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Sir, Sandrone *et al.* (2012, 2013) rediscovered, translated, and commented on the manuscripts of Angelo Mosso (1882, 1884), in which Mosso described his 'human circulation balance'; James (1890) described this as a 'delicately balanced table which could tip downwards either at the head or the foot if the weight of either end were increased'. Mosso claimed that the balance allowed him to observe changes in cerebral blood volume (CBV) associated with mental effort and emotional responses, and consequently the balance is regarded as the direct forerunner of modern non-invasive functional neuroimaging techniques. However, Sandrone *et al.* stated that 'we have no direct evidence that the balance was really able, as stated, to measure changes in cerebral blood flow during acts of cognition … despite its proven ability to measure blood volume changes in various organs (e.g. lungs, feet, hands)'.

In our laboratory, we recently constructed a balance similar to Mosso's, and using modern data collection and analysis methods that were unavailable to Mosso, we investigated whether the balance was sensitive to changes in CBV produced by modulating the level of mental activity. The construction and mechanism of our balance is depicted and explained in Fig. 1, and may be compared to Figs. 3 and 8 in Sandrone *et al.*, which show Mosso's apparatus. The balance is a class 1 lever, in which the moment of a force measured at the fulcrum is proportional to the magnitude of the force and its distance from the fulcrum. With a participant lying on the balance across the fulcrum, if mental activity produces a net shift of blood towards or away from the head then this will produce a slight change in the centre of mass of the participant relative to the fulcrum of the lever, consequently changing the force exerted at the end of the lever.

Mosso went to great lengths to keep his balance in equilibrium, i.e. able to tip back and forth rather than coming to rest on one end, and employed a counterweighting system to dampen the respiratory fluctuations, which have a large effect on the location of the centre of mass. Instead of mechanically dampening the respiratory fluctuations, our approach was to allow one end of the balance to rest stationary on a set of electronic scales and measure the variation in force exerted over time. Like Mosso, we additionally observed a slow increase in force exerted beneath the head as blood redistributed from the legs to the upper body when supine. Mosso removed this factor from his data by asking subjects to rest on the balance for one hour prior to the experiment so that the redistribution of blood was completed. Our alternative approach was to model the linear increase in force produced by this factor over time using regression, which allowed us to remove it from the data.

Initial recordings made with our apparatus of a participant at rest revealed an additional high frequency signal that Mosso's apparatus was not sensitive to - probably due

to the mechanical damping he applied. We assume that this signal arises from the heartbeat, and we largely removed its influence on our time-course data by temporal smoothing. The heartbeat and respiration signals present in the data are shown in Fig. 2a and 2c. Comparing these with panel A of Fig. 5 in Sandrone *et al.* illustrates that Mosso's balance was sensitive to respiration but not heartbeat.

Proof that the balance is sensitive to changes in CBV was provided by asking participants to hold their breath while lying on the balance. Breath-holding produces an increase in the level of CO_2 in the bloodstream (hypercapnia), which induces vasodilation and increases CBV (Kastrup *et al.*, 1999, Ito *et al.*, 2003). Conveniently, movement of the diaphragm ceases during breath holding. This allowed us to observe using the balance the well-established effect of hypercapnia on CBV, in the absence of the large oscillations produced by breathing. For the large majority of trials, breath-holding resulted in an increase in force exerted on the scales that was an approximately linear function of time (Fig. 2b and 2d). The size of the effect differed between individuals, and for a minority of individuals tested the effect was sometimes non-linear. Breath-holding tasks have been used in fMRI studies to try to isolate and control for variation in the fMRI BOLD response that is vascular rather than neural in origin (e.g. Handwerker *et al.*, 2007). The possibility of individual differences in CBV response to this task revealed by our initial exploration with the balance suggests that balance-based measurements could prove useful for refining breath-hold based methods of calibrating the fMRI BOLD signal.

