Supplementary information

Electrochemical Detection of Exogenously Administered Melatonin in the Brain

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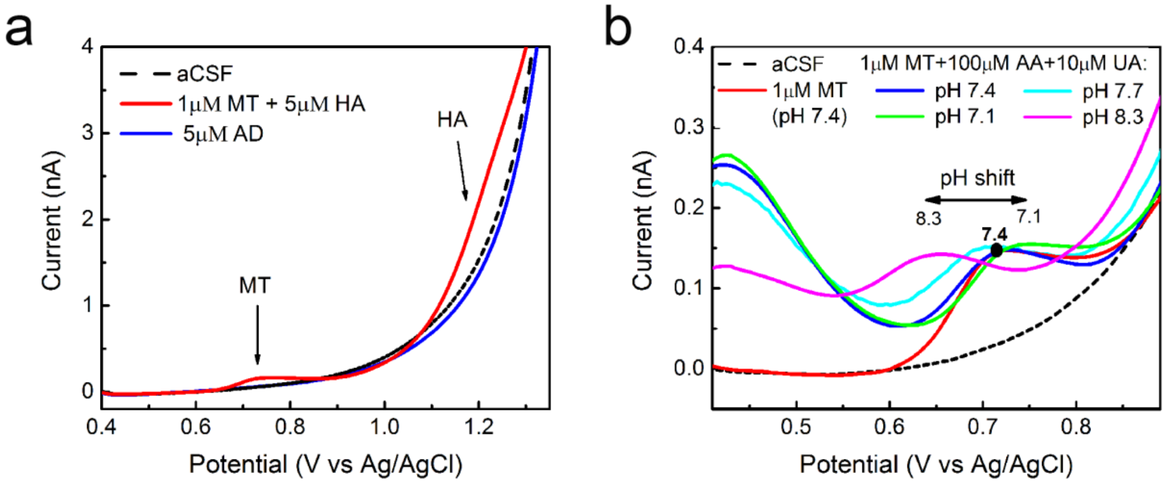

Supplementary Figure 1. a) SWVs (potential window from 0.4V to 1.4V) performed in aCSF (black), 5µM AD (blue), a mixture of 1µM MT and 5µM HA (red) respectively. AD do not present oxidation peaks using this SWV. HA present a peak around 1.3V, not interfering with MT detection. b) MT peak shift in presence of 100µM AA and 10µM UA, and subsequent pH adjustment. SWVs performed in aCSF (black), 1µM MT (red), a mixture of 1µM MT, 100µM AA and 10µM UA (green, blue, cyan, magenta) respectively. Following the AA addition, a MT peak shift has been observed (green), due to a change of the aCSF pH from 7.4 to 7.1. When the aCSF was pH adjusted to 7.4 (blue), the peak returned to the original position. For higher pH, i.e. pH 7.7 (cyan) and pH 8.4 (magenta), we observed a MT peak shift in the opposite direction. The potential of the MT peak is pH [1, 2] dependent.

Recently, *Venton* group further investigated the mechanism of HA oxidation and electropolymerization at carbon electrodes, demonstrating that HA oxidation requires a potential of at least +1.1 V vs Ag/AgCl, undergoing one-electron oxidation on an imidazole nitrogen that produces a radical, which subsequently dimerize, causing electropolymerization and fouling at the electrode surface.[3] Accordingly, when we extended the SWV potential window from 0.4 to 1.4 V, we observe a HA reaction around 1.3V, starting from concentration higher than 3µM (Supplementary Figure 1a, red). The HA oxidation peak is not well defined because it is at the limit of the safe water window for CFEs. We did not observe any AD oxidation peaks in the 0.4 -0.9 V potential window. Previous voltammetry studies performed using glassy carbon and pyrolytic graphite electrodes reported a single AD oxidation potential peak at a potential of 1.3 V versus Ag/AgCl at neutral pH. In FSCV studies, two oxidation peaks have been observed for AD at CFE electrodes, the primary one at 1.5V, shifted respect the slow voltammetry peak at 1.3 because of the fast scan rate, and a secondary one at ∼1.0 V. The secondary oxidation is likely due to a slow AD oxidation mechanism, that cannot be completed during the time of one single FSCV scan.[4] The majority of studies reported AD detection limits not sufficient for *in vivo* use (50−200 nM in the brain), in particular when planar carbon electrodes have been used in combination with slow electrochemical techniques[5-7]Single-wall carbon nanotubes modifications and nafion-carbon nanotubes coatings have been adopted to enhance the AD sensitivity of carbon based electrodes[6, 8]. Furthermore, negative holding potentials have been demonstrated to improve the AD absorption at CFE[4]. Thus, it is not surprising that, adopting a slow scan rate SWV at bare CFEs with a positive holding potential, we are not able to detect 1-5µM AD concentration also in the extended potential window (0.4-1.4 V), as can be observed by the SVW collected in 5µM AD (Supplementary Figure 1a, blue).

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