**Supplementary Materials for "A functional cerebral endothelium is necessary to protect against cognitive decline"**

Lianne J Trigiani, Miled Bourourou, María Lacalle-Aurioles, Clotilde Lecrux, Amy Hynes, Shoshana Spring, Darren Fernandes, John G Sled, Frédéric Lesage, Markus Schwaninger, Edith Hamel

**Supplementary Materials and Methods**

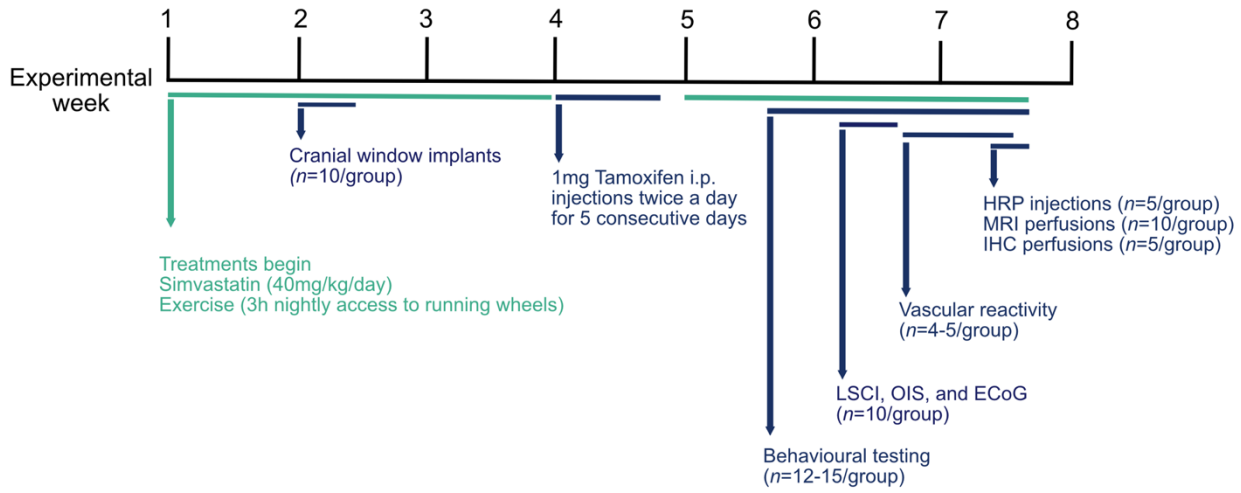
**Olfactory Discrimination Test.** Olfactory deficits have recently been identified as an early predictor of cognitive decline[1], so we explored whether Nemo$^{beKO}$ mice were able to discriminate between different odors using the protocol outlined by Yang & Crowley[2] and described briefly here. Mice were given 30min to habituate to their individual testing cages, equipped with corncob bedding and a wire cover prior to testing. Then, a Q-tip contained within a 15mL falcon tube was introduced in the cage where the water bottle is normally placed. Mice were presented with 5 different odors in triplicate for 2min each, with Q-tips being changed between each odor presentation. First, mice were exposed to deionized water, then to an almond scent (almond extract diluted 1:100 in water), then a banana scent (1:100 diluted banana extract in water), and then two different social odors obtained by wiping a Q-tip on the bottom of 2 different dirty cages (not changed for 1 week) containing mice of the same sex. This test can assess whether the mouse can smell, as well as its ability to distinguish similar and different odors. A habituation score, defined as a progressive decrease in olfactory investigation towards a repeated presentation of the same odor, was calculated as the sum of the time sniffing the last 2 presentations of the odor divided by the total time sniffing the odor. If the mouse recognized the odor, it would spend less time sniffing, resulting in a lower habituation score. A dishabituation score, defined as a reinstatement of sniffing when a novel odor stimulus was presented, was also calculated. More time would be spent sniffing if the novel odor was recognized as different from the previous odor, with a higher score indicating a greater ability to discriminate between odors.

**Wire Hang Test.** Motor deficits are often present in vascular dementia, and have been negatively correlated with white matter integrity (WM) in clinical settings.[3] An assessment of grip strength in Nemo$^{beKO}$ mice could help explain the pathology we observed in WM. To this end, mice were first placed on a wire cage top, which was then quickly but gently inverted and suspended above a cage with bedding for the mouse to land on, similar to the inverted screen test described by Deacon.[4] The latency for the mouse to release its grip was measured. Mice were given 3 trials/day over the course of 3 days, and the average of the trials was recorded each day.

