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Time to wake up: Studying neurovascular coupling and brain-wide circuit function in the un-anesthetized animal

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ABSTRACT

Functional magnetic resonance imaging (fMRI) has allowed the noninvasive study of task-based and resting-state brain dynamics in humans by inferring neural activity from blood-oxygenation-level dependent (BOLD) signal changes. An accurate interpretation of the hemodynamic changes that underlie fMRI signals depends on the understanding of the quantitative relationship between changes in neural activity and changes in cerebral blood flow, oxygenation and volume. While there has been extensive study of neurovascular coupling in anesthetized animal models, anesthesia causes large disruptions of brain metabolism, neural responsiveness and cardiovascular function. Here, we review work showing that neurovascular coupling and brain circuit function in the awake animal are profoundly different from those in the anesthetized state. We argue that the time is right to study neurovascular coupling and brain circuit function in the awake animal to bridge the physiological mechanisms that underlie animal and human neuroimaging signals, and to interpret them in light of underlying neural mechanisms. Lastly, we discuss recent experimental innovations that have enabled the study of neurovascular coupling and brain-wide circuit function in un-anesthetized and behaving animal models.

Introduction

The discovery of anesthetics has been a boon to mankind, allowing invasive surgical procedures to be performed with little pain. In the history of neurophysiology, anesthetics were widely used to immobilize animals and to reduce variability in neural responses due to behavioral and attentional changes. Neurophysiological studies using the anesthetized preparation has tremendously advanced our understanding of brain function. Anesthetics are also an important tool for investigating the phenomenon of consciousness and theory of mind (Alkire et al., 2008; Brown et al., 2010, 2011), given their remarkable ability to manipulate consciousness level.

Despite the significant role that anesthesia plays in neuroscience research, it has become increasingly clear that anesthetics produced a neurological 'state' unlike any natural physiological condition, but rather, anesthesia is more akin to a 'temporary, reversible coma' (Brown, 2010). As a consequence of this better understanding of the nature of the anesthetized brain, and improvements in experimental methodology, there has been a push in the neuroscience community to utilize awake animal models in neurophysiological experiments (Ferenczi et al., 2016). Such models can be particularly valuable to the neurovascular coupling and animal neuroimaging communities, because the interpretation of both task-based and resting-state functional magnetic resonance imaging (fMRI) data collected in un-anesthetized humans depends on our understanding of neurovascular coupling under normal physiological conditions, and consequently, it is critical for translational animal experiments to be done without anesthesia as well. Furthermore, the vast majority of animal models of brain disorders rely on behavioral assessment conducted in the awake condition. Therefore, it is essential to image un-anesthetized animals if the imaging data will be used to interpret behavioral measurements (Ferenczi et al., 2016; Liang et al., 2014).

In this review, we summarize a large body of literature showing that anesthetics produce profound changes in cerebral hemodynamics,

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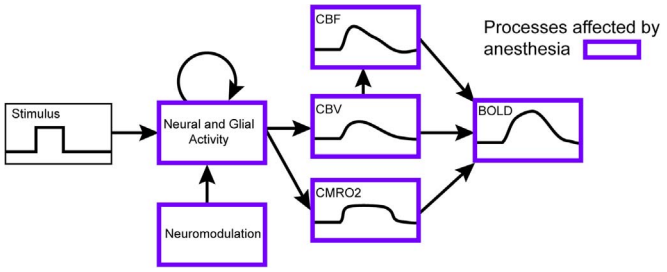

Fig. 1. Effects of anesthetics on hemodynamic responses. Schematic showing how a stimulus-generated changes in neural activity are converted into a detectable BOLD signal. Physiological processes affected by anesthesia are outlined in purple.

brain metabolism, neural activity, neurovascular coupling, and functional connectivity relative to the awake or sleeping states. Because of the large physiological differences between anesthetized and unanesthetized animals, it is inappropriate to generalize physiological experimental data from anesthetized animals to physiological observations in awake humans. Given that technical advances have made it possible to study neurovascular coupling and brain circuit function in awake, behaving animals, the time is right for the community to move towards paradigms using awake animals.

