Supplementary Material

Theoretical Foundation for Data Processing

The estimations of Δ[HbO], Δ[HHb] and Δ[CCO] from raw spectral data taken by the bb-NIRS were performed in MATLAB. First, from the experiments we obtained 15 spectra over the 15 min per person per treatment: 2 pre-TILS baseline readings, 8 TILS readings and 5 recovery readings. The relative optical density, ΔOD, was calculated at each wavelength λ as:

$$\Delta OD(\lambda) = \log_{10}[\frac{I_0(\lambda)}{I(\lambda)}], \quad (1)$$

where $I_0(\lambda)$ is the average of the 2 initial baseline spectral readings (i.e., the first two spectra collected in each experiment), and $I(\lambda)$ represents the 13 time-varying spectra (i.e., 8 readings during TILS and 5 readings post-TILS) acquired at each time point.

Based on the Modified Beer-Lambert Law (1), the relationship of $\Delta OD(\lambda)$ versus Δ[HbO], Δ[HHb] and Δ[CCO] at each λ correspond to a wavelength dependent factor *L(λ)*. And $\Delta OD(\lambda)/L(\lambda)$ could be expressed as a sum of optical absorbance contributed by HbO, HHb and CCO components, as given below (2):

$$\begin{bmatrix} \frac{\Delta OD(\lambda_1)}{L(\lambda_1)} \\ \frac{\Delta OD(\lambda_2)}{L(\lambda_2)} \\ \frac{\Delta OD(\lambda_3)}{L(\lambda_3)} \\ \dots \\ \frac{\Delta OD(\lambda_n)}{L(\lambda_n)} \end{bmatrix} = \Delta[HbO]*\begin{bmatrix} \varepsilon_{HbO}(\lambda_1) \\ \varepsilon_{HbO}(\lambda_2) \\ \varepsilon_{HbO}(\lambda_3) \\ \dots \\ \varepsilon_{HbO}(\lambda_n) \end{bmatrix} + \Delta[HHb]*\begin{bmatrix} \varepsilon_{HHb}(\lambda_1) \\ \varepsilon_{HHb}(\lambda_2) \\ \varepsilon_{HHb}(\lambda_3) \\ \dots \\ \varepsilon_{HHb}(\lambda_n) \end{bmatrix} + \Delta[CCO]*\begin{bmatrix} \varepsilon_{CCO}(\lambda_1) \\ \varepsilon_{CCO}(\lambda_2) \\ \varepsilon_{CCO}(\lambda_3) \\ \dots \\ \varepsilon_{CCO}(\lambda_n) \end{bmatrix}, \quad (2)$$

where Δ[HbO], Δ[HHb] and Δ[CCO] are relative concentration changes of HbO, HHb and CCO respectively; $\varepsilon_{HbO}(\lambda)$, $\varepsilon_{HHb}(\lambda)$ and $\varepsilon_{CCO}(\lambda)$ represent the extinction coefficients at each wavelength of HbO, HHb and CCO, which can be found in ref. (3); *L(λ)* is a wavelength dependent factor that denotes the effective pathlength of the detected photons through tissues at each wavelength. Furthermore, according to the Modified Beer-Lambert Law (1, 4), *L(λ)* can be further expressed as:

$$\begin{bmatrix} L(\lambda_1) \\ L(\lambda_2) \\ L(\lambda_3) \\ \dots \\ L(\lambda_n) \end{bmatrix} = r * \begin{bmatrix} DPF(\lambda_1) \\ DPF(\lambda_2) \\ DPF(\lambda_3) \\ \dots \\ DPF(\lambda_n) \end{bmatrix}, \quad (3)$$

where *r* is a constant that denotes the source-detector distance. In this study, we used source detector separation of 3 cm, so *r*=3. The wavelength dependence of L(λ) is caused by a wavelength-dependent differential pathlength factor, DPF(λ). Note that in this study, we regard DPF as a wavelength dependent vector rather than a constant across the wavelength range. By substituting Eq. (3) into Eq. (2) for multiple wavelengths, the estimation of Δ[HbO], Δ[HHb] and Δ[CCO] can be expressed in a matrix format as follows:

