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Full Communication

Electrochemical detection of amine neurotransmitters is drastically different in buffer solutions, *in vivo*, and cell culture systems

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ABSTRACT

Detection of neurotransmitters requires high sensitivity and temporal resolution, favoring electrochemical techniques for the sensing mechanism. However, electrochemical detection of amine neurotransmitters is highly dependent on electrode surface condition and thus, results obtained in clean buffer solutions are not directly applicable to the real measurement environment *in vivo* or *in vitro*. In these more complex electrolyte solutions, the presence of antioxidants and surface-adsorbing molecules drastically alters the redox characteristics of amine neurotransmitters, their precursors and metabolites. Accordingly, we surveyed their redox characteristics in the phosphate buffered saline (PBS), cerebrospinal fluid (CSF) and cell culture medium, with high-sensitivity electrodes made of single-walled carbon nanotube network.

The concentration of surface-fouling molecules was lowest in the PBS and highest in the culture medium. Accordingly, electrochemical reaction kinetics were facile in the PBS and sluggish in the culture medium. Surprisingly, analyte molecular structure had much more importance in the CSF compared to other electrolytes, however the reaction kinetics remained to be generally slower in the CSF compared to when measured in the PBS.

Whereas the CSF also contains L-Ascorbic acid and uric acid that are electrochemically active interfering molecules, they are either completely absent or can be omitted in the *in vitro* setting. On the contrast, the culture medium contains substantially higher concentration of surface-adsorbing molecules that causes more significant fouling of electrode and thus loss of sensitivity. As the *in vitro* brain-on-a-chip applications are rapidly being adopted, direct comparison of these different experimental settings was essential to understand their implications for electrochemical sensors.

1. Introduction

Dopamine (DA), norepinephrine (NOR-EPI), epinephrine (EPI) and serotonin (5-HT) play an important role in various bodily functions including cognition, memory, motivation, emotions, wakefulness, temperature, and movement [23,17,5,15]. To better understand the functions and importance of these neurotransmitters, their concentration and release profiles are desired to be monitored in real-time. Technologies for this has been available *in vivo* since the development of Fast-Scan Cyclic Voltammetry (FCSV) and has been thoroughly reviewed earlier [21]. However, the *in vitro* setting has become a more and more prevalent experimental condition due to the very recent advancements towards the generation of accurate *in vitro* nervous system models [30,29,12,24,16,1] for brain-on-a-chip applications [20].

Electrochemical techniques can provide sensitivity down to picomolar concentrations with temporal resolution in the millisecond range [20] and thus, they are often selected for the detection of neurotransmitters [21]. However, amine neurotransmitters have inner-sphere reaction nature [2,18,9,31] and thus their electrochemistry is highly dependent on the surface quality of electrodes [20]. The electrochemical reaction mechanisms has not been thoroughly studied for the biomolecules that were used in the present study. However, our results suggests that the amine neurotransmitters but also their precursors and metabolites all undergo inner-sphere reaction mechanism, as they show significantly decreased reaction kinetics inside the fouling electrolytes.

The electrolytes used in the present study were PBS, CSF, and cell

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culture medium, and their compositions are listed in Table 1. Whereas PBS is a highly clean buffer solution absent of surface fouling species or interfering molecules, both CSF and culture media contain different types of molecules that affect electrochemical detection of neurotransmitters. For example, the CSF contains L-Ascorbic acid (AA) at ≈200 μM concentration [25,26], which itself is electroactive molecule at the same potentials with neurotransmitters-of-interest [19,20] but also regenerates the oxidation products of amine neurotransmitters back into their reduced form and thus increases oxidation current amplitude [21]. In addition, the CSF contains 25 μM of uric acid together with aromatic molecules xanthine and hypoxanthine at combined 5 μM concentration [27], which all are a heterocyclic aromatic compound that can adsorb onto single-walled carbon nanotube (SWCNT) electrodes, and glucose at ≈3–3.5 mM concentration [13,4].

