developed a treadmill adapted to a primate chair (Primate Products Inc., Immokalee, USA) (Fig. 1). The animal was restrained to the chair by means of a collar. The treadmill was linked to a DC engine with a transmission shaft long enough to avoid electromagnetic artefact during electrophysiological MER. The treadmill was controlled by a home-designed software based on Labview system (National Instruments, Austin, USA) that allowed to tune the treadmill velocity and to synchronize the treadmill onset/offset with a visual preparation cue, electrophysiological recordings and video acquisition using Transistor–Transistor Logic inputs (Fig. 1).

2.2. Animals

Three cynomolgus monkeys (*M. fascicularis*)(CRP Port Louis, Mauritius) were used in this study (Monkey A: male, 9 years old, weight: 9 kg; Monkey B: male, 3 years old, weight: 5.5 kg; Monkey C: male, 3 years old, weight: 4.5 kg). All experiments were carried out in accordance with the recommendations of the European Community Council Directives of 1986 (86/609/EEC), the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Ethics Committee of Région Rhône-Alpes. Animals were kept with other congeners allowing social behaviours, in an air-conditioned room under standard conditions of temperature (23 ± 1 °C), humidity (65 ± 4%) and light (12 h light/dark cycle). They had access *ad libitum* to food and water and were given fresh fruits and vegetables every day. All animals included in this study naturally adopted bipedal posture in their cage or during locomotion with a handling stick and were therefore considered to be good subjects for the bipedal locomotion protocol.