Changes in CBV associated with mental activity would be much smaller than those induced by hypercapnia, and Mosso claimed that his apparatus was able to detect these small changes. To investigate whether our apparatus was sensitive to these smaller changes in CBV, we planned to use a periodic, sparse, event related experimental design. In a sparse design the interval between stimuli is of sufficient duration for haemodynamic responses caused by the stimuli to return to baseline. We refer to our design as periodic because the inter-stimulus interval was of fixed rather than variable duration. However, such designs are vulnerable to confounding by other periodic signals of similar frequency, and so we needed to confirm that the periodic frequency of our experiment differed from the frequencies of physiological processes in the body that might themselves cause shifts in the centre of mass. Visual inspection had already revealed the obvious periodic signals related to respiration and the cardiac cycle, but other lower frequency signals that could potentially confound our design could not be easily ruled out. To assess these directly we performed a 'resting state' study on 6 participants (5 female, age range 18-35) lasting 7.5 minutes; although chosen arbitrarily, this duration would allow us to detect potential confounding cycles of quite a low frequency using a Fast Fourier Transform. Slow linear drifts were removed from the data prior to the Fast Fourier Transform, but the data were not smoothed. The results (Fig. 3) confirmed the presence of periodic signals in the data at lower frequencies than respiration and suggested that the region of the power spectrum around 0.05 Hz was 'quiet', i.e. the resting state data did not naturally contain this temporal frequency. We chose 0.04 Hz as

the temporal frequency of stimulation for the final experiment. This frequency would not be confounded with the frequencies of physiological signals, and furthermore we were confident that the resulting 25 second cycle time would allow haemodynamic responses to return to baseline between stimulus presentations.

We conducted an investigation using 14 participants (13 female, age range 18-30) aimed at detecting changes in CBV induced by mental activity using the balance. Two of the participants had also taken part in the 'resting state' study. The experimental paradigm consisted of 22 trials, where each trial started with a 2 s stimulus, followed by 23 s of rest. In other words the period of the design was 25 s, the frequency was 0.04 Hz, and the duty cycle was 8.7%. It was reported by Mosso's daughter (1935) that he utilised what is now referred to in fMRI literature as a 'parametric modulation' of cognitive load. In contrast to this, our strategy was to manipulate the volume of sensory cortex activated by our 2 s stimulus. To this end we compared no stimulation, auditory stimulation, and combined auditory and visual stimulation. Each auditory stimulus was an excerpt from a different piece of music, presented through headphones at a comfortable level of intensity. During this condition participants had their eyes closed to prevent visual stimulation. For consistency, headphones were also worn in the no-stimulation condition, and eyes remained closed. In the combined auditory and visual condition the visualiser built into Windows Media Player was used to generate a visual display for each sound file, producing colourful geometric shapes that moved in synchronisation with the music. The order of the three experimental conditions was counterbalanced across participants. Data for each participant in each of the three conditions were averaged across the 22 repetitions of the 25 s experimental cycle, and the resulting plots were themselves averaged. As in electrophysiology, this form of trial averaging suppresses fluctuations that are not time locked to the stimulus, allowing induced signal components to be identified clearly. In our case, these fluctuations include the respiratory and cardiovascular signals that – by experimental design – were largely uncoupled from the neuronal stimulations. Results (Fig.4) showed a clear effect of cognitive load on the force exerted, which was larger for the condition targeting visual and auditory cortices in the brain than the one targeting only auditory cortex. The force exerted falls below the average value in the period immediately following stimulation, and then rises above the average value before falling again. The initial fall might be due to an increase in the volume of blood leaving the brain via the jugular vein, caused by an increase in the concentration of by-products of neural metabolism in the blood. The delayed rise might be caused by an increase in flow rate in the carotid artery, which replenishes supplies of glucose and oxygen.

To establish the statistical significance (and sensitivity) of our paradigm, we compared the three conditions (no stimulation, auditory stimulation and auditory-visual stimulation) using analysis of variance (ANOVA). Crucially, testing force levels as a function of time, i.e. as they are presented in Fig. 4, is a difficult problem in our setting – and in the analysis of hemodynamic and metabolic time-series in general. This is because there are