Contrasting the *in vivo* setting where AA concentration remains stable, AA is rapidly oxidized away in the culture medium and thus its interfering effect can be completely eliminated by experimental design [19]. However, it must be noted that the oxidized form of AA (dehydroascorbic acid) has been observed to adsorb strongly to carbon nanofibers [8] and thus, we can assume similar behavior on CNTs also due to similarities in the surface chemistry. The culture media do not contain uric acid but instead, contains highly complex formulation that also includes mono-, di- and triaromatic molecules at a total concentration of ≈900 μM, which compete for the adsorption sites at electrode surface [20]. Due to the complexity of cell culture media and the presence of various fouling molecules at high concentration, sensitivity of electrochemical sensors is severely compromised in the *in vitro* setting.

Whereas thorough characterization of amine neurotransmitters with their precursors and metabolites has been earlier done in the *in vivo* setting by using carbon fiber electrodes [21], this earlier characterization is not directly applicable to the *in vitro* setting because of significantly different electrolyte composition. In addition, electrochemistry of amine neurotransmitters is greatly dependent on the electrode material and thus, we cannot assume this earlier characterization done by carbon fiber electrodes to be applicable with our SWCNT electrodes that have been recently shown [20] to have exceptional analytical performance down to nanomolar concentration, also inside the highly fouling culture medium. To complement the earlier knowledge about *in vivo* electrochemistry of neurotransmitters, we characterized amine neurotransmitters with their precursors and metabolites in the greatly different experimental settings of clean electrolyte PBS and the *in vivo* replica with CSF, but also the cell culture medium.

Table 1

The composition of phosphate buffered saline (PBS), cerebrospinal fluid (CSF), and culture media are drastically different. Note that L-Ascorbic Acid oxidizes rapidly in the culture medium, however the oxidized form may still competitively adsorb on the electrodes. The total aromatic molecule calculation contains uric acid. Note that medium ion content and aromatic molecule concentration is dependent on the composition of medium, the triaromatic molecule concentration arising from phenol red.

	PBS	CSF	Culture medium
pH	7.4	7.3	7.4
Na$^+$	≈150 mM	135–150 mM [4]	≈125 mM
Cl$^-$	≈140 mM	115–130 mM [4]	≈105 mM
K$^+$	≈4 mM	≈3 mM [4]	≈5 mM
Ca^{2+}	0 mM	1.0–1.4 mM [4]	≈1.4 mM
Mg^{2+}	0 mM	1.2–1.5 mM [4]	≈0.8 mM
Glucose	0 mM	3 mM [13]	≈20 mM
Protein	0 mg/ml	≈0.4 mg/ml [22]	0.1–10 mg/ml [28]
L-Ascorbic Acid	0 μM	≈200 μM [25,26]	0–200 μM [20]
Uric Acid	0 μM	25 μM [27]	0 μM
Total			
Monoaromatic molecules	0 μM	≈50 μM [7]	≈835 μM [20]
Diaromatic molecules	0 μM	≈30 μM [27]	≈70 μM [20]
Triaromatic molecules	0 μM	0 μM	≈20 μM [20]

2. Materials and methods

Electrochemical recordings were performed with pristine single-walled carbon nanotube (SWCNT) electrodes, which were prepared from a SWCNT network film provided by Canatu Oy (Finland), and which has been thoroughly characterized earlier [10]. The SWCNTs in these films are synthesized by floating catalyst chemical vapor deposition, followed by collection onto a filter membrane from which they can be press-transferred onto a substrate-of-interest without the need of using dispersion agents. Thus, the process provides electrodes with highly clean SWCNT surface.

2.1. Electrode preparation

Preparation of the SWCNT electrodes from the SWCNT film has been introduced elsewhere earlier [19,20]. Briefly, glass coverslips (12-mm diameter, Fisher Scientific) were first cleaned sequentially in acetone, isopropanol, and deionized water, followed by drying with nitrogen gun. Next, these coverslips were silanized with hexamethyldisilazane (HMDS) inside a priming oven at 148 °C. Within few hours after silanization, the SWCNT film was press-transferred onto the glass coverslip and the network was then densified with 99.5 % ethanol (Etax Aa, Anora, Finland) and dried in air. Next, conductive silver paste (Electrolube) was added to one edge of the SWCNT film to generate mechanically robust electrical contact to the SWCNT electrode, after which conductive copper tape (Ted Pella) was contacted to the silver paste region to provide high-conductive path to the potentiostat once connected with crocodile pins. Because the coverslips are highly fragile, additional mechanical support was provided by a thin polyethylene terephlate film piece that was placed beneath the coverslip. To obtain well-defined electrode surface area, a 50-μm thick PTFE tape (Irpola) was used to insulated the SWCNT electrode everywhere except a 2-mm diameter hole that was pre-cut into the tape. As such, we obtained SWCNT electrodes with 2-mm diameter.