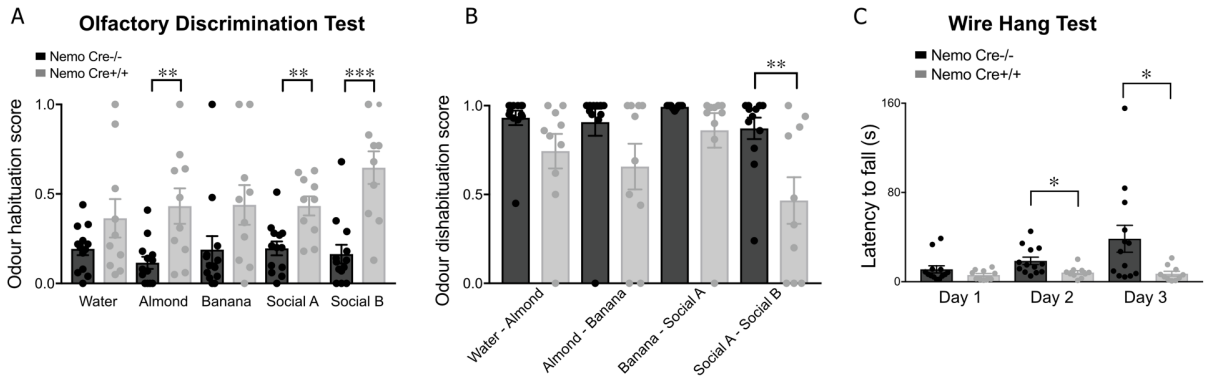
***Supplementary Table 1.*** **List of antibodies used for immunohistochemistry analysis**

| Antibody | Concentration | Source |
|---|---|---|
| rabbit anti-GFAP | 1:800 | DAKO, GA52461-2 |
| rabbit anti-Iba-1 | 1:300 | Wako, 019-19741 |
| rat anti-galectin-3 | 1:1500 | Cedarlane, CL8942AP |
| goat anti-collagen IV | 1:300 | Chemicon, AB769 |
| rabbit anti-TMEM-119 | 1:100 | Abcam, ab209064 |
| rat anti-CD45 | 1:150 | BD Bioscience, Clone 30-F11 |
| goat anti-ChAT | 1:250 | Millipore, AB144P |
| rabbit anti-SOM | 1:1500 | Peninsula, T4103 |
| guinea pig anti-PV | 1:500 | Synaptic System, 195004 |
| rabbit anti-pro-CCK | 1:300 | Gift from Dr. Andrea Varro, department of physiology University of Liverpool, UK |

GFAP: glial fibrillary acidic protein; Iba-1: onized calcium binding adaptor molecule 1; TMEM-119: Transmembrane Protein 119; CD45: Protein tyrosine phosphatase, receptor type, C; ChAT: choline acetyltransferase; SOM: somatostatin; PV: parvalbumin; CCK: cholecystokinin.




***Supplementary Figure 1.*** Experimental timeline for the first cohort of mice. Treatments began at 3 months of age for 1 month prior to tamoxifen injections to induce selective and transient brain endothelial cell dysfunction, during the 5 days of tamoxifen injections simvastatin and exercise treatments were stopped and reinstated thereafter. Mice used for optical imaging experiments were implanted with a cranial window over the barrel cortex 2 weeks prior to tamoxifen injections to allow for sufficient recovery. Eleven days following the first tamoxifen injection behavioural testing began for 14 days (Morris water maze, 3 chamber sociability test, and nesting), during which optical imaging (laser speckle contrast imaging (LSCI) and optical imaging of intrinsic signals (OIS)) (3 days), electrocorticogram (ECoG) (3 days) and vascular reactivity (5 days) experiments were performed on different mice. Mice used for behavioural experiments were then perfused for immunohistochemical (IHC) analysis or magnetic resonance imaging (MRI), or injected with horseradish peroxidase (HRP) to assess blood-brain barrier leakages.




***Supplementary Figure 2.*** In a separate cohort of mice, we found that Nemo$^{beKO}$ mice displayed problems of olfactory discrimination such that they had more difficulty recognizing an almond scent and two social odours after multiple presentations (A). They were also less able to discriminate between the two social odours as shown by a lower dishabituation score (B). Motor deficits were observed in the wire hang test whereby Nemo$^{beKO}$ mice had poorer grip strength compared to control Nemo$^{Fl}$ mice (C). *N* = 10-13 mice/group, unpaired sample t-tests were performed for each odor and each odour pairing, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

## References


1. Devanand D, Lee S, Manly J, et al. Olfactory deficits predict cognitive decline and Alzheimer dementia in an urban community. *Neurology*. 2015; 84: 182-9.
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4. Deacon RM. Measuring the strength of mice. *J Vis Exp*. 2013.