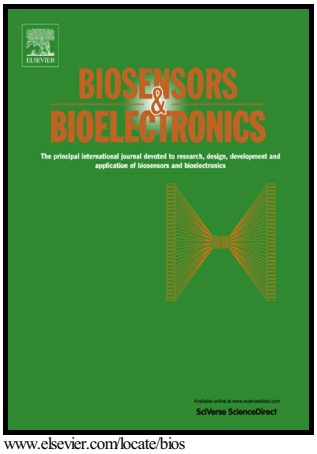


www.elsevier.com/locate/bios

Clinical implications and electrochemical biosensing of monoamine neurotransmitters in body fluids, *in vitro*, *in vivo*, and *ex vivo* models

Anupriya Baranwal, Pranjal Chandra

PII: S0956-5663(18)30691-2
DOI: https://doi.org/10.1016/j.bios.2018.09.002
Reference: BIOS10742

To appear in: *Biosensors and Bioelectronic*

Received date: 12 July 2018
Revised date: 25 August 2018
Accepted date: 1 September 2018

Cite this article as: Anupriya Baranwal and Pranjal Chandra, Clinical implications and electrochemical biosensing of monoamine neurotransmitters in body fluids, *in vitro*, *in vivo*, and *ex vivo* models, *Biosensors and Bioelectronic,* https://doi.org/10.1016/j.bios.2018.09.002

Clinical implications and electrochemical biosensing of monoamine neurotransmitters in body fluids, *in vitro*, *in vivo*, and *ex vivo* models

Anupriya Baranwal and Pranjal Chandra*
Laboratory of Biomedical Sensors and Nanobioengineering, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati -781039, Assam, India
***Corresponding author.** Tel.: +91 361 258 3207; fax: +91 361 258 2249. pchandra13@iitg.ernet.in

Abstract

Monoamine neurotransmitters (MNTs) belong to one of the most important groups of neurotransmitters in the central nervous system. They play crucial role in functioning of cardiovascular, renal, and hormonal systems along with establishing human brain-body integration. Abnormal level of MNT is associated with numerous psychotic (schizophrenia, depression, dementia, etc.) and neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's disease, etc.), therefore their sensitive and robust detection is of great clinical significance. Electrochemical detection (ECD) techniques have been paving the path in this direction, for more than four decades now. Keeping the immense importance of MNTs in mind, this review has been formulated to describe fundamentals of MNTs followed by their clinical significance in neuroscience. Herein, we have emphasized on the ECD of MNTs, in various matrices reported till date. In order to provide information on ECD of MNTs in clinically comparable systems, we have included a comprehensive discussion on sensor design and its analytical performance towards analyzing MNTs in the *in vitro*, *in vivo*, and *ex vivo* models. An extensive table is also incorporated to provide better understanding of the role of MNTs in various clinical conditions. Furthermore, this review briefly discusses the challenges faced with EC sensing techniques and loopholes present in current research works. Apart from this, three extended tables are also included in this review, to provide an insight into the amount of work done in ECD of MNTs in last ten years (2008–2018). These tables comprehensively discuss sensor fabrication strategies for determining MNTs in various matrices and models along with their mode of detection and analytical performance.

Abbreviation (Full name)

5-HT, Serotonin; AA, Ascorbic acid; ADHD, Attention deficit hyperactivity disorder; AgNP, Silver nanoparticle; AuNP, Gold nanoparticle; BRE, Biorecognition element; ca., Circa; CE, Capillary electrophoresis; CFE, Carbo fiber electrodes; CFME, Carbon fiber microelectrodes; ChA, Chronoamperometry; CL, Chemi-luminescence; CNF, Carbon nanofiber; CNS, Central

nervous system; CNT, Carbon nanotube; CP, Carbon paste; CPE, Carbon paste electrode; CV, Cyclic voltammetry; DA, Dopamine; pDAN, poly-1,5-diaminonaphthalne; DPV, Differential pulse voltammetry; EC, Electrochemical; ECD, Electrochemical detection; ECR, Eriochrome Cyanine R; EDTA, Ethylenediaminetetraacetic acid; Ep, Epinephrine; FA, Folic acid; FLD, Fluorescence detection; FSCV, Fast scan cyclic voltammetry; FSCAV, Fast-scan controlled-adsorption voltammetry; GCE, Glassy carbon electrode; GO, Graphene oxide; His, Histamine; HPLC, High performance liquid chromatography; IL, Ionic liquid; LC, Liquid chromatography; LOD, Limit of detection; LOQ, Limit of quantification; LR, Linear range; LSV, Linear sweep voltammetry; MCM-41, Silica based mesoporous material; ME, Microelectrode; MEA, Microelectrode array; MFB, Medial forebrain bundle; MIP, Molecular imprinted polymer; MNT, Monoamine neurotransmitter; MPC aptamer/SiNW-FET, DNA-aptamers on a multiple-parallel-connected silicon nanowire field-effect transistor; MS, Mass spectrometry; MWCNT, Multi walled carbon nanotubes; NAc, Nucleus accumbens; NE, Norepinephrine; NiO-NP, Nickel oxide nanoparticles; NM, Neuromodulator; NPV, Normal pulse voltammetry; NT, Neurotransmitter; OCD, Obsessive-compulsive disorder; PC12, Pheochromocytoma cell line; PEI, Polyethylenimine; PMB, Polymethylene blue; PtNPs, Platinum nanoparticles; rGO, Reduced graphene oxide; RSD, Relative standard deviation; SNR, Substantia nigra pars reticulata; SPCP, Screen printed carbon paste; SPE, Screen printed electrode; SWV, Square wave voltammetry; UA, Uric acid; UVD, Ultraviolet detection

Keywords
Human health; monoamine neurotransmitter; electrochemical biosensor; multiplex detection; neuronal disorder diagnosis; nanomaterials

1. Introduction

1.1 Biological significance of MNTs

Decades of incessant work in NT measurement has paved path for better understanding of the relationship between the neuronal chemistry and the complex brain function of an organism (Colliver and Ewing 2006). NTs are biologically important endogenous chemical messengers that relay information between neuron and other “target” cells (neuron, myocyte, or gland cells)

across chemical synapse (Hasanzadeh et al., 2017). Individual neurons can never act in isolation so, they rather interact with several (millions) other neurons to generate and execute highly ordered brain functions (Jeon et al., 2016). A variety of NTs (amino acids, monoamines, neuropeptides, neuro-purines, gaso-neurotransmitters, and trace amines) exist in the human brain where they come together to play a very crucial role in brain-body integration. Since, last four decades, these NTs have been enormously studied, however, reports existing on MNT detection are relatively more due to their self-reporting ability and high stability. The most common types of MNTs include DA, 5-HT, NE, Ep, and His (Perry et al., 2009). Owing to the presence of catechol group in their structure, DA, NE, and Ep belong to the group of catecholamines. These NTs are involved in mediating a wide range of physiological and homeostatic functions, which vary with the part of the brain being examined. DA is a NM which plays crucial role in motor coordination, motivational behavior, and regulation of cognitive processes such as attention and working memory (Tzschentke, 2001; Vallone et al., 2000). It is primary NT involved in reward pathways that is considered important in mediating effects of abusive drugs (Volkow et al., 2004). DA acts on a range of DA receptors located in various brain regions and in the periphery. Alterations in the optimal concentration of DA has been associated with various neurodegenerative (Parkinson's) and psychotic (Schizophrenia, and addiction) disorders (Perry et al., 2009). Apart from acting as NT, a few MNTs like NE and Ep behave as hormones and influence different parts of body including heart, lungs, and arteries of skeletal muscles (Moore and Bloom, 1979). Both MNTs play indispensable role in body's natural fight-or-flight response to stress and have various important medical uses as well (Mefford, 1988; Morilak et al., 2005). Having excess or deficiency of either of the two MNTs can leave noticeable effects on a person's health. The most common clinical conditions associated with NE include Parkinson's disease, ganglia neuroblastoma, depression, and attention deficit hyperactivity disorder (Moret and Briley, 2011) and with Ep include anxiety and stress (Schildkraut, 1965). In addition to DA, 5-HT also exists in CNS of an organism and play crucial role in complex behaviors; such as mood, appetite, sleep, cognition, perception, motor activity, temperature regulation, pain control, sexual behavior, and hormone secretion. It is also involved in a variety of physiological processes like regulation of blood pressure, smooth muscle function, and bone health (Kema et al., 2000; Saxena, 1995). Besides CNS, 5-HT is also reported to be present in blood platelets and the gastrointestinal mucosa and contribute in crucial functions. Therefore, imbalance in optimal level

of 5-HT can cause several psychotic-pathological disorders like, depression, anxiety, alcohol addiction, eating disorder, autism, psychosis, and obsessive-compulsive disorder (Naughton et al., 2000). Compared to other MNTs, His impacts lesser yet extremely essential functions in the CNS. It is involved in functions like arousal, control of pituitary hormone secretion, suppression of eating, and cognitive functions. The central role of His is associated with mediation of allergic reactions, gastric acid secretion, and inflammation in the periphery. Several studies have revealed that alterations in histaminergic system can lead to neurodegenerative (Alzheimer's and Parkinson's) and psychiatric diseases (Schizophrenia and vascular dementia) (Nuutinen and Panula, 2010; Torrealba et al., 2012). Since, the clinical conditions **(figure 1)** associated with MNTs are very cumbersome and severely affect the patients' life quality, methodologies that can provide analytical understanding of the relationship between MNTs and organ functions are of pressing need. To enable ease of comparison and understanding of the MNTs' biological function, their concentration ranges in various matrices and their association with different neuronal disorders, a summary **table 1** has been incorporated.

1.2. Strategies for MNT detection

In last several decades, various analytical techniques have been exploited for the analysis of MNTs in different systems. The widely explored conventional techniques for the purpose include LC (Zhu et al., 2011), CE (Chicharro et al., 2004; Parrot et al., 2004), and HPLC (He et al., 2016b; Yang et al., 2014b). These chromatographic techniques have been coupled with other detection systems like MS (Gottås et al., 2015; He et al., 2016b), UVD (Thomas et al., 2015), FLD (He et al., 2016a), ECD (Allen et al., 2017; Ferry et al., 2014), and CL (Holland et al., 2014) to detect MNTs. Although these techniques enable successful detection of MNTs, but they (other than ECD techniques) suffer from numerous limitations. For example, HPLC-MS and CE-MS techniques require bulky instrumentation, trained personnel, and often result in relatively higher LOD values as compared to that of ECD and FLD techniques (Perry et al., 2009; Zhao and Suo, 2008). Their requirement for heavy sophisticated instrumentation system presents them with inability of miniaturization and hence, limit their usage in routine tests and point-of-care applications. UVD technique lacks sensitivity and selectivity for MNTs and CE encounters

similar electrophoretic behavior between MNTs and interfering compounds (Vlčková and Schwarz 2007) limiting their application in clinical sample analysis. Laser-induced fluorescence detection and CL techniques often require tedious procedure of sample preparation (e.g. derivatization) and their performance is occasionally compromised in realistic biological samples (Cai and Zhu 2010). These limitations, therefore suggest that there is room for improvement and development of better techniques. In the view of this, several sensing and biosensing techniques have been developed to ensure detection and observation of a wide range of analytes (Chandra 2016). Analytical devices engendering EC sensing are extremely powerful tools as they can allow quick, inexpensive, selective, and sensitive detection of the target biomolecule in both *in vivo* and *in vitro* systems (Bansal et al., 2017; Mahato et al., 2018; Si and Song 2018; Yadav et al., 2014). Additionally, these techniques can also enable biomolecule detection in a miniaturized analytical device which suggests their suitability for point-of-care testing (Chandra 2013; Chandra 2016; Mahato et al., 2016; Mahato et al.,, 2016; Turner 2000, 2013).