2.2. Electrochemistry

All electrochemical procedures were carried out at 37 °C temperature with Reference 620 (Gamry) potentiostat, using sterile 12-well or 24-well plates (VWR) as the container for electrolytes. An Ag/AgCl pseudoreference electrode was prepared from silver wire (99.9 % purity, Alfa Aesar) by immersion into 10 % sodium hypochlorite solution (FF-Chemicals) for 45 min, while a platinum wire was used as counter electrode (99.95 % purity, Alfa Aesar).

2.2.1. Electrolytes and analytes

Three different electrolytes were chosen with different concentration of fouling and interfering molecules. Dulbecco's Phosphate Buffered Saline (PBS, Gibco) was selected as a highly clean buffer solution, which is often used to benchmark neurotransmitter sensor performance. Rat cerebrospinal fluid was acquired from BioIVT (RAT00CSF-0001024, Sprague Dawley Rat Cerebrospinal Fluid Gender Unspecified Pooled) to mimic the *in vivo* environment without performing animal experiments. Cell culture medium was prepared to mimic the *in vitro* environment.

To characterize the analytes in the *in vitro* environment, we prepared N2B27 cell culture medium that can be used to culture human midbrain organoids when further supplemented with dibutyryl cyclic adenosine monophosphate, L-Ascorbic acid, and growth factors at small concentration [6]. As instructed, we prepared the culture medium under sterile conditions by first mixing DMEM/F-12 (Gibco) and Neurobasal (Gibco) at 1:1 ratio, followed by supplementing with N-2 supplement (Gibco) at 1:200 dilution and B-27 supplement (Gibco) at 1:100 dilution, GlutaMAX (Gibco) at 1:100 dilution, and Penicilin-Streptomycin (Gibco) at 1:100 dilution. As we have earlier characterized the full organoid medium without observing any redox-active species after overnight incubation [19,20], we did not further supplement the N2B27 medium. However, to obtain similar condition as in the *in vitro* setting, the culture medium was incubated overnight inside a humidified incubator (37 °C, 5 % CO_2). All recordings with the culture medium were performed within 30 min to avoid the pH shifting above pH 8, which occurs under ambient conditions without the presence of 5 % CO_2 [14].

Electrolyte solutions were not deaerated in order to recapitulate the real system where biological samples are measured. Concentrated analyte stock solutions were prepared into sterile Dulbecco's Phosphate Buffered Saline without magnesium and calcium (PBS, Gibco) and were used immediately after preparation, as many of the analytes are prone to autoxidation in aqueous solutions. All analytes were acquired from Sigma-Aldrich: 3,4-Dihydroxy-L-phenylalanine (L-DOPA), Dopamine hydrochloride (DA), 3,4-Dihydroxyphenylacetic acid (DOPAC), L-Norepinephrine hydrochloride (NOR-EPI), (±)-Epinephrine hydrochloride (EPI), Homovanillic acid (HVA), Serotonin hydrochloride (5-HT), and 5-Hydroxyindole-3-acetic acid (HIAA).

2.2.2. *Hydrophilization of electrodes*

The SWCNT electrodes are inherently superhydrophobic, preventing wetting of the electrodes and thus electrochemical characterization. To improve wettability, a mild electrochemical oxidation treatment was performed in PBS by using chronoamperometry at +1.4 V (vs. Ag/AgCl) holding potential for 30 s, followed by a stabilization treatment at +0.4 V (vs. Ag/AgCl) for 60 s, and lastly cycling between −0.2 V and +0.6 V (vs. Ag/AgCl) potential at 500 mV/s for 10 cycles. After this, the electrodes were maintained in PBS at room temperature until being recorded.

2.2.3. *Cyclic voltammetry*

Cyclic voltammetry was used for electrochemical characterization of amine neurotransmitters and their precursors and metabolites, using three different scan rates (100 mV/s, 200 mV/s, and 400 mV/s). The potential window was selected to be between −0.2 V and +0.6 V (vs. Ag/AgCl).