$$\begin{bmatrix} \Delta[HbO] \\ \Delta[HHb] \\ \Delta[CCO] \end{bmatrix} = \frac{1}{r} * \begin{bmatrix} \varepsilon_{HbO}(\lambda_1) & \varepsilon_{HHb}(\lambda_1) & \varepsilon_{CCO}(\lambda_1) \\ \varepsilon_{HbO}(\lambda_2) & \varepsilon_{HHb}(\lambda_2) & \varepsilon_{CCO}(\lambda_2) \\ \dots & & \\ \varepsilon_{HbO}(\lambda_n) & \varepsilon_{HHb}(\lambda_n) & \varepsilon_{CCO}(\lambda_n) \end{bmatrix}^{-1} \begin{bmatrix} \frac{\Delta OD(\lambda_1)}{DPF(\lambda_1)} \\ \frac{\Delta OD(\lambda_2)}{DPF(\lambda_2)} \\ \dots \\ \frac{\Delta OD(\lambda_n)}{DPF(\lambda_n)} \end{bmatrix}. \quad (4)$$

In order to accurately solve Δ[HbO], Δ[HHb] and Δ[CCO] using Eq. (4), we would need to know DPF(λ) in the wavelength range of our measurements. It is known that appropriate or accurate selection/estimation of wavelength-dependent DPF is crucial for accurate estimation of chromophore concentrations (5). In this study, DPF(λ) values were assumed to be time-invariant because of given stable brain optical properties. Based on diffusion theory with the semi-infinite boundary geometry (6), DPF(λ) can be determined by

$$DPF(\lambda) = \frac{\sqrt{3\mu_s'(\lambda)}}{2\sqrt{\mu_a(\lambda)}} * \frac{r\sqrt{3\mu_a(\lambda)\mu_s'(\lambda)}}{r\sqrt{3\mu_a(\lambda)\mu_s'(\lambda)}+1} \quad (5)$$

where μa(λ) and μs'(λ) are the estimated absorption and reduced scattering coefficients across the wavelength range of interest. The following steps list sequential procedures for quantifying μa(λ) and μs'(λ) of the human forehead across all the subjects.

(1) In the beginning of each experiment, each subject's right forehead was measured with a frequency-domain OxiplexTS tissue oximeter, which could provide absolute concentrations of [HbO] and [HHb] at the measurement site as well as absolute values of μa and μs' at 750, 785, 811, and 830 nm.

(2) The measured concentrations of [HbO] and [HHb] were multiplied by wavelength-dependent extinction coefficients, $\varepsilon_{HbO}(\lambda)$ and $\varepsilon_{HHb}(\lambda)$, respectively, in the spectral range of 740-900 nm. This operation would generate a spectrum of absorption coefficient across 740-900 nm, based on $\mu a(\lambda) = \varepsilon_{HbO}(\lambda)[HbO] + \varepsilon_{HHb}(\lambda)[HHb]$.

(3) In the meantime, according to Mie Theory (7), light scattering coefficients across 740-900 nm can be expressed by $\mu s'(\lambda)=k\lambda^{-b}$, where *k* and *b* are constants that are associated with the size and density of light scatterers. In this study, *k* and *b* were obtained by fitting this equation to the four measured μs' values at 750, 785, 811, and 830 nm in step (1).

(4) We then interpolated and extrapolated the μs'(λ) values in 740-900 nm by $\mu s'(\lambda)=k\lambda^{-b}$.

(5) Then, by substituting the quantified μa(λ) and μs'(λ) values across 740-900 nm back into Eq. (5), we were able to obtain DPF(λ) of the subject's forehead within 740-900 nm for further data processing.

Next, a multiple linear regression analysis was implemented in 740-900 nm (with a total of 161 wavelengths) to fit for Δ[HbO], Δ[HHb] and Δ[CCO] based on Eq. 4 using a MATLAB-based function.

References:

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