Since MNTs detection via. EC sensing techniques is an enormous and incessantly advancing field, plethora of research work has been published over the years, so we do not claim to include all the published work in this review. We have focused on last ten years' (2008–2018) work and tried to present a comprehensive overview of the progress made in sensing strategies during this time. It is well-known that ECD techniques have been used for recognition of >80 NTs; however, this review focuses on detection of NTs that remain electroactive within the potential window of the interstitial (Robinson and Justice, 2013) and biological fluids (e.g. serum, plasma, urine, and cerebral fluids) (Hasanzadeh et al., 2017), specifically MNTs. Numerous reviews (Hasanzadeh et al., 2017; Jeon et al., 2016; Moon et al., 2018; Perry et al., 2009; Sharma et al., 2018) focusing on analyses of NTs' via. different techniques have been published in recent years. The information presented in these reports is quite valuable; however, several crucial aspects remain unaddressed. For instance, these articles lack comprehensive information on the neurological systems and their relation with MNTs, which is an important aspect to understand if one wishes to design clinically relevant analytical system. Most of these reports fall short to describe MNTs detection in the *in vitro, in vivo,* and *ex vivo* models which is inevitable to ascertain the correlation of neurotransmission with organisms' behavior, patients' disease state, and drug concentrations in the intact brain. Additionally, these reports rarely discuss simultaneous detection of MNTs, which is extremely important to know if, one wishes to

have a better understanding of the neurological system and neuronal disorders.

The present review explicitly focuses on these unaddressed issues and discusses them comprehensively. Herein, great emphasis is given on the clinical aspects of MNTs along with their multiplex detection in various body fluids. In order to ascertain better understanding of MNT detection in clinically comparable systems, a detailed information on their detection in realistic systems like *in vitro, in vivo*, and *ex vivo* is also included. Furthermore, this review encompasses a comprehensive table indicating biological function of MNTs, their involvement in neurological disorders, and concentration ranges in biological fluids and different regions of human brain. Ultimately, this review briefly touches upon various challenges faced with ECD techniques and gaps present in recent research works and hence, guides its readers in the direction of developing strategies to overcome these challenges in future.

2. Multiplex electrochemical sensing of MNTs in body fluids

Most of the published reports in last few decades emphasize exclusively on single MNT detection (Jackowska and Krysinski 2013; Sharma et al., 2018 Valentini et al., 2014) or on detection of a MNT in presence of interfering molecules (Jackowska and Krysinski 2013; Sajid et al., 2016). Although, these methodologies generate critical data, however, they do not provide any information on how MNTs may affect each other or alter concurrently in response to a stimulus. Since, neurological processes and neurological disorders usually involve several NTs and NMs, therefore it is very crucial to have such analytical techniques which can directly map and quantitate multiplex signaling molecules in single analysis. Owing to their extremely low concentration, multiplex MNTs detection with other coexisting MNTs, their precursor amino acids and metabolites in biological samples calls for highly selective and sensitive approaches. Histochemical, immuno-histochemical, and ligand-based assays are the indirect methods that have been used traditionally for recording MNTs level (Felten et al., 1990; Shim et al., 2001). These approaches often suffer from selectivity limitation as they cannot distinguish between an MNT from its metabolites and/or its precursors. Direct methods generally include various chromatographic and EC sensing techniques (Perry et al., 2009). Although these methods enable simultaneous detection of multi-analytes, however, most of these methodologies suffer from numerous limitations. Hence, development of a reliable methodology for multiplex detection of MNTs and related compounds is highly imperative in both investigative and medical

neuroscience field. To address the limitations faced with abovementioned techniques, EC biosensing techniques have been used as a technique of choice. The overall concept behind ECD of MNTs has been discussed briefly in the **supplementary information section-1 (SI-1).** In the view of clinical importance of multiplex detection of MNTs, the following sections have been grouped according to their different combinations detected in various body fluids utilizing diverse sensor designs.

2.1. Sensing of DA and 5-HT

Simultaneous detection of DA and 5-HT is very important from (patho)physiological aspect (Zhou et al., 2013) and in last one decade myriad reports have come up with an aim to detect them simultaneously. For instance, Rand *et al.* developed a carboxylated (-COOH) carbon nanofiber sensing probe for the simultaneous detection of DA and 5-HT in a single experimental setting (Rand et al., 2013). The LOD values obtained from DPV analysis for DA and 5-HT was found to be 50 nM and 250 nM, respectively. Not only this, the modified sensing probe also acted as an effective platform and provided better sensitivity and selectivity towards analytes when compared with conventional CPE, CNT-modified GCE, and graphene-modified GCE. This effectiveness of CNF electrode came from combination of high conductivity with higher number of –COOH functional group, that led to improved electron transfer and subsequently mediated selective differentiation of MNTs. The results obtained by this group are of immense value, however, they have not presented detection of DA and 5-HT in real samples, which is extremely important for a sensor to be successful commercially and thus, shows that there is room for improvement. In the view of this important issue, Hasanzadeh *et al.* developed MCM-41modified electrode **(figure 2A)** for the simultaneous detection of DA and 5-HT in human serum (Hasanzadeh et al., 2013). The use of MCM-41 was very advantageous as it enabled excellent charge transport and provided better sensitivity towards MNT detection in the biological sample. The LR values obtained for DA and 5-HT was 0.2–103 nM and 0.2–94.4 nM, respectively with a similar LOD of 0.1 nM for both the analytes. An RSD of 3.2% and 2.0% was obtained for DA and 5-HT, respectively, indicating good reproducibility of the designed sensor. Wider LR and LOD value lower than the basal extracellular concentration of MNTs claims that this EC sensing system can be used for *in vivo* biosensing application. Apart from aforementioned reports, numerous other reports on simultaneous ECD of DA and 5-HT

(Abbaspour and Noori 2011; Atta et al. 2011; Wang et al., 2016; Zhou et al. 2013) have been published. Besides this, there have also been reports on simultaneous ECD of these MNTs in presence of a metabolite and drug (Atta et al. 2012) or an interferent (Sun et al., 2018). More elaborate discussion on these reports has been presented in **table 2.1.**

2.2. Sensing of catecholamines

Ep and DA are extremely crucial catecholamines as they play a critical role in maintaining the health of humans (Mittal et al., 2017) as well as other mammals (Manouchehri et al., 2013). Since, these MNTs are structurally alike and typically co-exist with other interferents in the biological samples, their simultaneous EC detection becomes a challenging task. With an aim to overcome this issue, Figueiredo-Filho *et al.* developed a novel EC sensor by incorporating a GCE modified with NiO-NPs and CNTs in a dihexadecylphosphate film **(figure 2 B)** (Figueiredo-Filho et al., 2014). This modified sensor was used to perform simultaneous detection of DA and Ep in various biological samples like cerebrospinal fluid, human serum, and lung fluid. On performing DPV analysis at the modified electrode, spectacularly separated reduction peak potential at about ca. 360 mV was generated along with low value of LOD: 0.5 nM for DA and 0.82 nM for Ep. In addition, no significant interference was evident from real sample matrix suggesting that the proposed sensor can be successfully employed for intricate biological sample analysis. Not only this, the modified electrode showed high stability as it allowed repeatable (n=30) analysis of target MNTs in biological sample with high sensitivity. The RSD value for DA and Ep detection in human body fluids was found to be 3.2% and 1.2%, indicating negligible fouling of the modified electrode surface. Although, the electro-catalytic activity of CNT matrices and conjugated polymers individually display good results; however, there seems to be dearth of some properties like sensitivity for different EC techniques, the stability of the modified electrode, and electrocatalysis for multiple analyte detection. To mitigate this issue, several new strategies have been developed, one such strategy involves combination of both polymer and CNT in order to give composite films. CNTs possess unique π-conjugative morphology with highly hydrophobic surface that allows them to interact with other aromatic organic compounds and give away composite films (Li et al., 2004; Zhang et al., 2005). These composite films ensure large surface area with enhanced electrocatalytic activity towards analytes ensuing their detection (Yang et al., 2013). A number of reports have come up in past

which incorporate CNTs with polymer for the simultaneous detection of multiple analytes. For instance, Yogeswaran and Chen reported fabrication of a novel EC sensor by modifying GCE with a composite film which contained MWCNTs and PMB (Yogeswaran and Chen 2008). At near physiological pH, the composite film exhibited spectacular electro-catalytic activity by bringing out successful oxidation of DA, Ep, and AA. The developed composite film offered several other advantages too, like ease of electrode fabrication, prolonged stability of the modified electrode, and highly reproducible results. The modified sensing probe was reported to give an LOD of 69.8 µM and 67 µM for Ep and DA, respectively. In another study, Cincotto *et al.* showed simultaneous detection of DA and Ep by exploiting a AgNP decorated hybrid material for electrode modification (Cincotto et al., 2014). The hybrid material which was composed of mesoporous silica and GO allowed the modified electrode to have outstanding properties like low LOD, high conductivity, good repeatability (RSD = 3.2%) and reproducibility (RSD = 2.5%). This sensing system generated LOD value of 0.26 µM and 0.27 µM for DA and Ep, respectively and enabled MNT detection with high selectivity in human urine samples. Tsai *et al.* used ionic liquid (IL) generated Pd-Au nanoparticles to modify GCE. The modified electrode was efficient enough to detect Ep (LOD = 5 µM), DA, and UA simultaneously (Tsai et al., 2012). Use of ionic liquid (green solvent) for reduction purpose prevented the use of toxic solvents, making whole approach environment-friendly. Apart from reports on simultaneous ECD of Ep and DA, several other reports have come up with their aim to simultaneously detect other combinations of catecholamines. For instance, DA, Ep, and NE; and Ep and NE (Goyal and Bishnoi 2011). Former combination of catecholamine has been studied in the presence of a metabolite L-DOPA (Atta et al. 2010) and interferents UA and AA (Chitravathi and Munichandraiah 2015). More elaborate discussion on these reports has been incorporated in **table 2.2** by including information on the type of catecholamines that were detected, sensing probe fabrication strategies, interferent studies, and the analytical performance of the sensing probes.

2.3. Sensing of NE and 5-HT

Owing to their distinguished biological functions in a mammalian CNS and association with numerous disease conditions (Mandel, 2013), both NE and 5-HT are also are very crucial form analytical point of view. Numerous reports have been published which studied their individual detection using various EC sensing techniques (Ran et al., 2017; Samdani et al., 2017; Samdani et al., 2016). In this direction, Mazloum-Ardakani and Khoshroo developed a highly selective EC sensor for the simultaneous detection of NE and 5-HT (Mazloum-Ardakani and Khoshroo, 2014). They made use of a novel nano-composite material composed of benzofuran derivative functionalized with MWCNTs and IL for the purpose of electrode modification. An excellent electro-catalytic action was observed with a potential difference of 210 mV towards analytes, which is sufficient enough for their simultaneous detection. In addition to ease of electrode fabrication and accurate detection of MNT concentration in human serum, this novel sensing platform also ensured low value of LOD, 49 nM and 2 μM for NE and 5-HT, respectively and high reproducibility (RSD = 1– 4%). To enhance the suitability of EC sensing probes in biological sample analysis and further lower the LOD for NE and 5-HT, Wang *et al.* developed a MWCNTs-ZnO/chitosan composite modified SPE (Wang et al. 2015). On SWV analysis, the modified sensing probe showed greatly enhanced current with peak poential separation at about 90 mV (NE) and 280 mV (5-HT). The peak currents of NE and 5-HT were found to be linearly dependent on their concentrations in the range of 0.5–30 μM and 0.05–1 μM, with an LOD of 0.2 μM and 0.01 μM, respectively. The modified sensing probe remained stable for at least 3 month at 4°C and successfully detected the level of NE and 5-HT in rat cerebrospinal fluid. Yao *et al.* reported ECR-modified GCE for the simultaneous detection of 5-HT and NE (Yao et al., 2009). The fabricated system resulted in LOD of 0.05 μM and 1.5 μM with LR of 0.05–5 μM and 2–50 μM for 5-HT and NE, respectively in presence of excess interfering compounds, UA and AA. Comparing the sensor performance in abovementioned examples, it can be seen that both the sensing probes result in LOD values similar to that of basal concentration of MNTs in body fluids. This finding suggests that these sensors have ability to be commercialized to monitor the levels of NE and 5-HT. At the moment, only few reports are available on the simultaneous ECD of NE and 5-HT and most reports do not present comparison between existing reports and lack

any information on detection of these NTs in complex biological samples.