2.3. *Data processing and analysis*

Electrochemical measurement data was analyzed in Echem Analyst software (Gamry). First, background trace of the electrolyte itself was subtracted away from analyte-containing traces. After this, redox characteristics were analyzed to determine the peak potentials and onset potentials. Cyclic voltammograms without background-subtraction are available in the Supplementary Information.

3. Results and discussion

Electrochemical characterization was done with cyclic voltammetry. Electrochemical onset potential denotes to the potential, where electrochemical reaction begins to occur. Due to the possibility of different analytes being present at significantly different concentrations, from the viewpoint of selectivity it may be necessary to understand both the peak potentials but also onset potentials. Whereas peak potential is affected by analyte concentration unless kinetics are extremely rapid, the onset potential remains to be unaffected by concentration. In Table 2, we have listed both the onset and peak potential of the analytes in all three different electrolytes. Briefly, both the onset and peak potentials were lowest in PBS and highest in culture medium.

Peak amplitude analysis would provide information about electrode fouling and sensitivity in different electrolytes. However, such analysis was not applicable here because the CSF had unstable background current (Supplementary Fig. S1), due to rapid auto-oxidation of AA [19], and thus it was not possible to accurately analyze the Faradaic current of analytes. It should thus be further investigated *in vivo*.

Table 2
Oxidation potentials of amine neurotransmitters and their precursors and metabolites, as measured in phosphate buffered saline (PBS), cerebrospinal fluid (CSF), or cell culture medium. Onset potential denotes to the potential, where analyte begins to undergo electrochemical oxidation. Analytes were recorded at 10 μM concentration, temperature being maintained at 37 °C. Note that Ascorbic Acid oxidation potentials were only determined in the CSF.

	Onset potential			Peak potential		
	100 mV/s	200 mV/s	400 mV/s	100 mV/s	200 mV/s	400 mV/s
PBS						
L-DOPA	+31	+39	+43	+143	+154	+163
Dopamine	+15	+22	+24	+105	+107	+112
DOPAC	+6	+9	+11	+177	+179	+195
NOR-EPI	+19	+27	+30	+128	+129	+128
EPI	+1	+4	+9	+103	+110	+117
HVA	+317	+323	+340	+409	+420	+431
5-HT	+132	+147	+160	+263	+277	+283
HIAA	+134	+153	+165	+286	+287	+288
CSF						
L-DOPA	+116	+132	+147	+293	+335	+379
Dopamine	−8	−7	−4	+108	+114	+122
DOPAC	+120	+139	+153	+355	+388	+419
NOR-EPI	+21	+28	+41	+165	+184	+194
EPI	−10	−2	+5	+108	+127	+151
HVA	+230	+243	+258	+426	+450	+465
5-HT	+128	+132	+136	+220	+230	+239
HIAA	+111	+121	+131	+272	+284	+300
Ascorbic acid	+1	+33	+50	+254	+274	+295
Culture medium						
L-DOPA	+132	+151	+165	+455	+461	+472
Dopamine	+16	+27	+45	+186	+198	+210
DOPAC	+124	+143	+177	+465	+476	+492
NOR-EPI	+68	+87	+113	+272	+290	+310
EPI	+28	+52	+70	+275	+298	+317
HVA	+337	+348	+368	+484	+501	+512
5-HT	+184	+187	+193	+275	+284	+295
HIAA	+212	+232	+251	+415	+426	+437

In the background-subtracted traces (Fig. 1), many CSF recordings appear to show downward curves at potentials above +200 mV (vs. Ag/AgCl), sometimes even causing the total current to become negative. After analyzing the oxidation current signal from the CSF background itself over time (Supplementary Fig. S1), we can conclude this redox current anomaly to arise from the auto-oxidation of AA [19] in the presence of easily-oxidized biomolecules such as those present in the CSF itself but also the analytes studied here. We did not observe such anomaly in the PBS nor in the culture medium, as the latter had already been stabilized overnight in the incubator.

