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Characterizing dynamic properties of retinal vessels in the rat eye using high speed imaging

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A R T I C L E I N F O

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A B S T R A C T

Purpose: The dynamic properties of retinal vessels including pulse wave propagation and pulsatility index provide new perspective in retinal hemodynamic analysis. In this study we utilize a high speed imaging system to capture these characteristics in the rat eye for the first time.

Methods: Retinal video images of 9 Wistar-Kyoto (WKY) rats were captured at a rate of 250 frames per second for 10 s with a 50° field of view using a high speed camera (Optronis, Kehl, Germany). Two recordings were taken from each rat at the same sites for repeatability analysis. The electrocardiogram (ECG) was measured simultaneously with retinal images. Arterial retinal pulse wave velocity (rPWV) and arterial/venous pulse amplitude were calculated from recorded images. Arterial measurements were repeated in another normotensive strain of the same age (Sprague–Dawley, $n = 4$).

Results: The average WKY rPWV was 11.4 ± 6.1 cm/s. The differences between repeated measures were not significant (-2.8 ± 2.9 cm/s, $p = 0.2$). Sprague–Dawley animals had a similar rPWV (9.8 ± 2.2 cm/s, $p = 0.61$). The average arterial and venous pulse amplitude was 7.1 ± 1.5 μm and 8.2 ± 2.0 μm respectively. There was a positive correlation between rPWV and heart rate in the WKY groups ($r^2 = 0.32$). A positive correlation was also obtained between arterial and venous diameter and their pulse amplitude ($r^2 = 0.67$ and $r^2 = 0.37$ respectively).

Conclusion: rPWV was associated with heart rate. Higher pulsation amplitude was also correlated with larger vessel diameter. High speed imaging of retinal vessels in the rat eye provides an accurate and robust method to study dynamic characteristics of these vessels and their relationship with ocular and systemic abnormalities.

Introduction

The eye is the only organ which provides direct, non-invasive access to its internal anatomical structure. Retinal vessels provide a unique opportunity to study ocular, systemic and neurological disorders. Several studies have shown that reduced retinal arterial caliber is strongly related to higher blood pressure (Ikram et al., 2004; Wong et al., 2007), whereas a larger retinal venous caliber is consistently associated with higher levels of cholesterol and clinical signs of atherosclerosis (De Jong et al., 2007; Liew et al., 2008a). Retinal venous dilation is also observed in the early stages of diabetes (Nguyen et al., 2007). Other studies have reported an association between static measures of ocular hemodynamic parameters including intraocular pressure (IOP), vessel caliber and cerebrospinal fluid pressure (CSFp) with risk of systemic diseases (Berisha et al., 2007; Liew et al., 2008b; Wostyn et al., 2008). However, little is known of the dynamic characteristics of the ocular circulation.

Dynamic properties of retinal vessels such as pulse wave propagation speed or pulsatility index may vary in different disease processes. The elastic properties of the arteries allow a pressure wave to travel along it. Propagation of this pulse wave is associated with the stiffness of the arteries, such that higher speeds of pulse wave propagation, or pulse wave velocity (PWV), are associated with stiffer arteries, as described in the Moens–Korteweg equation (Nicholas et al., 2011). Arterial blood pressure and heart rate are both known to affect PWV (Gribbin et al., 1976; Lantelme et al., 2002; Tan et al., 2012). Earlier studies have reported that higher arterial stiffness is a major factor in hypertension (Avolio, 1987; Blacher et al., 1999). A nine year follow-up on 1331 individuals reported that the chances of cognitive decline was 40% greater for subjects with middle values of carotid-femoral PWV and 59% greater for subjects with high carotid-femoral PWV, compared with low carotid-femoral PWV (Zeki Al Hazzouri et al., 2013). Mehrabian et al., 2012 studied cognitive dysfunction and arterial stiffness in type 2 diabetes. Their results confirmed that large artery stiffness was increased and was associated with cognitive impairment in type 2 diabetes.

While a large number of studies have shown the importance of central aortic PWV in relation to various diseases, little has been published on PWV in microvessels. The Dynamic Vessel Analyzer (DVA, Imedos, Germany) is the only commercially available device to non-invasively

Abbreviations: PWV, pulse wave velocity; rPWV, retinal pulse wave velocity; IOP, intraocular pressure; DVA, Dynamic Vessel Analyzer; WKY, Wistar-Kyoto; SD rats, Sprague–Dawley rats; CSFp, cerebrospinal fluid pressure; HR, heart rate; OPA, ocular pulse amplitude; BP, blood pressure.