2.4. Sensing of Ep and 5-HT

Both Ep and 5-HT play crucial role in the emotional system of a mammalian brain and FA is considered essential for their production. Hence, their simultaneous detection by means of EC sensing techniques is of paramount importance. In this context, Narayana *et al.* studied simultaneous detection of Ep and 5-HT in the presence of FA at a GCE modified by poly-serine and MWCNTs (Narayana et al., 2015). On DPV analysis, the modified sensing probe allowed them to detect Ep with low LOD of 0.6 μM and LOQ of 2 μM and 5-HT with an LR of 5 μM to 30 μM in synthetic as well as human serum sample. On 20 separate experiments on the same electrode, the RSD was found to be 4.86%, indicating good reproducibility and negligible electrode poisoning. Mazloum-Ardakani *et al.* in a different study presented a strategy to fabricate a novel EC sensor by modifying carbon paste (CP) with graphene sheets and used this sensor for simultaneous detection of Ep and 5-HT (Mazloum-Ardakani et al., 2017). The modified CPE not only exhibited enhanced chemical properties but also provided an easy approach for electrode fabrication, offering an LOD of 11 μM, with wide LR of 0.1 to 1000 μM for Ep, and low ohmic resistance which subsequently aided in generating a stable response which was reproducible. Administration of neurological drugs has been shown to influence the concentration of MNTs. Owing to this fact, a brief discussion on ECD of MNTs in the presence of various drugs like acetaminophen, nicotine, paracetamol, etc. has been discussed in **SI-2.**

Monitoring of fluctuations in the concentration of MNTs in biological fluids provide crucial information; however, their analyses in the *in vitro* conditions would be quite imperative to provide a better understanding of the brain functioning and its disorders. The following section deals with *in vitro* detection of MNTs by means of various EC sensing techniques.

3. Electrochemical sensing of MNTs in the *in vitro* models

Exocytosis (release or secretion) of MNTs and other vesicular contents into the extracellular environment is a vital process by which living cells participate in neuronal signaling. Owing to its high sensitivity and suitable temporal resolution, investigation of secretory MNTs in the *in*

vitro model has been performed by using single-cell amperometry technique. Some of the non-synaptic *in vitro* models used for the purpose include beige mouse mast cells, PC12 cells, human pancreatic beta cells, and chromaffin cells (Colliver and Ewing 2006). A number of groups have reported the EC analysis of extracellular MNTs concentration at the level of a single cell and in most instances, a similar experimental protocol which is as follows is carried out (i) electrode is positioned onto a cell in culture with an aim to minimize interferences from nearby cells, (ii) to trigger the exocytosis of the MNTs from a neuronal cell, chemical stimulants like potassium ion (K^+) and/or nicotine, etc. are administered, (iii) the positioned electrode (biosensor) is operated at a constant potential and (iv) the MNTs released from neuronal cell get oxidized and the resulting current provides analytical signal for their (MNT) detection. The overall phenomenon involving exocytosis of MNTs from a single neuronal cell and their detection using various EC biosensing techniques has been illustrated in **figure 3**.

In a study performed by van Kempen *et al.* exocytosis of catecholamines from PC12 and mouse adrenal chromaffin cells was investigated by using CFE (van Kempen et al., 2011). They used this EC system to reveal that the exocytosis of MNTs occurs in three distinct modes *viz.* (i) complete membrane distention, (ii) kiss-and-run, and (iii) kiss-and-stay exocytosis. Most of the single-cell amperometry systems incorporate CFE to record the exocytosis events. Owing to their small size and ability to give rapid response, these electrodes ensure high signal to noise ratio and offer high temporal resolution. In addition to the benefits that these electrodes have to offer, certain limitations have also been reported *viz.* (i) to obtain statistically significant data, experiments are performed on multiple single cells which becomes time taking for single cell experimentations (Mosharov and Sulzer 2005) and (ii) EC recording of exocytosis along with fluorescence imaging of the vesicle involved in the process is difficult to perform (An and Almers 2004). In order to overcome these limitations, Berberian *et al.* fabricated surface patterned platinum microelectrodes insulated with fused silica (Berberian et al., 2009). This system allowed recording of single vesicular exocytosis of MNTs from chromaffin cells with high resolution and high signal to noise ratio. They also proposed that this system could also be suitable for parallel electrode arrays to allow recording of single events from a large number of cells. Using a similar strategy, Lin *et al.* used arrays of carbon-ring microelectrode for ECD of catecholamine exocytosis from a single PC12 cell. They recorded exocytotic events for 180 s

occurring at a single cell after treating it with K^+ (stimulant) by using amperometry. The transient current showed EC oxidation of catecholamines released from the intracellular vesicle (Lin et al., 2012). Microelectrode array-based analysis is usually exhausting and require sophisticated instrumentation and expertise. Additionally, development of these systems requires high production cost ca. $100 per chip and lack reusability. This dictates the need to develop systems which are inexpensive, reusable, and allow quick high-throughput analysis. In this direction, Yakushenko *et al.* developed a reliable microelectrode for the detection of catecholamine in simple yet robust experimental setting by using SPCP (Yakushenko et al., 2012). This system has shown the ability to culture PC12 cells followed by the on-chip detection of catecholamine precisely. Since the devised system made use of materials worth only 20 cents and enabled quick MNT detection, therefore it may serve as an inexpensive on-chip laboratory demonstration model for studying the intricate biological phenomenon. Several other groups have also investigated catecholamine release by monitoring exocytosis on chromaffin cells (Picollo et al. 2016; Kisler et al. 2012; Carabelli et al. 2010; Gao et al. 2009) and MN9D cells (Dong et al. 2008). In their respective experiments, these groups stimulated both the cell lines by using high K^+ ion containing solution and reported the EC monitoring of catecholamines. A detailed information on these reports including type of sensing probes used for monitoring catecholamines and their analytical performance in terms of LOD and LOQ has been compiled in **table 3.1**. Another biosensing system was reported by Ariano *et al.*, for monitoring the neuronal activity of GT1-7 cells, a neuronal cell line. They made use of hydrogen terminated diamond electrode for recording the extracellular electrical activity of the cultured neuronal cells (Ariano et al., 2009). The representative scheme of the experimental setup has been shown in **figure 4.** The ability of developed system to detect spontaneous extracellular electrical activity can be used to monitor the changes in the extracellular medium such as ion substitution or addition of pharmacological agents, e.g. channel blockers. The results obtained by this group are very encouraging, however, absence of MNT's detection shows the need to extend the work in this direction. Considering this, Li *et al.* developed a biosensing system for detection of released MNTs from a single cell by modifying DNA-aptamers on a multiple-parallel-connected silicon nanowire field-effect transistor (MPC aptamer/SiNW-FET). The real-time recording was carried out by stimulating the release of MNT by creating hypoxic conditions for the PC12 cells (Li et al., 2013). This system showed improved detection of exocytosed DA with a LOD of $<10^{-11}$ M.

Additionally, this system was highly selective towards the MNTs under investigation as it was able to distinguish between the analyte and other chemical analogs like AA, Ep, NA, catechol, tyrosine, and phenylalanine effectively. Not limiting to this, the MPC aptamer/SiNW-FET system also revealed that high intracellular concentration of Ca^{+2} ion is essential to trigger the secretion of DA. A recent report published by Mir *et al.* studied GO/AuNPs/pDAN-EDTA probe mediated ECD of *in vitro* (PC12 cell line) DA levels under the stimulatory influence of K^+ ions. The result obtained by this group showed excellent sensitivity (LOD = 5nM) and selectivity with no interference from AA and UA (Mir et al., 2015). There have also been other reports on the EC monitoring of DA exocytosis from PC 12 cells (Kruss et al. 2017; Alatraktchi et al. 2014, Zhang et al. 2010) and detailed information on these reports has been incorporated in **table 3.2**.

Apart from PC 12 cells, bovine adrenal chromaffin cells have also been investigated for DA exocytosis (Huang et al. 2017; Kim et al. 2013; Ayers et al. 2010). A variety of sensing probes were designed to enable successful detection of DA, details of which have been discussed in **table 3.2**. In addition to DA, there have been reports on ECD of exocytosed NE from different neuronal cell lines. In one such example, Ges *et al.* developed a planar thin film Pt probe modified with electrochemically deposited iridium oxide films in order to ensure mechanistically rich data with increased throughput towards catecholamines, (Ges et al., 2012). The modified sensing probe detected release of catecholamines, NE in this case, from adrenal chromaffin cells trapped in a microfluidic network with an LR of 0–400 μM and a sensitivity of 23.1 ± 0.5 mA/M mm^2. Owing to its excellent analytical performance and utility of integrated microfluidic network, the modified sensing probe enabled real-time quantitative detection of catecholamines released from small populations of chromaffin cells. Another report on *in vitro* ECD of NE was published very recently where PC 12 cell line has been used as a model system (Emran et al. 2018). A C-, N-doped NiO broccoli-like hierarchy containing nano-tubular arrangements was used as highly sensitive and selective sensing system. The homogenous doping and anisotropic dispersion of C- N nanospheres along the entire NiO broccoli head nanotubes lead to the development of abundant electroactive sites in the interior tubular vessels and outer surfaces. Presence of such electroactive sites enabled ultrasensitive detection of NE exocytosed from K^+ ion stimulated PC12 cells with an LOD of ~6 nM.

Examination of MNTs in the *in vitro* system is imperative; however, their real-time

analyses in the *in vivo* models are crucial to enhance the knowledge and provide better understanding of the intricate human biological system and associated diseases. Thorough knowledge and better understanding of such systems can aid in paving means to develop novel detection methods and finding remedies for neuronal diseases.

4. Electrochemical sensing of MNTs in the *in vivo* models

Brain is an intricate organ which has heterogeneous fluid environment due to the presence of several chemical entities at varying concentration. This change in chemical concentration usually occurs on a very narrow time-scale of second or millisecond (Bard and Rubenstein 1996). Therefore, a quintessential technology to examine the neurochemistry of the brain should be able to mediate quick assessments to meet the timescale at which neural signaling ensues. It should also be able to detect and distinguish between chemicals from spatially distinct sites without disturbing the local neuronal environment. In this section, crucial developments and findings from recent studies towards achieving these objectives by using *in vivo* voltammetry technique has been highlighted.

In vivo voltammetry has been used as a common terminology to designate a set of EC techniques that are established to record multiple MNTs in the brain. These techniques can vary depending on the type of electrode, the rate of scanning, and the mode of scanning (pulse or continuous). In last few decades, several reports have come up with their objective to detect multiple MNTs in the *in vivo* models and in all the reports the experimental protocol followed is somewhat similar. For instance, first the animal is weighed and then anaesthetized using anesthetic agent e.g. urethane. Second, holes are drilled in the animal skull at desired location(s) according to the stereotaxic coordinates referenced from bregma and then MEs are placed. Third, MNT release is electrically elicited by placing bipolar electrodes in the desired areas of brain and finally the concentration of MNTs is monitored. Hashemi *et al.* studied the simultaneous release of 5-HT and His in the rat SNR post electrical stimulation of 5-HT projections in MFB (Hashemi et al., 2011). The histological verification of the location of the stimulating and CFME placement in the brain tissue has been shown in **figure 5,** where the stimulating electrode was placed in the MFB and the CFME was placed in the SNR. The MFB stimulation led to the simultaneous

release of 5-HT and an additional species in SNR. FSCV based electrochemical study confirmed this additional species to be His. This report highlighted the importance of *in vivo* voltammetry technique in exploring the dynamics of 5-HT, as it enabled selective recording of MNTs with high temporal resolution.