The measurement environment had a clear trend for the electrolyte's effect on analyte redox reactions: Electrolytes that contain fouling reagents had more severe impact on those analytes that showed more sluggish kinetics already in PBS. In the PBS electrolyte that was absent of fouling molecules, we observed very sharp oxidation peaks for the neurotransmitters, which indicates strong adsorption of the analytes but also rapid apparent reaction kinetics for their oxidation reaction (Fig. 1). This is also numerically verified by analyzing the difference between onset and peak potentials (Table 2). Contrasting that, L-DOPA but also the metabolites DOPAC and HVA showed much broader oxidation peaks with strong diffusion tail that indicates limited adsorption tendency to the SWCNTs but also slightly slower reaction kinetics in PBS. Contrasting these monoaromatic neurochemicals, both serotonin and HIAA showed sharp oxidation peaks in PBS that indicates their adsorption capability to the SWCNTs and facile reaction kinetics.

Once the electrochemical recordings were performed inside the respective electrolytes for *in vivo* and *in vitro* instead of a clean buffer solution, we observed a decrease in oxidation reaction kinetics.

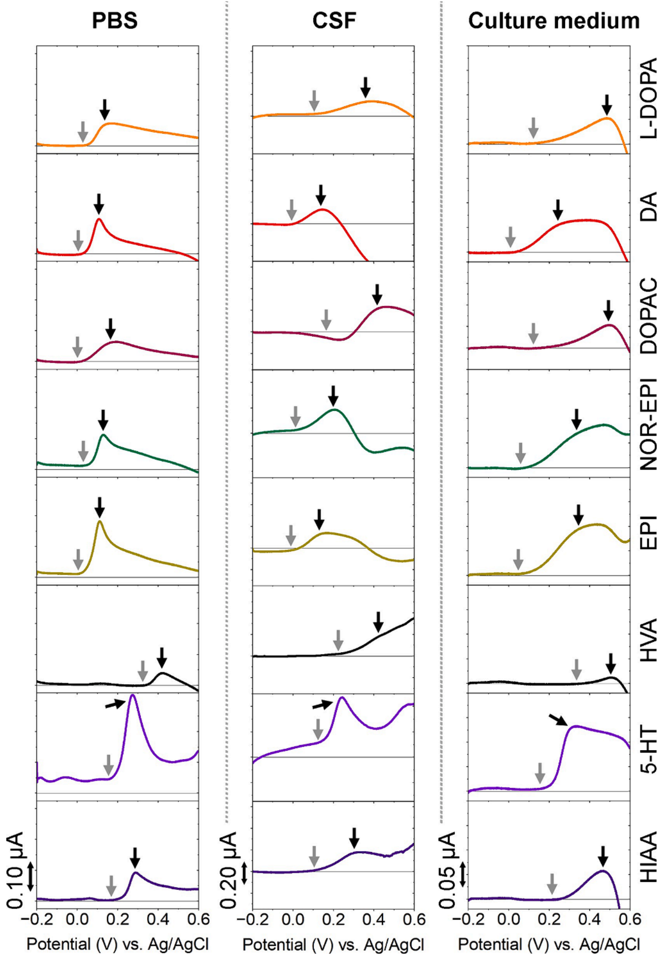

Fig. 1. Drastically different electrochemical oxidation characteristics can be observed for amine neurotransmitters and their precursors and metabolites, depending on the electrolyte composition. Straight horizontal line depicts the zero-current level, whereas gray arrows denote the oxidation onset and black arrows the oxidation peak potential. DOPAC onset was determined based on the reverse scan. All traces are background-subtracted and were recorded at 200 mV/s scan rate. Some of the recordings in CSF contain inverse peak between +0.2 V and +0.4 V (vs. Ag/AgCl) potential, arising from autoxidation of L-Ascorbic acid in the presence of easily-oxidated biomolecules. Note different scale in different electrolytes.

Surprisingly, the presence of fouling molecules impacted much more severely the precursor and metabolite analytes compared to neurotransmitters (Fig. 2). Already in the mildly-fouling CSF, we observed that L-DOPA oxidation peak shifted from +154 mV to +335 mV (vs. Ag/AgCl) and DOPAC from +179 mV to +388 mV (vs. Ag/AgCl) potential, compared to when measured in the PBS, whereas dopamine oxidation peak potential shifted only very slightly from +107 mV to +114 mV (vs. Ag/AgCl). Contrasting that, the serotonin metabolite HIAA showed practically no change in its oxidation peak potential (+277 mV vs. +284 mV (vs. Ag/AgCl)), whereas we surprisingly observed a cathodic shift (+277 mV vs. +230 mV (vs. Ag/AgCl)) in the oxidation potential of serotonin.

