Laminar Fluid Ejection for Olfactory Drug Delivery: In-Vitro and In-Vivo Tests

Supplementary Information

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Study 3: Detailed fMRI Methods

MRI Acquisition: Magnetic resonance imaging was completed at the Moncton MRI clinic (Moncton, NB, Canada) using standard sequences on a 3T Siemens Skyra, VD13. Anatomical T1-weighted Magnetization-Prepared Rapid Acquisition Gradient Echo (MPRAGE) images were acquired for each subject [TR = 1900ms, TE = 2.99ms, Flip Angle = 9 degrees, Voxel Size = 0.45 x 0.45 x 0.9mm, Dimensions = 448 x 512 x 160]. Resting state functional T2*-weighted Blood Oxygenation Level Dependent (BOLD) images [TR = 2600ms, TE = 30ms, Flip Angle = 90 degrees, Voxel Size = 3 x 3 x 3mm, Dimensions = 80 x 80 x 45] were acquired for each subject at baseline and again 15 minutes and 60 minutes following delivery of insulin. Each resting state functional scan consisted of 180 volumes acquired over the course of 7 minutes and 48 seconds while the participants lay still with their eyes open in the scanner.

MRI Preprocessing: MRI data was preprocessed using the open-source, standardized fMRIPrep pipeline (v22.0.2) (Esteban et al. 2019). Preprocessing steps of the anatomical images included correction for intensity nonuniformity, skull stripping, segmentation of gray matter, white matter, and cerebrospinal fluid (CSF) masks, and normalization to Montreal Neurological Institute (MNI) template space. Preprocessing steps for the functional images included slice time correction, head motion correction (calculation of framewise displacement and 6-degrees of motion parameters and their temporal derivatives), registration to the subjects T1w image, resampling to 2mm isotropic voxel size, normalization to 2mm MNI template space, and spatial smoothing (3mm Full width at Half Maximum (FWHM) Gaussian kernel).

fMRI Denoising: Functional MRI data were denoised using a standard denoising pipeline implemented in the CONN Toolbox (v22.a) and SPM (v12.7). Denoising included the regression of potential confounding effects characterized by white matter time series (5 CompCor noise components), cerebrospinal fluid time series (5 CompCor noise components), motion parameters and their first order derivatives (12 factors total), outlier scans (below 12 factors), session and task effects (linear detrending) and their first order derivatives (12 factors), and quadratic effects (3 factors) within each functional run. This was followed by bandpass frequency filtering of the BOLD timeseries between 0.008Hz and 0.09Hz. Compcor noise components within the white matter and CSF were estimated by computing the average BOLD signal as well as the largest principal components orthogonal to the BOLD average, motion parameters, and outlier scans within each subject's eroded segmentation masks. From the number of noise terms included in this denoising strategy, the effective degrees of freedom of the BOLD signal after denoising were estimated to range from 353.9 to 365.9 (average 361.5) across all subjects.

O. Esteban *et al.*, "fMRIPrep: a robust preprocessing pipeline for functional MRI," *Nat. Methods*, vol. 16, no. 1, pp. 111–116, Jan. 2019, doi: 10.1038/s41592-018-0235-4.