Apart from single microelectrode, MEA has also been developed to monitor MNTs present in a restricted area of the brain, i.e., rat striatum (Zachek et al., 2010). FSCV analysis was performed on MEA to observe different neurochemical events, such as heterogeneity of electrically elicited DA release and uptake, drug-mediated changes in DA concentration within a single brain region and across brain regions within 1 mm. For pharmacological studies, raclopride and cocaine were used in the striatum and the consistency of the result was confirmed by similar study on multiple animals. Various other groups also investigated DA release in different regions of brain under *in vivo* condition, electrochemically (Taylor et al. 2017; Vreeland et al., 2015; Yang et al. 2016a; Zachek et al. 2010). A comprehensive discussion on these reports has been incorporated in **table 4((A) 4.1).** Unfortunately, in most *in vivo* detection systems, biocompatibility and electrode fouling are two of the major and frequently encountered issues. To overcome these issues, Özel et al. developed a chitosan modified CFE for the *in vivo* analysis of 5-HT (Özel et al., 2011). On DPV analysis, the fabricated biosensing probe displayed excellent capability to reject interferences coming from AA at the physiological concentration and thus, enabled selective detection of 5-HT with an LOD of 1.6 nM and LR of 2–100 nM in the living embryonic intestine of zebrafish. In addition to this, high reproducibility (RSD=6.5%) and no electrode fouling was observed due the application of chitosan in the sensing matrix. Park *et al.,* investigated the release of NE and DA in different regions of brain including NAc shell (Park et al. 2010), ventral bed nucleus of the stria terminalis and NAc shell (Park et al., 2011; Park et al., 2015), and rostral and caudal NAc shell (Park et al., 2017). They made use of glass-sealed CFME with exposed tip length of 75−100 μm and diameter 7 μm in aforementioned regions of living brain for FSCV mediated MNT release analysis. More detailed information on these reports has been incorporated in **table 4.2.** Besides voltammetry, there have also been reports on chromatography coupled ECD techniques for MNT detection. In few such examples, ultra-high performance liquid chromatography (UHPLC) coupled with ECD (Van Schoors et al. 2016; Van Schoors et al. 2015; Parrot et al. 2011) and capillary UHPLC-ECD (Ferry et al. 2014) techniques have been employed to analyze DA, NE, and 5-HT in various *in vivo* systems. Details of these *in vivo*

MNTs monitoring systems have been discussed comprehensively in **table 4.3**.

5. Electrochemical sensing of MNTs in *ex vivo* models

The very first report on ECD of MNTs in the *ex vivo* (brain slices) model came in the early 1980s with an aim to provide quantitative data and help to address shortcomings and benefits encountered with ECD in the *in vivo* models (Schenk et al., 1983). Owing to the several technical benefits associated with this approach, *ex vivo* voltammetric techniques (FSCV, FSCAV, etc.) have become very popular and have added more value to the work done *in vivo*. The technical benefits associated with usage of *ex vivo* systems are (i) prevention of confounding effects arising from usage of anesthetics, (ii) intrinsic reduction/simplification of intricate neural network present in the model during an experiment, and (iii) preclusion of the worry associated with the ability of any given compound to cross the blood-brain-barrier or plausible toxicity that a given compound may have on the whole animal. These benefits combined together, widen the range of compounds which can be studied in an *ex vivo* model, due to ease of controlling the suffusing fluid composition and minimizing interferences coming from compounds like AA, UA, etc.

Generally, to initiate any experiment in *ex vivo* model, the first requisite is brain slice preparation from a whole intact brain. For this, the brain is quickly removed from an animal and immediately kept in cold physiological saline solution. A tissue section is then cut containing the desired portion from the whole brain and slices of the tissue are prepared. Ever since the first report, different voltammetric techniques have been used for experiments on *ex vivo models*, however, FSCV is one of the most widely used technique. The main intention of performing experiments on *ex vivo* models roughly fall into: (i) investigating release of NT and its uptake in different sections of brain (Burrell et al., 2015; Smith et al., 2017; Wakabayashi et al., 2016), (ii) comparing the release and uptake of NTs within different regions of brain (Ferris et al., 2013; Salinas et al., 2016; Siciliano et al., 2014), and (iii) examining the presynaptic regulation of NTs release under the influence of pharmacologically manipulating auto-receptors (Karayannis et al., 2014; McGinnis et al., 2016; Shin et al., 2017; Siciliano et al., 2018). This article does not cover findings from these studies in detail, as it is beyond the scope of this article; however, discusses

them briefly to give an insight into the recent developments in this direction.

Over the years, CFEs have been exploited for investigating MNTs in various models due to its ability to exhibit high absorption, display efficient electrical conductivity, and small size (McCreery 2008). Keeping these benefits in mind, Patel and Rice reported CFE mediated EC examination of axonal (forebrain slice) and somato-dendritic (midbrain slice) DA release with an LOD of ~30–50 nM (Patel and Rice 2013). Though, the results obtained by this group were encouraging, the need to examine exact tonic level of DA was still persisting. To overcome this issue, a recent study employed a modified FSCV technique called FSCAV on CFE to examine the exact tonic level of DA in an *ex vivo* model (Burrell et al., 2015). This study is the first ever report to have successfully investigated the prolonged levels of DA extracellularly in brain slices by using an EC technique. Another interesting report on ECD of MNTs in *ex vivo* model was published by Kaplan *et al.* to study the impairment in the release of DA and 5-HT post treatment with carboplatin, a chemotherapeutic agent (Kaplan et al., 2016). Based on their study, they were able to justify that the chemotherapy treatment significantly affected the release of MNTs. Though, CFEs are very attractive detectors for *ex vivo* models, but there are certain issues associated with these electrodes which necessitate the need to develop better electrodes. For instance, development of an electrode which possesses high sensitivity, resists surface fouling and exhibits rapid electron transfer kinetics. In this direction, Zestos *et al.* developed a PEI-CNT fiber electrode for highly sensitive and selective detection of MNTs in an *ex vivo* model (Zestos et al., 2014). They compared their results with the results of poly-(vinyl alcohol) CNT fiber electrodes and reported PEI-CNT fiber electrode to have higher sensitivity with a lower LOD of 5 nM for DA. Additionally, PEI-CNT fibers exhibited lower over-oxidation potential and showed excellent antifouling properties. Apart from single electrode, there have been reports on MEAs for monitoring the concentration MNTs. Wang *et al.* fabricated a MEA by micro-electromechanical system in order to develop a probe which could enable rapid, sensitive, and reliable real-time sensing of NE secretion (Wang et al., 2017). To improve the analytical performance, MEA was modified with electrodeposited rGO and PtNPs which showed wider LR of 5–250 nM, lower LOD of 0.08 μM and high sensitivity of 1.03 nA μM^{-1}. DPV analysis of real-time NE secretion from the locus coeruleus brain slice was done by using fabricated MEA. Not only this, they also monitored spike firing from the hippocampal brain slice and reported that the fabricated MEA exhibits potential to be used in spatially resolved delivery of trace

neurochemicals and electrophysiological activities of a variety of biological tissues. Apart from aforementioned reports, other studies have also been published with an aim to measure both the release of MNT and electrophysiological responses (Suzuki et al., 2013), to improve the analytical performance of sensor (Fang et al., 2013), and enhance its temporal and spatial resolution (Maina et al., 2012). Detailed information on these reports including sensing probe fabrication, analytical performance, and the type of brain tissue used for study has been incorporated in **table 4 (B)**.

6. Challenges faced with electrochemical sensing techniques and future directions

The major challenge that lies in any modification strategy is the achievement of consistent electrode composition in the solution interface, during and post each measurement. EC sensors incorporating modified electrodes display an adequate potential to apply them in analytical applications in real time. Still, why these sensors are restricted to proof-of-concept is a matter of concern that needs proper addressing. In our view, the fundamental requirement would be to explore appropriate strategies that can bring out integration of modified electrodes into a compact system. The probability of developing such intricate sensing systems are still far from being realized due to inadequate information on biocompatibility and poisoning of the sensing probe modification materials. Albeit modified electrodes are considered rather advanced from the development standpoint, but a majority of original articles do not provide any information on structural changes that occur on a sensor surface post analytes' analyses. Therefore, future works need to be directed towards addressing these issues. In recent years, a new class of *in vitro* model: organ-on-chip has been introduced which can efficiently combine the benefits of *in vivo* and *in vitro* models of different tissues and organs (Bhatia and Ingber, 2014). This so-called chip is actually a microfluidic device in which a tissue can be cultured in an engineered environment, mimicking its *in vivo* microenvironment (van der Meer and van den Berg, 2012; Moraes et al., 2012). An advantage of this organ-on-chip platform is that imaging systems and sensors with real-time readouts can also be integrated, which makes use of this device highly valuable (Bhatia and Ingber, 2014). With the ability to replicate organ-level functions and potential to allow the study of (patho)physiology on a higher level (van der Helm et al., 2016), this device can be used to study the dynamics of MNTs in the brain and open new avenues in neuroscience. Therefore,

attempts should be made towards developing brain-on-chip models to better understand neurological processes, their disorders, and effect of neurological drugs.

7. Conclusions

The ultimate aim of the neuronal studies has always directly or indirectly been to have better insight into the complex mammalian neuronal circuitry. Since Adams' revolutionary work ca. 40 years ago, several advancements in EC sensing techniques have been documented which has pushed EC sensing techniques to the forefront of most-widely used techniques today. The surge in the application of ECD techniques for neuronal studies lies in the attractive benefits associated with this technique, such as rapid, inexpensive, highly selective, and point-of-care analysis. Amongst all NTs, MNTs belong to the group of highly investigated NTs by means of ECD techniques due to their inherent electroactive nature. Years of incessant work in this direction has offered a great deal of information on neuroscience and has also shown the clinical implication of EC sensors as diagnostic tools. Though, the development made in this field is appreciable, there are still several issues at which present work staggers. Therefore, in order to approve EC sensors as a top method for monitoring MNTs in real systems, it is imperative to address these aspects with great attention. Nevertheless, the unparalleled efforts put forth by the scientific community in this direction allow great optimism that this goal will be achieved in the near future.

Conflict of interest

Authors declare absence of any commercial or financial relationships which can be construed as a conflict of interest on their part.

Acknowledgments

This work is supported by a DBT research grant, project number BT/PR16634/NER/95/225/2015 awarded to Dr. Pranjal Chandra by Government of India.