While we cannot provide a conclusive explanation for the different behavior between catecholamines and L-DOPA and DOPAC, we attribute this to arise from the electrode surface fouling due to competitively adsorbing molecules, especially as L-DOPA and DOPAC adsorption tendency was limited to the SWCNT electrodes already in the PBS. Due to the already more sluggish kinetics for L-DOPA and DOPAC in the PBS, their electrochemical reaction can be expected to be more susceptible to the presence of surface fouling molecules, including those with higher aromatic nature. Although the CSF contains only very little amount of fouling species such as proteins and amino acids, it contains mono-aromatic (≈50 µM) and diaromatic (≈30 µM) molecules but also glucose at high concentration (≈3 mM), which can bind to the SWCNT electrode surface and hinder analyte adsorption. In addition, L-DOPA and DOPAC are more hydrophilic molecules compared to the catecholamines, which could contribute to the observed difference under mildly fouling environment. The effect of analyte hydrophilicity on its adsorption strength should however be verified in the future by additional studies, as this hydrophilicity hypothesis is in stark contrast with what has been observed for the adsorption of phenolic compounds on CNTs in the absence of foulants [11].

Apparent reaction kinetics were further decreased once the measurements were done inside the highly fouling culture medium. All monomoamine neurotransmitters showed a significant anodic shift in their oxidation peak potential (dopamine from +107 mV to +198 mV; norepinephrine from +129 mV to +290 mV; epinephrine from +110 to +290 mV vs. (Ag/AgCl), whereas the oxidation peak of both L-DOPA and DOPAC had shifted to potentials above +450 mV (vs. Ag/AgCl). Concerning the final metabolic product of catecholamines, HVA, the oxidation peak always occurred at very high potential irrespective of the electrolyte composition (PBS +420 mV; CSF +450 mV; Culture medium +501 mV (vs. Ag/AgCl)). For HIAA we observed no significant change in peak oxidation potential in the CSF, however inside the culture medium we observed a substantial shift to +426 mV (vs. Ag/AgCl) potential. Unexpectedly, serotonin oxidation potential remained largely unchanged at +284 mV potential.

As a summary, the introduction of electrolytes with higher fouling tendency caused a substantial anodic shift for the analyte oxidation potentials, with the exception of serotonin and HIAA. This could be resulting from the different molecule structure, as serotonin and HIAA both are heterocyclic aromatic molecules whereas the other analytes consist only one aromatic ring. Accordingly, stronger adsorption interaction can occur for the heterocyclic aromatic molecules onto SWCNTs [3,11,20] and thus the presence of mildly fouling molecules is not as severe. We also surprisingly observed a cathodic shift for serotonin once the measurement was performed inside CSF compared to PBS, which was not present for other analytes except the onset potential of dopamine. It is possible that the culture medium also contains some of the constituents that generated cathodic shift for serotonin in CSF, as its oxidation was largely unaffected in culture medium but HIAA oxidation kinetics were greatly decreased. Thus, this hypothesized cathodic shift could partly compensate for the anodic shift that would otherwise occur to serotonin due to electrode fouling in culture medium.

Observing a significant shift in the peak oxidation potential for L-DOPA and DOPAC, but not for the neurotransmitters, starkly contrasts what has been observed with carbon fiber electrodes [21]. However, this difference arises from the electrode type. Our SWCNTs were highly pristine [10] and thus enable stronger π-electron coupling interaction between the analytes and electrode, compared to carbon fiber electrodes. This pristinity of SWCNTs could promote adsorption strength of glucose, ascorbic acid, and other interfering aromatic molecules, whose significantly larger concentration results into competitive adsorption that is unfavorable from the viewpoint of L-DOPA and DOPAC adsorption. Nevertheless, it remains to be unclear why the peak potentials of neurotransmitters were not largely affected by the presence of CSF, although molecule hydrophilicity may affect the adsorption process.