profound serial correlations and low-frequency fluctuations that render classical independence assumptions – about the residuals – invalid. We therefore summarized the magnitude of condition-specific responses using the relative spectral power at the frequency of our stimulation (0.04 Hz). This was defined as the absolute value of the Fourier transform at 0.04 Hz squared, expressed as a percentage of the total power in each time-series. The ensuing measures were subjected to analysis of variance (after applying a log transform to render them more normally distributed). This enabled us to test for significant differences between the conditions in terms of the frequency specific fluctuations induced by our paradigm. As predicted the relative power at 0.04 Hz was highest in the audio-visual condition (1.37 % (SD 2.17)), and lower for auditory (0.49 %(SD 1.40)). However, the relative power in the 'no stimulation' condition, which was predicted to be the lowest, was similar to that in auditory (0.51 %(SD 0.52)). The overall ANOVA established a significant difference in power among the three conditions ($F_{(2,26)}$ = 6.2; p = 0.006, and a post-hoc t-test showed that audio-visual stimulation induced greater power than auditory alone ($t_{(13)}$ = 3.9; p = .002). The unpredicted similarity between relative power at 0.04 Hz in the 'no stimulation' and auditory conditions can be explained by the fluctuations in force over peristimulus time present in the 'no stimulation' condition (Fig. 4). The cause of these fluctuations is discussed further below, but it is important to note that, in the average peristimulus time plot, the fluctuations present in the 'no stimulation' condition are out of phase with those in the other two conditions. This is something that power spectra, and therefore the statistical tests that we report above, are insensitive to. It also suggests that the 'no stimulation' fluctuations in force differ in origin from those in the auditory and auditory-visual conditions. Finally, to provide a robust quantitative measure of the condition-specific effects, we repeated the relative power analysis on the grand average of the time-series over subjects. Note that the power of the average is not the average of the power - and therefore this quantitative assessment is relatively immune to inter-subject variations in power. The results of this analysis correspond closely to what is suggested by inspection of Fig. 4, i.e. the least relative power at the experimental frequency is present in 'no stimulation' (0.23 %), the power is greater than this in auditory (0.84 %), and highest in auditory-visual (1.44 %).

There are several explanations of the fluctuations in force over peristimulus time in the 'no stimulation' condition (Fig. 4). First, our averaging procedure may not have fully suppressed slow frequency fluctuations in the time-series. Alternatively, the subject may be expressing omission related responses or may also have entrained their breathing or heart-rate variability to the duty cycle of our experimental paradigm. This is an interesting observation that has also been observed in non-human primate neurophysiology (Cardoso *et al*, 2012.). These slow fluctuations maybe related to heart rate variability signals or Mayer waves that attend peripheral autonomic control (see also Zheng *et al*., 2001). However, inspection of individual participant peristimulus time plots revealed that only four participants showed fluctuations without stimulation, and these may have

disproportionately influenced the ANOVA reported above that compared power at 0.04 Hz between experimental conditions.

Overall, this initial exploration provides proof that Mosso's balance is capable of detecting small changes in CBV associated with variation in the amount of neural activity taking place in the brain. This historic forefather of modern neuroimaging may yet find a place in the repertoire of modern techniques for assessing cerebral haemodynamics. In particular, if appropriately calibrated the balance might yield a useful measure of global cerebral blood flow change. Such a measure would be a useful complement to assessment of changes in regional blood flow using BOLD fMRI. For example, the balance could indicate which of two cognitive tasks produced the greatest overall change in CBV.

Acknowledgements

References
Cardoso, M M, Sirotin, Y B, Lima, B, Glushenkova, E, Das, A. (2012). The neuroimaging signal is a linear sum of neurally distinct stimulus-and task-related components. Nature Neuroscience. 2012; 15: 1298-1306.

Handwerker DA, Gazzaley A, Inglis BA, D'Esposito M. Reducing vascular variability of fMRI data across aging populations using a breathholding task. Human Brain Mapping. 2007;28(9):846-59.

Ito H, Kanno I, Ibaraki M, Hatazawa J, Miura S. Changes in human cerebral blood flow and cerebral blood volume during hypercapnia and hypocapnia measured by positron emission tomography. Journal of Cerebral Blood Flow & Metabolism. 2003;23(6):665-70.

James W. The principles of psychology.: MacMillan; 1890.

Kastrup A, Krüger G, Glover GH, Neumann-Haefelin T, Moseley ME. Regional variability of cerebral blood oxygenation response to hypercapnia. Neuroimage. 1999;10(6):675-81.