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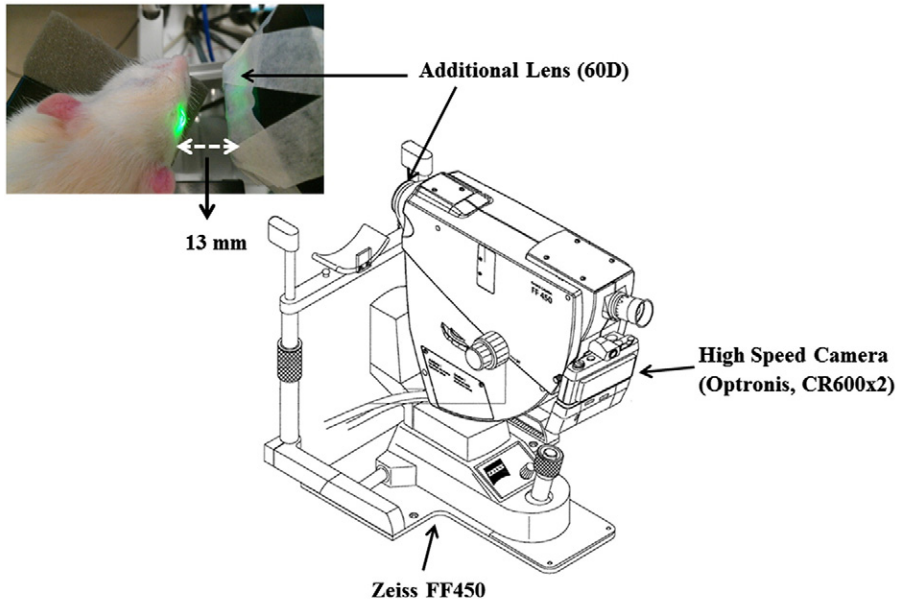

Fig. 1. Sketch of imaging setup used to image the rat retina.

measure dynamic changes in microvessels in real time, specifically, the retinal vessels in humans. However, the DVA measures retinal vessel diameter changes at a rate of 25 Hz and results contain high frequency noise. Kotliar et al. (2011,2013) have used the DVA to measure the retinal arterial pulse at proximal and distal locations of the optic disk to calculate retinal PWV (rPWV). They have applied three techniques to over-come the limitations of the DVA including: 1. Filtering at the heart rate (HR) frequency, 2. Filtering at higher multiples of HR, and 3. Applying a slope of linear regression to distance against phase shift at narrow and wide sections of an artery. Results show that methods 2 and 3 have a significant association with systemic blood pressure ($p < 0.001$).

The veins within the eye are the only veins in the human body which can be seen to pulsate. The amplitude of these pulsations is increased when the IOP is increased or the CSFp is decreased (Golzan et al., 2011; Levine, 1998). Different studies have linked the degree of these pulsations to risk of glaucoma development and progression (Graham et al., 2012; Kain et al., 2010). Morgan et al. (2004) reported a 54% presence of retinal venous pulsation in glaucoma subjects compared with 98% in normal subjects. The amplitude of venous pulsation in relation to IOP has also been used as a non-invasive approach to estimate CSFp (Golzan et al., 2012a).

Measurement of waveform shape parameters in retinal vessels has been limited to human studies. However, application of the technique to animals would open up research avenues in models of disease and aging. However, animals, specifically rats, have significantly higher heart rates compared to humans (i.e. 300–700 BPM) and therefore require a higher sampling frequency to record these dynamic changes. In this study we utilize a high speed camera to capture dynamic changes of retinal vessels in the rat eye including rPWV and vessel pulsatility. Analysis of dynamic characteristics of retinal vessels in the rat eye will provide a platform for further studies investigating the relationship between properties of retinal vessels and ocular and systemic abnormalities, using different animal models of disease.

Methods

Animal preparation

A total of thirteen rats were included in this study. Nine Wistar-Kyoto (WKY, 280 ± 30 g) males aged twelve weeks and an additional four Sprague–Dawley (SD, 320 ± 30 g) males aged twelve weeks were used. The animals were anesthetized with urethane (ethyl carbamate, 1.3 g/kg). The depth of anesthesia was assessed regularly by assessing the reflex response to noxious stimuli (hindpaw pinch) or tactile stimuli (corneal stroking). A random eye was selected and a single drop of tropicamide 0.5% (Alcon, Fort Worth, Texas, USA) was administered to dilate the pupil. All animals were then placed in front of the fundus camera for high speed retinal imaging. The eye surface was moisturized using a 0.9% saline solution prior to recording of

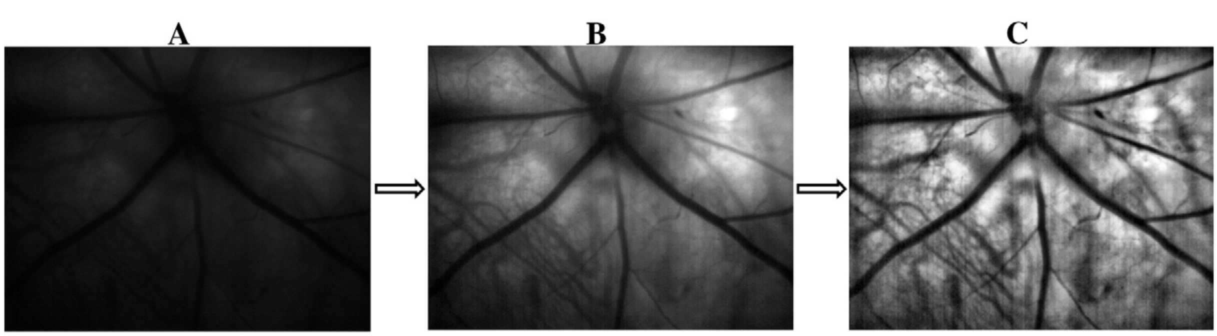

Fig. 2. A. Original image captured using high speed camera, B. Adjusted brightness and contrast, C. Applied CLAHE algorithm.