References

Abbaspour, A., Noori, A., 2011. Biosens. Bioelectron., 26(12), 4674-4680.
Alatraktchi, F.A., Bakmand, T., Dimaki, M., Svendsen, W.E., 2014. Sensors, 14(11), 22128-22139.
Allen, S.A., Rednour, S., Shepard, S., Pond, B.B., 2017. Biomed Chromatogr., 31(11), 1-8
An, S.J., Almers, W., 2004. Science, 306(5698), 1042-1046.
Ariano, P., Giudice, A.L., Marcantoni, A., Vittone, E., Carbone, E., Lovisolo, D., 2009. Biosens. Bioelectron., 24(7), 2046-2050.
Atta, N.F., El-Kady, M.F., Galal, A., 2010. Anal. Biochem., 400(1), 78-88.
Atta, N.F., Galal, A., Ahmed, R.A., 2011. J. Electrochem. Soc., 158(4), 52-60.
Atta, N.F., Galal, A., Azab, S.M., 2012. J. Electrochem. Soc. 159(10), H765-H771.
Ayers, S., Berberian, K., Gillis, K.D., Lindau, M., Minch, B.A., 2010. IEEE transactions on biomedical circuits and systems, 4(2), 86-92.
Bansal, S., Jyoti, A., Mahato, K., Chandra, P., Prakash, R., 2017. Electroanalysis, 29(11), 2665-2671.
Bard, A.J., Rubenstein, I., 1996. Electroanalytical chemistry: a series of advances. CRC Press.
Baselt, R.C., (1989). CRC PRESS, INC., BOCA RATON, FL(USA). 1989.
Berberian, K., Kisler, K., Fang, Q., Lindau, M., 2009. Anal. Chem., 81(21), 8734-8740.
Bhatia, S.N., Ingber, D.E., (2014). Nat. Biotechnol., 32(8), 760.
Burrell, M.H., Atcherley, C.W., Heien, M.L., Lipski, J., (2015). ACS Chem. Neurosci., 6(11), 1802-1812.
Cai, H.-L., Zhu, R.-H., 2010. Anal. Biochem, 396(1), 103-111.
Carabelli, V., Gosso, S., Marcantoni, A., Xu, Y., Colombo, E., Gao, Z., Vittone, E., Kohn, E., Pasquarelli, A., Carbone, E., 2010. Biosens. Bioelectron., 26(1), 92-98.
Chandra, P., 2013. J. Bioanal. Biomed, 5, e122.
Chandra, P., 2016. Nanobiosensors for personalized and onsite biomedical diagnosis. The Institution of Engineering and Technology.
Chandra, P., Zaidi, S.A., Noh, H.-B., Shim, Y.-B., 2011. Biosens. Bioelectron., 28(1), 326-332.
Chicharro, M., Sánchez, A., Zapardiel, A., Rubianes, M., Rivas, G., 2004. Anal. Chim. acta, 523(2), 185-191.
Chitravathi, S., Munichandraiah, N., 2015. J. Electrochem. Soc., 162(7), B163-B172.
Cincotto, F.H., Canevari, T.C., Campos, A.M., Landers, R., Machado, S.A., 2014. Analyst, 139(18), 4634-4640.
Colliver, T.L., Ewing, A.G., 2006. Encyclopedia of Analytical Chemistry, 1-25.
Dong, Y., Heien, M.L., Maxson, M.M., Ewing, A.G., (2008). J. Neurochem., 107(6), 1589-1595.
Elhomsy, G., 2014. In: Staros, E.B. (Ed.).
Emran, M.Y., Mekawy, M., Akhtar, N., Shenashen, M.A., EL-Sewify, I.M., Faheem, A., El-Safty, S.A., 2018. Biosens. Bioelectron., 100, 122-131.
Fang, H., Pajski, M.L., Ross, A.E., Venton, B.J., 2013. Anal. Methods, 5(11), 2704-2711.
Faraone, S.V., Bonvicini, C., Scassellati, C., 2014. Curr. Psychiatry Rep., 16(11), 497.
Felten, D.L., Felten, S.Y., Sladek, J.R., Notter, M.D., Carlson, S.L., Bellinger, D.L., Wiegand, S.J., 1990. J. Microsc. 157(3), 271-283.
Fernández-Novoa, L.a., Cacabelos, R., 2001. Behav. Brain Res., 124(2), 213-233.
Ferris, M.J., Calipari, E.S., Yorgason, J.T., Jones, S.R., 2013. ACS Chem. Neurosci., 4(5), 693-703.
Ferry, B., Gifu, E.-P., Sandu, I., Denoroy, L., Parrot, S., 2014. J. chromatogr. B, 951, 52-57.
Figueiredo-Filho, L.C., Silva, T.A., Vicentini, F.C., Fatibello-Filho, O., 2014. Analyst, 139(11), 2842-2849.
Friedman, B.S., Steinberg, S.C., Meggs, W.J., Kaliner, M.A., Frieri, M., Metcalfe, D.D., 1989. Am. J. Med., 87(6), 649-654.
Gao, Y., Bhattacharya, S., Chen, X., Barizuddin, S., Gangopadhyay, S., Gillis, K.D., 2009. Lab Chip, 9(23), 3442-3446.
Ges, I.A., Currie, K.P., Baudenbacher, F., 2012. Biosens. Bioelectron. 34(1), 30-36.

Gottås, A., Ripel, Å., Boix, F., Vindenes, V., Mørland, J., Øiestad, E., 2015. J. Pharmacol. Toxicol. Methods, 74, 75-79.
Goyal, R.N., Bishnoi, S., 2011. Talanta, 84(1), 78-83.
Hasanzadeh, M., Shadjou, N., de la Guardia, M., 2017. Trends Anal. Chem., 86, 107-121.
Hasanzadeh, M., Shadjou, N., Omidinia, E., 2013. J. Neurosci. Methods, 219(1), 52-60.
Hashemi, P., Dankoski, E.C., Wood, K.M., Ambrose, R.E., Wightman, R.M., 2011. J. Neurochem., 118(5), 749-759.
He, M., Wang, C., Wei, Y., 2016a. Talanta, 147, 437-444.
He, Y., Zhao, X.-E., Zhu, S., Wei, N., Sun, J., Zhou, Y., Liu, S., Liu, Z., Chen, G., Suo, Y., 2016b. J. Chromatogr. A, 1458, 70-81.
Holland, B.J., Conlan, X.A., Stevenson, P.G., Tye, S., Reker, A., Barnett, N.W., Adcock, J.L., Francis, P.S., 2014. Anal. Bioanal. Chem., 406(23), 5669-5676.
Horakova, Z., Keiser, H.R., Beaven, M.A., 1977. Clin. Chim. Acta, 79(2), 447-456.
Huang, M., Delacruz, J.B., Ruelas, J.C., Rathore, S.S., Lindau, M., 2017. Pflügers Archiv., 1-11.
Jackowska, K., Krysinski, P., 2013. Anal. Bioanal. Chem., 405(11), 3753-3771.
Jeon, J., Hwang, I., Chung, T.D., 2016. Biomed. Eng. Lett., 6(3), 123-133.
Kaplan, S.V., Limbocker, R.A., Gehringer, R.C., Divis, J.L., Osterhaus, G.L., Newby, M.D., Sofis, M.J., Jarmolowicz, D.P., Newman, B.D., Mathews, T.A., 2016. ACS Chem. Neurosci., 7(6), 689-699.
Karayannis, T., Au, E., Patel, J., Kruglikov, I., Markx, S., Delorme, R., Héron, D., Salomon, D., Glessner, J., Restituito, S., 2014. Nature, 511(7508), 236.
Kema, I.P., de Vries, E.G., Muskiet, F.A., 2000. J. Chromatogr. B Biomed. Sci. Appl. 747(1-2), 33-48.
Kim, B.N., Herbst, A.D., Kim, S.J., Minch, B.A., Lindau, M., 2013. Biosens. Bioelectron., 41, 736-744.
Kisler, K., Kim, B.N., Liu, X., Berberian, K., Fang, Q., Mathai, C.J., Gangopadhyay, S., Gillis, K.D., Lindau, M., 2012. J. Biomater. Nanobiotechnol., 3(2A), 243.
Kruss, S., Salem, D.P., Vuković, L., Lima, B., Vander Ende, E., Boyden, E.S., Strano, M.S., 2017. Proc. Natl. Acad. Sci., 201613541.
Li, B.-R., Hsieh, Y.-J., Chen, Y.-X., Chung, Y.-T., Pan, C.-Y., Chen, Y.-T., 2013. J. Am. Chem. Soc., 135(43), 16034-16037.
Li, Q., Zhang, J., Yan, H., He, M., Liu, Z., 2004. Carbon, 42(2), 287-291.
Lin, Y., Trouillon, R.l., Svensson, M.I., Keighron, J.D., Cans, A.-S., Ewing, A.G., 2012. Anal. Chem., 84(6), 2949-2954.
Mackay, A., Yates, C., Wright, A., Hamilton, P., Davies, P., 1978. J. Neurochem., 30(4), 841-848.
Mahato, K., Kumar, S., Srivastava, A., Maurya, P.K., Singh, R., Chandra, P., 2018. Electrochemical Immunosensors: Fundamentals and Applications in Clinical Diagnostics. Handbook of Immunoassay Technologies, pp. 359-414. Elsevier.
Mahato, K., Prasad, A., Maurya, P., Chandra, P., 2016. J. Anal. Bioanal. Tech., 7, e125.
Mahato, K., Baranwal, A., Srivastava, A., Maurya, P.K., Chandra, P., 2016. Techno-Societal 2016, International Conference on Advanced Technologies for Societal Applications, pp. 421-431. Springer.
Maina, F.K., Khalid, M., Apawu, A.K., Mathews, T.A., 2012. J. Vis. Exp. (59).
Mandel, S., 2013. Neurodegenerative Diseases: Integrative PPPM Approach as the Medicine of the Future. Springer Science & Business Media.
Manouchehri, N., Bigam, D.L., Churchill, T., Rayner, D., Joynt, C., Cheung, P.-Y., 2013. Pediatric Res., 73(4-1), 435.
Mazloum-Ardakani, M., Brazesh, B., Hosseinzadeh, L., Khoshroo, A., 2017. J. Anal. Chem., 72(6), 689-698.
Mazloum-Ardakani, M., Khoshroo, A., 2014. J. Electroanal. Chem. 717, 17-23.
McCreery, R.L., 2008. Chem. Rev, 108(7), 2646-2687.
McGinnis, M.M., Siciliano, C.A., Jones, S.R., 2016. J. Neurochem., 138(6), 821-829.
Mefford, I.N., 1988. Prog. Neuropsychopharmacol. Biol. Psychiatry, 12(4), 365-388.
Mefford, I., Oke, A., Adams, R., Jonsson, G., 1977. Neurosci. Lett., 5(3-4), 141-145.
Mir, T.A., Akhtar, M.H., Gurudatt, N., Kim, J.-I., Choi, C.S., Shim, Y.-B., 2015. Biosens. Bioelectron.,