Fig. S1. Tech-99 Flow Time Series

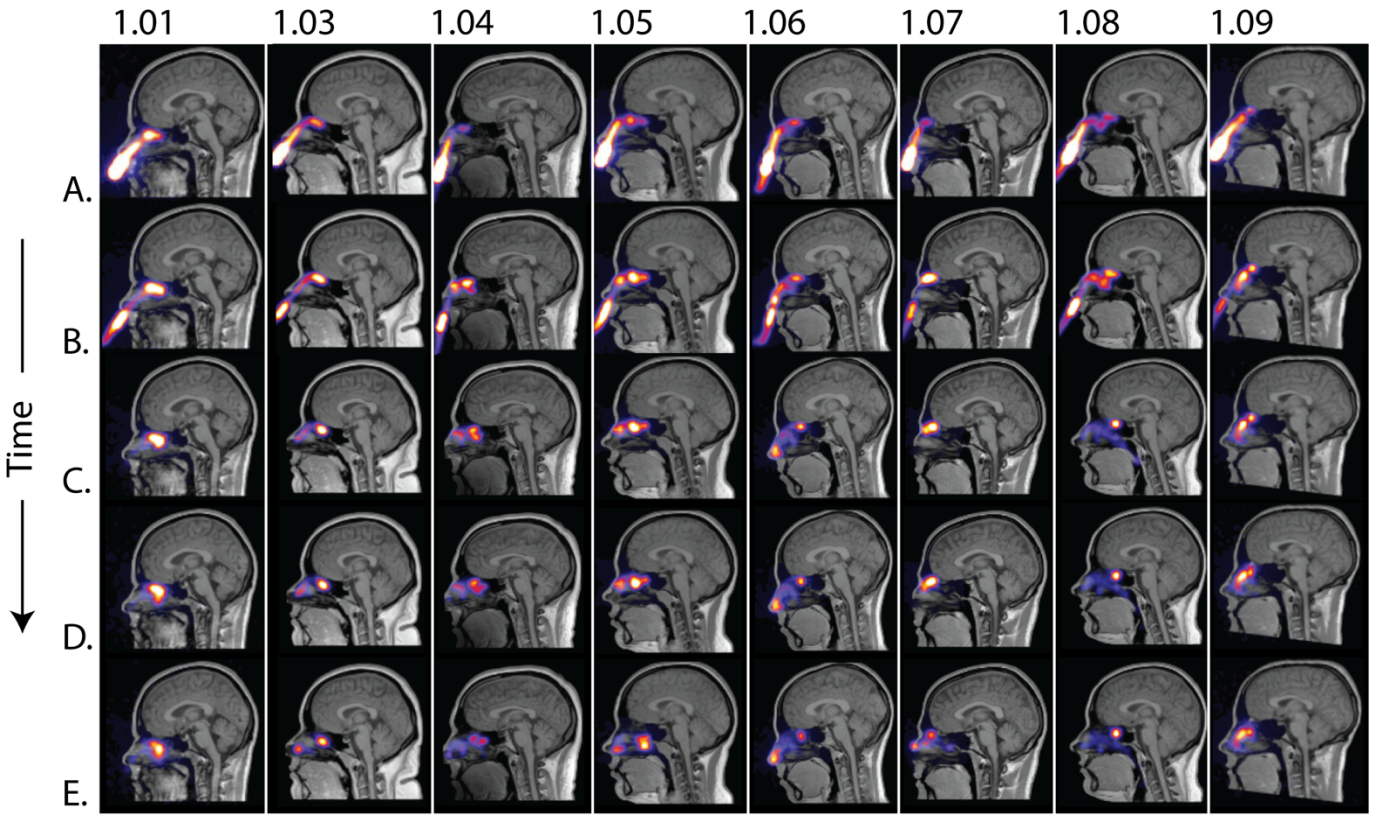

Fig. S1. Flow time series for LFE delivery of technetium-99 for each participant. (A) Bolus delivery: t=0s. (B) Cannula removed. (C) Median hold time. (D) Migration commenced. (E) Final frame.

Fig. S2. fMRI data for Block 2: Mucolox + Insulin

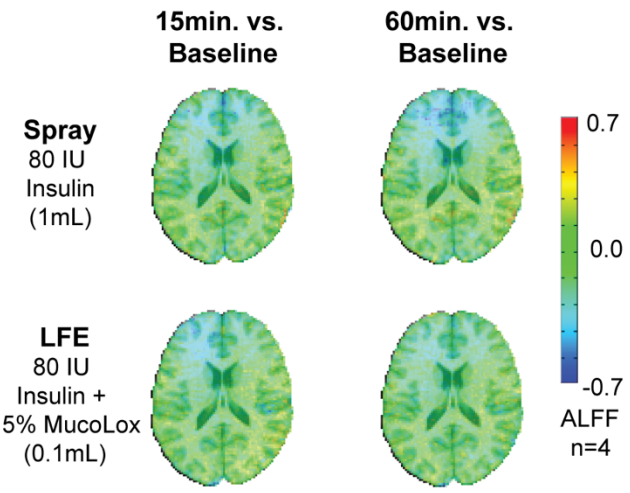

Fig. S2. *Block 2 (Yes MucoLox):* Group average fMRI results for Block 2. Four subjects were assigned to Block 2 of the study, receiving insulin via conventional spray and a mixture of insulin and 15% MucoLox via the novel LFE method. ALFF maps at 15 min. and 60 min. post insulin administration are shown here. Results demonstrate that with the addition of MucoLox (a mucoadhesive polymer), LFE administration was no longer associated with prolonged decrease in prefrontal brain activation. LFE = Laminar Fluid Ejection. ALFF = Amplitude of Low Frequency Fluctuations.