Physiological basis of the BOLD signal, and its sensitivity to anesthetics

The changes in oxygenation that underlie the blood-oxygenation-level dependent (BOLD) fMRI signal are due to an 'oversupply' of oxygenated blood to active areas of the brain (Buxton, 2012; Kim and Ogawa, 2012) (Fig. 1). Neural activity directly and indirectly leads to the release of vasoactive substances (Attwell et al., 2010; Cauli and Hamel, 2010), which relax contractile tissue around arteries (Kim and Ogawa, 2012) and potentially capillaries (Hall et al., 2014; Hartmann et al., 2015; Stefanovic et al., 2008, Fernández-Klett et al., 2010; Hill et al., 2015). Because the resistance of a blood vessel depends on its diameter, these dilations cause a sharp reduction of vascular resistance, which increases blood velocity and flux. This increase in arterial diameter and blood flow is relatively rapid, occurring within less than a second following the stimulation (Chen et al., 2011; Drew et al., 2011; Gao et al., 2015; Kim et al., 2013). Arterial dilation and increased blood flow result in an elevated influx of oxygenated hemoglobin that exceeds the oxygen demand of the surrounding neural tissue (Fox and Raichle, 1986). Once the oxygenated blood transits through the capillary bed and into the draining veins, the increase in venous blood oxygenation can be detected using BOLD fMRI (Kim and Ogawa, 2012) (Fig. 1). The BOLD signal depends on an interaction between local neural metabolic activity (which may not perfectly match up with the spike rate of neurons), increases in blood flow, volume and oxygenation. Because of the transit time, the BOLD signal is delayed relative to the increase in blood volume (Hirano et al., 2011; Silva et al., 2007a). A slow dilation of the veins underlies the "balloon" or "windkessel" models of the BOLD responses (Buxton et al., 2004; 1998; Mandeville et al., 1999; Miller et al., 2001). If the stimulus is sustained for tens of seconds, a slow dilation of veins is observed (Drew et al., 2011; Huo et al., 2015a; Kim and Kim, 2011; Mandeville et al., 1999), though the venous dilation seems to fail under some anesthetics (Drew et al., 2011; Hillman et al., 2007; Lee et al., 2001). Studying the relationship between neural activity and vascular dynamics (neurovascular coupling) is crucial for interpreting brain functional imaging signals. Substantial amount of previous work using anesthetized animals has significantly contributed to our understanding of neurovascular coupling and mechanisms of neuroimaging signals. However, as all of the processes that underlie the BOLD response are affected by anesthesia (Fig. 1) (Aksenov et al., 2015; Martin et al., 2006b; Pisauro et al., 2013; Alkire et al., 2000; Lyons et al., 2016; Sellers et al., 2015; Ferezou et al., 2007; Nimmerjahn et al., 2009; Thrane et al., 2012; Chapin and Woodward, 1981; Crane et al., 1978; Cazakoff et al., 2014; Constantinople and Bruno, 2011; de Kock and Sakmann, 2009; Dudley et al., 1982; Ueki et al., 1992; Ferenczi et al., 2016), measurements made in the awake animal are expected to improve the quantification of neurovascular coupling and facilitate the translation over into the awake human studies.

Anesthetics attenuate the amplitude and increase the lag of the hemodynamic responses

Importantly, though anesthesia reduces neural activity, it will disproportionally suppress the measured hemodynamic (BOLD, cerebral blood volume (CBV), and cerebral blood flow (CBF)) responses (Logothetis et al., 2001; Goense and Logothetis, 2008; Pisauro et al., 2013; Aksenov et al., 2015) relative to neural activity. The different components of the hemodynamic and neural responses that lead to the BOLD signal (CBV, cerebral metabolic rate of oxygen ($CMRO_2$), etc.) can be differentially affected by anesthesia. While CBF, CBV, and $CMRO_2$ are all affected by anesthesia, this does not mean that the changes cancel out, and that the BOLD impulse response function will not be affected by anesthesia. In fact, as we will discuss below, the BOLD impulse response is greatly affected by anesthesia. For instance, in the somatosensory cortex, fentanyl and/or isoflurane decrease multiunit neural activity and the BOLD response, however, the decrease in the BOLD response is substantially larger than the decrease in multi-unit or LFP neural activity (Aksenov et al., 2015), implying a reduction in BOLD hemodynamic response function (HRF) amplitude. In addition, Pisauro et. al. directly measured multi-unit activity (MUA) and CBV responses in awake and anesthetized mice (Pisauro et al., 2013), and found that the spike rates to visual stimulation in awake animals were slightly increased relative to anesthetized animals (~20%), but the CBV response was increased to a much larger extent (~100%). This disproportional change in CBV and neural responses again means that the amplitude of the CBV HRF is reduced. A similar effect of anesthesia on the amplitude of the CBV and CBF HRFs is also seen when neural activity is measured using the local field potential (LFP) (Martin et al., 2006b).

Anesthesia also causes a *slowing* in the BOLD, CBV, and CBF HRFs. Pisauro et al., observed a 2 s delay in the CBV HRF in anesthetized mice relative to awake animals (Pisauro et al., 2013). This delay cannot be accounted for by a slight slowing in neural dynamics (~100 ms) caused by anesthesia. The anesthetic-induced lag in the neural response is too short (~100 ms) to account for the increase in the lag of the CBV change (~2 s) without a change in the *shape* of impulse response function that relates neural activity to CBV changes. As two impulse response functions with different shapes are by definition not the same, these neural recordings and CBV measurements clearly show a change in the HRF relating MUA to CBV by anesthesia. The same group of researcher also reanalyzed the BOLD data of Logothetis et al. (2001) and Goense and Logothetis, 2008 and found a similar slowing of the BOLD hemodynamic response in the anesthetized monkey relative to the awake monkey (Pisauro et al., 2013), which is a clear demonstration that anesthesia alters the shape of the BOLD HRF relative to the awake condition. Further, Martin and colleagues observed a similar slowing in the CBV, CBF and oxygenated hemoglobin responses in anesthetized rats relative to awake rats (Martin et al., 2006b). To our knowledge, every published comparison of neurovascular coupling between the awake and anesthetized condition has found substantial differences in every aspect of the hemodynamic response considered (CBV, BOLD, etc.). Taken together, these literature studies have found the BOLD, CBV and CBF HRFs are slowed in speed and decreased in amplitude by anesthesia (Fig. 2).

Critically, an accurate, quantitative understanding of the relationship between neural activity and hemodynamic signals is critical for interpreting neuroimaging data. Handwerker et al. (2004) showed that