68, 421-428.
Mittal, R., Debs, L.H., Patel, A.P., Nguyen, D., Patel, K., O'Connor, G., Grati, M.h., Mittal, J., Yan, D., Eshraghi, A.A., 2017. J. Cell. Physiol., 232(9), 2359-2372.
Moon, J.-M., Thapliyal, N., Hussain, K.K., Goyal, R.N., Shim, Y.-B., 2018. Biosens. Bioelectron., 102, 540-552.
Moore, R., Bloom, F., 1979. Annu. Rev. Neurosci. 2(1), 113-168.
Moraes, C., Mehta, G., Lesher-Perez, S.C., Takayama, S., 2012. Ann. Biomed. Eng., 40(6), 1211-1227.
Moret, C., Briley, M., 2011. Neuropsychiatr. Dis. Treat. 7(Suppl 1), 9.
Morilak, D.A., Barrera, G., Echevarria, D.J., Garcia, A.S., Hernandez, A., Ma, S., Petre, C.O., 2005. Prog. Neuropsychopharmacol. Biol. Psychiatry. 29(8), 1214-1224.
Mosharov, E.V., Sulzer, D., 2005. Nat. Methods, 2(9), 651-658.
Narayana, P., Reddy, T.M., Gopal, P., Reddy, M.M., Naidu, G.R., 2015. Mater. Sci. Eng. C, 56, 57-65.
Naughton, M., Mulrooney, J.B., Leonard, B.E., 2000. Hum. Psychopharmacol. Clin. Exp. 15(6), 397-415.
Noh, H.-B., Chandra, P., Moon, J.O., Shim, Y.-B., 2012. Biomaterials, 33(9), 2600-2607.
Nuutinen, S., Panula, P., 2010. Histamine in neurotransmission and brain diseases. Histamine in Inflammation, pp. 95-107. Springer.
Özel, R.E., Wallace, K.N., Andreescu, S., 2011. Anal. Chim. Acta, 695(1), 89-95.
Pacak, K., Palkovits, M., 2001. Endocrine reviews, 22(4), 502-548.
Park, J., Aragona, B.J., Kile, B.M., Carelli, R.M., Wightman, R.M., 2010. Neuroscience, 169(1), 132-142.
Park, J., Bucher, E.S., Budygin, E.A., Wightman, R.M., 2015. Pain, 156(2), 318.
Park, J., Takmakov, P., Wightman, R.M., 2011. J. Neurochem. 119(5), 932-944.
Park, J.W., Bhimani, R.V., Park, J., 2017. ACS Chem. Neurosci. 8(9), 1913-1924.
Parrot, S., Neuzeret, P.-C., Denoroy, L., 2011. J. Chromatogr. B, 879(32), 3871-3878.
Parrot, S., Sauvinet, V., Riban, V., Depaulis, A., Renaud, B., Denoroy, L., 2004. J. Neurosci. Methods, 140(1), 29-38.
Patel, J.C., Rice, M.E., 2013. Monitoring axonal and somatodendritic dopamine release using fast-scan cyclic voltammetry in brain slices. Dopamine, pp. 243-273. Springer.
Pearl, P.L., 2013. Handbook of clinical neurology, 113, 1819-1825.
Perry, M., Li, Q., Kennedy, R.T., 2009. Anal. Chim. Acta, 653(1), 1-22.
Picollo, F., Battiato, A., Bernardi, E., Marcantoni, A., Pasquarelli, A., Carbone, E., Olivero, P., Carabelli, V., 2016. Anal. Chem., 88(15), 7493-7499.
Ping, J., Wu, J., Wang, Y., Ying, Y., 2012. Biosens. Bioelectron., 34(1), 70-76.
Ran, G., Chen, X., Xia, Y., 2017. RSC Adv., 7(4), 1847-1851.
Rand, E., Periyakaruppan, A., Tanaka, Z., Zhang, D.A., Marsh, M.P., Andrews, R.J., Lee, K.H., Chen, B., Meyyappan, M., Koehne, J.E., 2013. Biosens. Bioelectron., 42, 434-438.
Robinson, T.E., Justice, J.B., 2013. Microdialysis in the neurosciences: techniques in the behavioral and neural sciences. Elsevier, Netherlands.
Sajid, M., Nazal, M.K., Mansha, M., Alsharaa, A., Jillani, S.M.S., Basheer, C., 2016. Trends Analyt. Chem. 76, 15-29.
Salinas, A.G., Davis, M.I., Lovinger, D.M., Mateo, Y., 2016. Neuropharmacology, 108, 275-283.
Samdani, K.J., Joh, D.W., Rath, M.K., Lee, K.T., 2017. Electrochim. Acta, 252, 268-274.
Samdani, K.J., Samdani, J.S., Kim, N.H., Lee, J.H., 2016. Biosens. Bioelectron., 81, 445-453.
Saxena, P.R., 1995. Pharmacol. Ther. 66(2), 339-368.
Schenk, J.O., Miller, E., Rice, M.E., Adams, R.N., 1983. Brain Res., 277(1), 1-8.
Schildkraut, J.J., 1965. Am J Psychiatry, 122(5), 509-522.
Sharma, S., Singh, N., Tomar, V., Chandra, R., 2018. Biosens. Bioelectron., 107, 76-93
Shim, S.H., Kim, S.J., Sugahara, K., Sugita, S., Tanaka, H., 2001. Experim. Anim. 50(5), 445-449.
Shin, J.H., Adrover, M.F., Alvarez, V.A., 2017. J. Neurosci., 37(46), 11166-11180.
Si, B., Song, E., 2018. Chemosensors, 6(1), 1.
Siciliano, C.A., Calipari, E.S., Jones, S.R., 2014. J. Neurochem., 131(3), 348-355.
Siciliano, C.A., Saha, K., Calipari, E.S., Fordahl, S.C., Chen, R., Khoshbouei, H., Jones, S.R., 2018. J.

Neurosci., 38(2), 484-497.
Smith, S.K., Lee, C.A., Dausch, M.E., Horman, B.M., Patisaul, H.B., McCarty, G.S., Sombers, L.A., 2017. ACS Chem. Neurosci., 8(2), 272-280.
Sun, D., Li, H., Li, M., Li, C., Dai, H., Sun, D., Yang, B., 2018. Sens. Actuators B Chem. 259, 433-442.
Suzuki, I., Fukuda, M., Shirakawa, K., Jiko, H., Gotoh, M., 2013. Biosens Bioelectron. 49, 270-275.
Taylor, I.M., Robbins, E.M., Catt, K.A., Cody, P.A., Happe, C.L., Cui, X.T., 2017. Biosens. Bioelectron., 89, 400-410.
Thomas, J., Khanam, R., Vohora, D., 2015. Pharma. Biol., 53(10), 1539-1544.
Torrealba, F., Riveros, M.E., Contreras, M., Valdes, J.L., 2012. Front. Syst. Neurosci. 6, 51.
Tsai, T.-H., Thiagarajan, S., Chen, S.-M., Cheng, C.-Y., 2012. Thin Solid Films, 520(7), 3054-3059.
Turner, A.P., 2000. Science, 290(5495), 1315-1317.
Turner, A.P., 2013. Chem. Soc. Rev., 42(8), 3184-3196.
Tzschentke, T., 2001. Prog. Neurobiol. 63(3), 241-320.
Valentini, F., Ciambella, E., Conte, V., Sabatini, L., Ditaranto, N., Cataldo, F., Palleschi, G., Bonchio, M., Giacalone, F., Syrgiannis, Z., 2014. Biosens. Bioelectron., 59, 94-98.
Vallone, D., Picetti, R., Borrelli, E., 2000. Neurosci. Biobehav. Rev. 24(1), 125-132.
van der Helm, M.W., van der Meer, A.D., Eijkel, J.C., van den Berg, A., Segerink, L.I., 2016. Tissue barriers, 4(1), e1142493.
van der Meer, A.D., van den Berg, A., 2012. Integr. Biol., 4(5), 461-470.
van Kempen, G.T.H., van den Berg, R., Eilers, P., Westerink, R., 2011. Biophys. J., 100(4), 968-977.
Van Schoors, J., Lens, C., Maes, K., Michotte, Y., Smolders, I., Van Eeckhaut, A., 2015. J. Chromatogr. B, 998, 63-71.
Van Schoors, J., Viaene, J., Van Wanseele, Y., Smolders, I., Dejaegher, B., Vander Heyden, Y., Van Eeckhaut, A., 2016. J. Pharma. Biomed. Anal., 127, 136-146.
Vlčková, M., Schwarz, M.A., 2007. J. Chromatogr. A, 1142(2), 214-221.
Volkow, N.D., Fowler, J.S., Wang, G.-J., Swanson, J.M., (2004). Mol. Psychiatry, 9(6), 557.
Vreeland, R.F., Atcherley, C.W., Russell, W.S., Xie, J.Y., Lu, D., Laude, N.D., Porreca, F., Heien, M.L., 2015. Anal Chem. 87(5), 2600-2607.
Wakabayashi, K.T., Bruno, M.J., Bass, C.E., Park, J., 2016. Analyst, 141(12), 3746-3755.
Wang, L., Song, Y., Zhang, Y., Xu, S., Xu, H., Wang, M., Wang, Y., Cai, X., 2017. J. Micromech. Microeng. 27(11), 115001.
Wang, S., Wang, Y., Min, Q., Shu, T., Zhu, X., Peng, A., Ding, H., 2016. Int. J. Electrochem. Sci. 11, 2360-2376.
Wang, Y., Wang, S., Tao, L., Min, Q., Xiang, J., Wang, Q., Xie, J., Yue, Y., Wu, S., Li, X., 2015. Biosens. Bioelectron. 65, 31-38.
Xu, H., Wang, D., Zhang, W., Zhu, W., Yamamoto, K., Jin, L., 2006. Anal. Chim. Acta, 577(2), 207-213.
Yadav, S.K., Agrawal, B., Chandra, P., Goyal, R.N., 2014. Biosens. Bioelectron., 55, 337-342.
Yakushenko, A., Schnitker, J., Wolfrum, B., 2012. Anal. Chem., 84(10), 4613-4617.
Yang, C., Trikantzopoulos, E., Nguyen, M.D., Jacobs, C.B., Wang, Y., Mahjouri-Samani, M., Ivanov, I.N., Venton, B.J., 2016a. ACS Sens., 1(5), 508-515.
Yang, X., Feng, B., He, X., Li, F., Ding, Y., Fei, J., 2013. Microchim. Acta, 180(11-12), 935-956.
Yang, X., Hu, Y., Li, G., 2014b. J. Chromatogr. A, 1342, 37-43.
Yao, H., Li, S., Tang, Y., Chen, Y., Chen, Y., Lin, X., 2009. Electrochim. Acta, 54(20), 4607-4612.
Yogeswaran, U., Chen, S.-M., 2008. Sens. Actuators B Chem., 130(2), 739-749.
Zachek, M.K., Park, J., Takmakov, P., Wightman, R.M., McCarty, G.S., 2010. Analyst, 135(7), 1556-1563.
Zestos, A.G., Jacobs, C.B., Trikantzopoulos, E., Ross, A.E., Venton, B.J., 2014. Anal. Chem., 86(17), 8568-8575.
Zhang, B., Heien, M.L., Santillo, M.F., Mellander, L., Ewing, A.G., 2010. Anal. Chem., 83(2), 571-577.
Zhang, M., Gong, K., Zhang, H., Mao, L., 2005. Biosens. Bioelectron., 20(7), 1270-1276.
Zhao, X.-E., Suo, Y.-R., 2008. Talanta, 76(3), 690-697.

Zhou, J., Sheng, M., Jiang, X., Wu, G., Gao, F., 2013. Sensors, 13(10), 14029-14040.
Zhu, K.Y., Fu, Q., Leung, K.W., Wong, Z.C., Choi, R.C., Tsim, K.W., 2011. J. Chromatogr. B, 879(11), 737-742.

Figure captions

Figure 1: Pictorial representation of the release of MNTs in synaptic cleft and their association with plausible neurodegenerative and psychotic disorders.

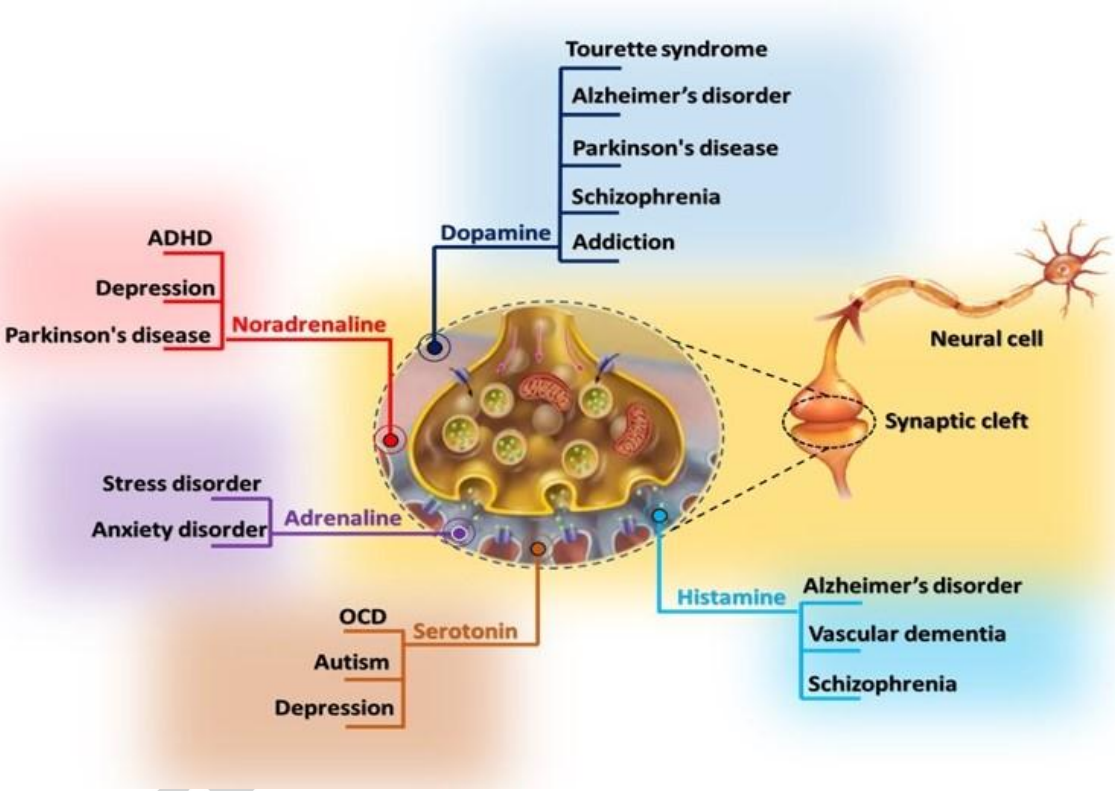


Figure 2: Schematic representation of sensing probe modification by (A) immobilization of MCM–41–NH_2 onto the GCE for detection of DA and 5-HT, adapted from (Hasanzadeh et al., 2013) with permission; (B) using NiO-NPs and MWCNTs within a dihexadecylphosphate film to modify GCE for detection of Ep and DA, adapted from (Figueiredo-Filho et al., 2014) with permission.