4. Conclusion

Thorough characterization for the electrochemistry of amine neurotransmitters has been already done elsewhere [21] earlier, however such characterization has not been done for the *in vitro* setting. Due to the significant differences in the composition of CSF and cell culture medium, those earlier results cannot be extrapolated to concern the *in vitro* setting also. Furthermore, the *in vivo* setting is often mistakenly

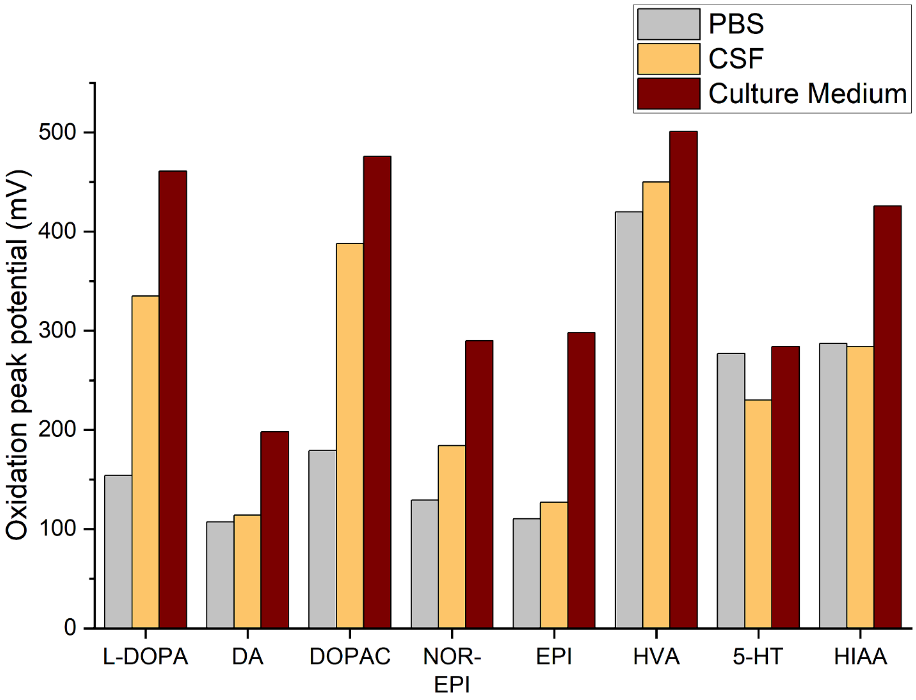

Fig. 2. Large variation was observed for the shift in peak oxidation potential for the analytes in different electrolytes. The oxidation peak potentials (vs. Ag/AgCl) were obtained with cyclic voltammetry by using 200 mV/s scan rate.

assumed to be the most complex environment for electrochemical recordings of neurotransmitters. To elucidate the differences between these experimental settings, we surveyed the electrochemical response of amine neurotransmitters in culture medium and compared that to the CSF and clean buffer solution PBS.

Electrolyte composition was found to drastically alter the electrochemical characteristics of amine neurotransmitters and their precursors and metabolites. All presented analytes showed rapid kinetics in the clean buffer solution, whereas the kinetics decreased notably in the presence of electrolytes with fouling components. Whereas the oxidation reaction kinetics for neurotransmitters remained relatively rapid also in the CSF, the precursors and metabolites were much more affected. In the culture medium, reaction kinetics of all analytes except serotonin were heavily affected. These results indicate that selectivity of SWCNT-based neurotransmitter sensors is improved in the CSF and culture medium, as the peak oxidation potential between analytes-of-interest and the precursors and metabolites becomes separated.

To highlight the sensitivity of electrochemical detection to electrode surface condition, monoamine neurotransmitters could be distinguished from L-DOPA and DOPAC when measured in the CSF and culture medium, which was not possible in the highly clean PBS solution absent of surface-adsorbing molecules.

CRediT authorship contribution statement

Samuel Rantataro: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Laura Ferrer Pascual:** Investigation, Formal analysis. **Tomi Laurila:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The raw data required to reproduce these findings is available to download from [Rantataro, Samuel (2024), “Electrochemical detection of amine neurotransmitters is drastically different in buffer solutions, in vivo, and cell culture systems”, Mendeley Data, V1, DOI: 10.17632/cm6xjbm36c.1].

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.elecom.2024.107732.

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