Mosso A. Applicazione della bilancia allo studio della circolazione del sangue nell'uomo. Reale Accademia delle Scienze di Torino. 1882;17:534-5.

Mosso A. Applicazione della bilancia allo studio della circolazione sanguigna dell'uomo. Atti della R Acad Lincei Mem Cl Sci Fis Mat Nat. 1884;19:531-43.

Mosso M. Un cercatore d'ignoto. Milan: Baldini & Castoldi - Editori; 1935.

Sandrone S, Bacigaluppi M, Galloni MR, Martino G. Angelo Mosso (1846-1910) J Neurol 2012; 259: 2513-4.

Sandrone S, Bacigaluppi M, Galloni MR, Cappa SF, Moro A, Catani M, et al. Weighing brain activity with the balance: Angelo Mosso's original manuscripts come to light. Brain. 2013.

Zheng, Y, Johnston, D, Berwick, J, & Mayhew, J. Signal source separation in the analysis of neural activity in brain. Neuroimage. 2001; 13(3), 447-458.

Figure 1. The balance consisted of a wooden board, reinforced longitudinally with 2 lengths of angle iron. A low friction pivot point (A) was created in the middle of the board by cutting an inverted 'V' into the 2 lengths of angle iron. This allowed the board to be mounted on a third perpendicular piece of angle iron with a ground upper edge, the whole system comprising a 'knife edge' bearing. To stop the board from tipping excessively, a stop (B) was positioned at one end of the board, which was always the end where the participants' legs and feet were located. When a participant was first placed on the balance it was ensured by the use of weights that the barycentre was to the foot end of the fulcrum so that the balance rested on the stop. Electronic scales (C) with 0.001 newtons resolution, and a sample rate of 10 Hz were then positioned underneath the head. The head was positioned at the far end of the balance regardless of the height of the participant. Weight was then removed from above (B) until the scales registered approximately 2 newtons, indicating that the barycentre was now towards the head end of the fulcrum. To allow presentation of visual stimuli a mirror (D) was positioned above the participant that allowed the participant to view a computer screen (E). Participant comfort was enhanced by provision of a pillow and 2 layers of foam mats. The left-hand photo shows the whole apparatus. Note that during data collection the pillow was positioned at the end of the balance, so that the head was above the scales. The right-hand image shows the fulcrum mechanism (A).

Figure 2. The force exerted on the scales positioned beneath the head as a function of time while a participant was at rest (A). A large oscillation produced by motion of the diaphragm relative to the fulcrum is clearly visible. Smoothing the data with a 3 s Hanning window reduces high frequency oscillations that we attribute to components of the cardiac cycle. During a 20 s breath hold after exhalation (B) the diaphragm oscillation ceases and a steady increase in force exerted is observable as blood was redistributed away from the fulcrum towards the head. In (C) and (D), comparable observations are presented for a second participant. Change in newtons is relative to the mean of the data series.

Figure 3. Representative power spectrums from Fast Fourier Transform of 3 participants' data who took part in the resting state experiment. Panels (A), (C), and (E) show frequencies up to 5 Hz. Spikes in the power spectrum at around 2-4 Hz are probably caused by components of the cardiac cycle. A large peak at around 0.15-0.30 Hz is caused by the periodic motion of the diaphragm during respiration. Panels (B), (D), and (F) zoom in on the lower temporal frequencies revealing a concentration of power around at 0.01-0.02 Hz. Based on analysis of 6 participants we concluded that the balance would be suitable to detect an uncontaminated experimental signal with a frequency of 0.04 Hz (Fig. 4).

Figure 4. Peristimulus time plots averaged across 14 participants from the functional experiment. Stimulation occurred between 0 and 2 s for the auditory (red) and combined visual and auditory (green) conditions. In the baseline condition (blue) there was no stimulation. The end of the stimulation period is indicated by the dashed line. Raw data for each participant was preprocessed by removing the linear drift and demeaning, followed by smoothing out the respiration and cardiac signals using a 12 s Hanning window. Event related averages were created for each condition for each individual to feed forward to the group average. For each data series change in force exerted is relative to the mean of that data series. Error bars represent +/- 1 SE based on between participant variation.