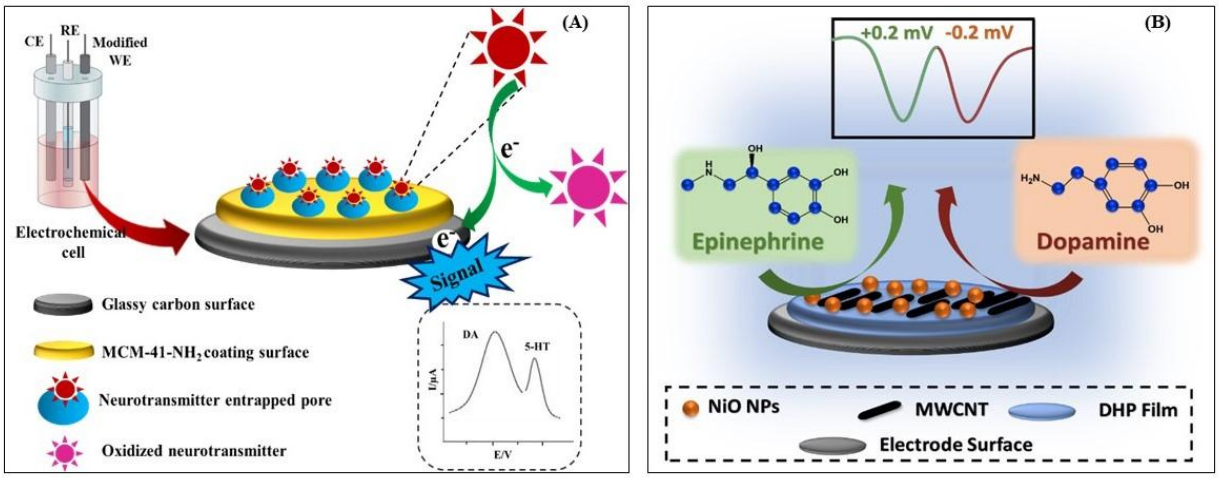


Figure 3: Schematic representation of single-cell exocytosis of neurotransmitters from intracellular synaptic vesicles post administration of stimulatory molecules (K^+, nicotine, etc.) and their electrochemical detection by using various techniques.

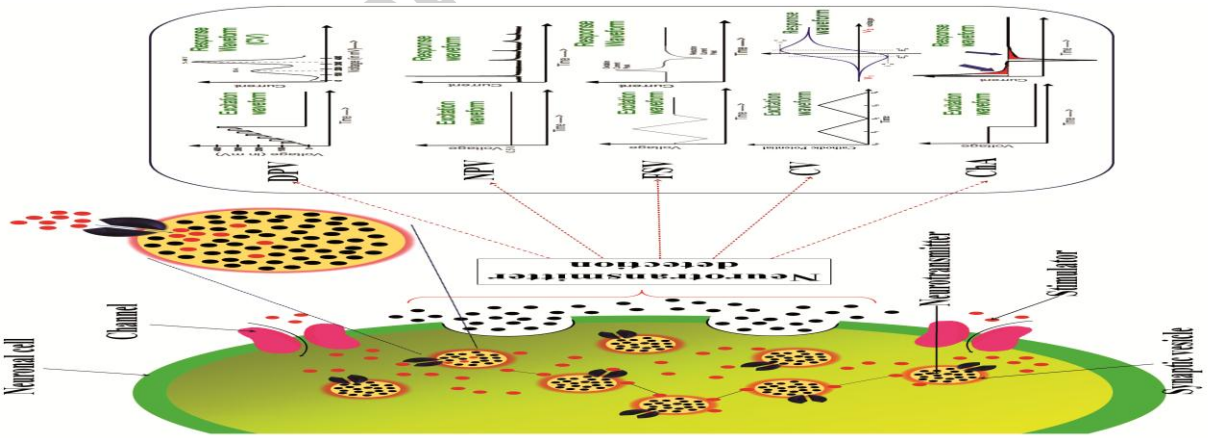


Figure 4: Schematic representation of the diamond-based biosensor fabrication for studying the neuronal activity of GT1-7 cells. Adapted with permission from (Ariano et al., 2009).

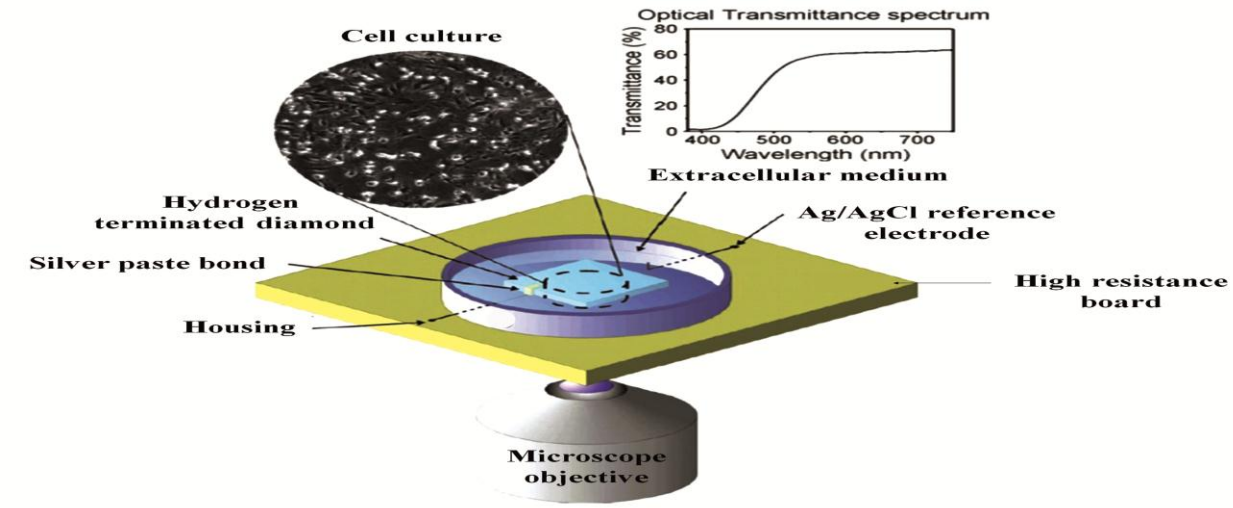


Figure 5: *In vivo* detection setup for analysis of 5-HT and His. Microscopic images of living mouse brain to show placement of (A) stimulation electrode in MFB and (b) CFE in SNR. Reprinted with permission from (Hashemi et al., 2011).

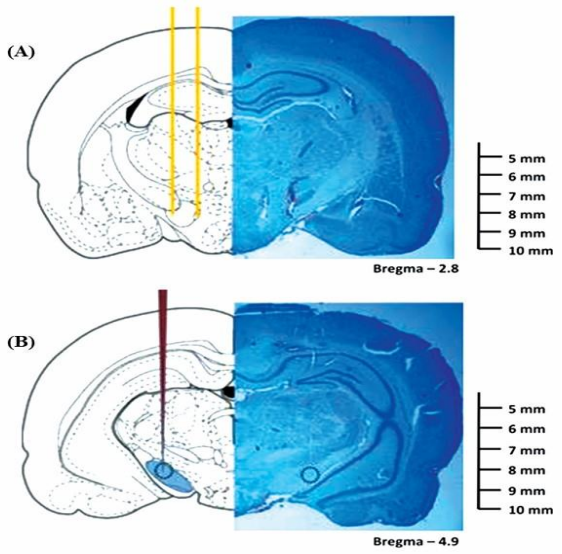
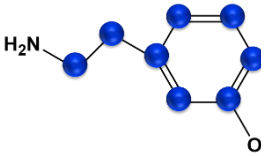
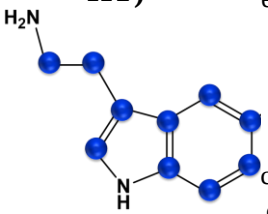
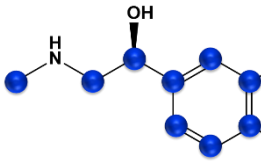
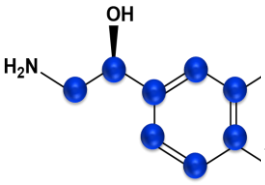


Table captions

Table 1: Role of MNTs, their normal concentration range, and related disorders.

Type of Monoamine neurotransmitter	Biological function	Normal concentration range in biological fluids			Related disorders	Concentration (ng/g of tissue) of MNT in the specific region of human brain	References
Dopamine (DA) 	DA effects a number of executive functions inside brain, like motor coordination, memory, perception, motivation, sexual pleasure, and reward system. It also affects the cardiovascular and renal systems	**Plasma** <0.13 nM (ambulatory adult) <0.065 nM (resting adult) <0.39 nM (age 3 – 15 years)	**Blood** 0.01 - 0.48 nM	**Urine (24h)** 523 – 2472 nM (3 – 8 years) 334 – 3100 nM (9 – 12 years) 332 – 4218 nM (13 – 17 years) 340 – 3139 nM (>17 years)	It is known to play central role in both neurodegenerative (Parkinson's disease) and psychotic disorders (schizophrenia, and addiction)	100 (Caudate) 185 ± 67 (Nucleus accumbens) 16 ± 4 (Substantia nigra) 17± 5 (Hypothalamus) 29 ± 11 (Olfactory area)	(Elhomsy 2014; Mackay et al., 1978)

Serotonin (5-HT) 	5-HT impacts every part of body, from emotion to motor coordination. It not only influences sleep and eating habits but also reduces depression, regulates anxiety, stimulates nausea and maintains bone health	0–22 NPML (this value doesn't change with age, sex, or race of an individual)	600 – 1000 nM	10.50 – 36.60 µmol	Its imbalanced concentration (low level) cause psychotic-pathological disorders like, depression, anxiety disorder, alcohol addiction, eating disorder, autism, psychosis, and obsessive-compulsive disorder	100 (Caudate) 308 ± 72 (Substantia nigra) 322 ± 108 (Pons) 371 ± 86 (Mid-brain) 134 ± 24 (Thalamus) 175 ± 61 (Hypothalamus) 94 ± 8 (Amygdala) 4 ± 28 (Orbital frontal cortex) 14 ± 3 (Convexity frontal cortex) 38 ± 7 (Cingulate gyrus) 27 ± 8 (Sensory cortex) 13 ± 4 (Motor cortex) 16 ± 4 (Parietal cortex) 29 ± 7 (Calcarine cortex) 30 ± 5 (Hippocampal cortex) 15 ± 6 (Temporal cortex) 15 ± 3 (Cerebellar cortex)	(Elhomsy 2014; Mackay et al., 1978)

						12 (Nucleus accumbens) 93 (Olfactory area)	
Epinephrine  **(Ep)**	Ep mediates fight or flight action in emergency conditions. Its influence is basically restricted to metabolic and broncho-dilation effects on organs that are not mediated by direct sympathetic interventions	0.02 - 0.46 nM	0.02 – 0.46 nM	0 – 2.5 µg (<1 year) 0 – 3.5µg (1 year) 0 – 6 µg (2 – 3 years) 0.2 – 10 µg (4 – 9 years) 0.5 – 20 µg (10 – 15 years) 0 – 20 µg (>16 years)	High level of Ep is associated with anxiety and stress disorders	15 – 40 (Aqueductal gray) 35 – 45 (fourth ventricle) Ca. 20 (Reticular formation) 5 (Superior colliculus) 0 – 2 (Pons, substantia nigra, inferior olive and cerebellum)	(Mefford et al., 1977; Pacak and Palkovits 2001)
Norepinephrine (NE) 	NE neuronal system when active influences large areas of brain and effects are manifeste	0.45 – 2.49 nM	0.45 and 2.49 nM	15 – 80 µg	NE system dysfunction leads to Parkinson's disease, ganglia neuroblastoma, depression, and attention deficit	19 ± 10 (Pons) 60 ± 48 (Nucleus accumbens) 15 ± 7 (Mid Brain) 12 ± 8 (Thalamus)	(Faraone et al. 2014; Mackay et al., 1978; Xu et al., 2006)

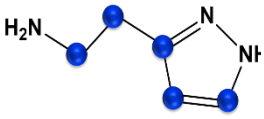
	d in the form of readiness, arousal, and vigilance. It also mediates several vascular activities like increase in heart rate, dilation of blood vessels, and increased blood pressure				hyperactivity disorder	100 (Hypothalamus) 19 ± 10 (Olfactory area) 33 ± 22 (Mammillary body)	
Histamine (His) 	It is responsible for creating vigilance, forgetting memories and learnings, arousal, circadian rhythms, vasodilation, and neuro-endocrine regulation	20 – 694 pg/ml	12 – 105 ng/ml	120 – 150 µg/g creatinine (till 5 years) 70 – 330 µg/g creatinine (6 – 16 years) 30 – 200 µg/g creatinine (>16 years)	Fluctuation in its concentration from optimal level causes Schizophrenia, vascular dementia, and Alzheimer's disorder	Ca. 175 (Frontal cortex) Ca. 115 (Temporal cortex) Ca. 125 (Occipital cortex) Ca. 110 (Nucleus accumbens)	(Fernández-Novoa and Cacabelos 2001; Friedman et al. 1989; Horakova et al. 1977; Mazurkiewicz-Kwilecki and Nsonwah, 1989)

Table 2: Multiplex detection of MNTs in real samples.

Sensing probe fabrication	Analytes	Interferent	Sample	Detection method	Analytical Performance	Reference
2.1 Simultaneous detection of Dopamine and Serotonin						
NiO/CNT/PEDOT composite with a coaxial tubular nanostructure was deposited on a GCE through electrochemical processes. Synergistic effect of NiO, CNT, and PEDOT resulted in three well-separated oxidation peaks.	DA, 5-HT, and Tryptophan	AA, UA, Lysine, Cysteine, Glucose, Guanine, Ca^{2+}, Mg^{2+}, K^{+}, etc.	Human blood serum	CV and DPV	LOD = 0.026 μM (DA), 0.063 μM (5-HT), and 0.210 μM (Tryptophan) LR = 0.03 – 20 μM (DA), 0.3 – 35 μM (5-HT), and 1 – 41 μM (Tryptophan)	(Sun et al., 2018)
A MWNTs-SiO_2-chitosn modified SPE was fabricated. Use of chitosan based composite	DA and 5-HT	AA, UA, CA, Glucose, Ca^{2+}, and Mg^{2+}	Rat cerebrospinal fluid	CV, DPV, and SWV	LOD = 0.2 μM (DA) and 0.01 μM (5-HT) LR = 1 –	(Wang et al., 2016)

efficiently improved electron transfer rate and enhanced the accumulation efficiency.					20 μM (DA) and 0.1-1 μM (5-HT).	
Covalently bonded GO-P was prepared by condensation reaction of GO and P. GO-P suspension coated GCE was electrochemically reduced to give ERGO-P/GCE which showed wide LR with excellent stability and reproducibility.	DA and 5-HT	AA	Synthetic mixture sample	CV and DPV	LOD = 3.5 × 10^{-2} μM (DA) and 4.9 × 10^{-3} μM (5-HT) LR = 0.1 – 500 μM (DA) and 0.1 – 300 μM (5-HT)	(Han et al. 2014)
GCE was modified with CS through a simple casting	DA and 5-HT	AA	Vitamin C injection, DA hydrochlor	DPV	LOD = 2.0 μM (DA), 0.7 μM (5-HT) and	(Zhou et al. 2013)

procedure and resulted in high selectivity, excellent electrochemical activitiy, and wider LR towards target analytes.			ide injection, and synthesized samples		0.6 µM (AA) LR = 20 – 150 µM (DA) and 40 –750 µM (5-HT)	
A highly sensitive AuNP decorated Nafion/CP electrode was developed which resulted in wider LR and enhanced sensitivity.	DA, 5-HT, L-DOPA, and AC	AA and UA	Synthetic mixture sample and human urine	CV and DPV	LOD =1.45 × 10^{-9} M (DA) and 2.84 × 10^{-6} M (5-HT) LR = 2.0×10^{-7} – 2.0×10^{-5} M (L-DOPA) and 5.0×10^{-5} – 3.0×10^{-3} M (DA)	(Atta et al. 2012)
β-CD/poly(N-acetylaniline)/ CNT composite	DA and 5-HT	AA and UA	Bovine serum	CV and DPV	LOD = 0.996 µM (DA) and 0.995 µM	(Abbaspour and Noori 2011)

modified CP Electrode was developed. Synergistic effect of MWCT in addition to the pre-concentrating effect of β-CD resulted in excellent electrocatalytic activity towards analytes.					(5-HT)	
A PEDOT modified Pt electrode was developed in presence of SDS. Presence of SDS in medium played a crucial role in electrostatic attraction of analytes towards the polymeric surface and caused repulsion	DA and 5-HT	AA, UA, and glucose	Human urine (5-HT) and Synthetic mixture sample	CV and LSV	LOD = 48 nM (DA) and 71 nM (5-HT)	(Atta et al. 2011)

towards the interfering molecules.						

2.2 Simultaneous detection of Catecholamines

Electrochemically activated CPE was prepared by successive potential cycling of CP in a 0.1 M NaOH solution. Strong electrocatalytic activity towards target analytes with apparent reduction of overpotentials was observed.	DA, Ep, NE, UA, and AA	NaCl, KCl, $ZnCl_2$, $MgCO_3$, Glucose, Sucrose, and Urea	Synthetic mixture sample, dopamine injection, human urine and vitamin C tablets	DPV	LOD = 0.08 μM (DA), 0.08 μM (Ep), 0.07 μM (NE), 0.1 μM (UA), and 6.0 μM (AA)	(Chitravathi and Munichandraiah 2015)
A hybrid material of mesoporous silica (SiO_2) and GO was obtained by sol–gel process and later decorated with	DA and Ep	AA and UA	Synthetic mixture sample and human urine sample	SWV	LOD = 0.26 μM (DA) and 0.27 μM (Ep)	(Cincotto et al. 2014)

AgNPs. This composite material was used to modify GCE which allowed target analysis with high sensitivity.						
MWNT modified EPPGE was developed to simultaneously detect Ep and NE. Modified electrode reaction followed diffusion a controlled pathway.	Ep and NE	UA, AA, and DA	Human blood plasma, and urine sample of smokers	SWV	LOD = 0.15×10^{-9} M (Ep) and 0.90×10^{-10} M (NE) LR = 0.5–100 nM (Ep and NE).	(Goyal and Bishnoi 2011)
Electrodeposition of Pd_{nano} on PMPy film-coated Pt electrode was carried out and the electroactivity of modified electrode was found to be dependent on	DA, Ep, NE and L-DOPA	AA and UA	Human Urine and Serum	CV and DPV	LOD = 12 nM (DA), 7000 nM (AA), and 27 nM (UA)	(Atta et al. 2010)

the electrosynthesis conditions of the PMPy film and Pd_{nano}.						

2.3 Simultaneous detection of Norepinephrine and Serotonin

MWNTs-ZnO suspension solution was casted on SPE and dried for 30 minutes. Later chitosan was coated to give away working MWNTs-ZnO/chitosan SPE. Modified electrode was stable up to 3 months and allowed detection of analytes with excellent selectivity and sensitivity.	NE and 5-HT	Glucose, Ca^{2+}, Mg^{2+}, and CA	Rat cerebrospinal fluid	SWV	LOD = 0.2 μM (NE) and 0.01 μM (5-HT) LR = 0.5 – 30 μM (NE) and 0.05 – 1 μM (5-HT)	(Wang et al. 2015)
A nanocomposite consisting of	NE and 5-HT	UA and AA	Human serum	CV and DPV	LOD = 49 nM (NE) and	(Mazloum-Ardakani and

benzofuran derivative-functionalized MWNTs and ionic liquid was developed for modifying GCE. Modified sensing probe showed excellent electrocatalytic activities toward the oxidation of target analytes.					2 μM (5-HT) LR = 0.1 – 30 μM and 30 – 1000 μM (NE) and 5 – 900 μM (5-HT).	Khoshroo, 2014)
GCE was modified by electrodeposition of ECR and exhibited excellent electrocatalytic activity towards analytes. Modified sensing probe allowed simultaneous detection of analytes at physiological	NE and 5-HT	K^+, Zn^{2+}, Ca^{2+}, NO^{3-}, Cl^-, SO_4^{2-}, sodium citrate, starch, lactose or gelatin	Complex sample	CV and DPV	LOD = 0.05 μM (5HT) and 1.5 μM (NE) LR = 2 – 50 μM (NE) and 0.05 – 5 μM (5-HT)	(Yao et al., 2009)

pH.				

Table 3: *In vitro* electrochemical detection of MNTs.

Sensing probe fabrication	Cell lines	Stimulatory solution	Analytical Performance	References
3.1 Detection of Catecholamine				
Single-crystal diamond substrate embedded microstructured graphitic 4 × 4 multielectrode array	Bovine chromaffin cells	KCl solution	LOD = 0.37 µM	(Picollo et al. 2016)
ITO, transparent glass electrodes and DLC electrode on ITO substrate fabricated by selected area photolithography	Bovine adrenal chromaffin cells	KCl, NaCl, $MgCl_2$, $CaCl_2$, and glucose in HEPES/KOH buffer	NR	(Kisler et al. 2012)
MEAs fabricated using microfabrication procedure using optical lithography followed by dry etching	Mouse chromaffin cells	NaCl, $MgCl_2$, HEPES/NaOH, glucose, KCl, and $CaCl_2$	NR	(Carabelli et al. 2010)
Microfluidic channels using photolithography and patterned etching	Bovine adrenal chromaffin cells	NaCl, KCl, $CaCl_2$, $MgCl_2$, glucose, and HEPES/NaOH (pH 7.2)	NR	(Gao et al. 2009)
CFME	MN9D cells	High K^+ ion containing	NR	(Dong et al. 2008)

		solution		

3.2 Detection of Dopamine

IC devices based on CMOS sensor array fabricated at MOSIS through the ON Semiconductor C5F/N process	Bovine adrenal chromaffin cells	KCl, NaCl, $MgCl_2$, $CaCl_2$, and glucose in HEPES/NaOH buffer	LOD = ~7000 molecules LR = 0.0033 – 9.48 pC Detection time = 10 ms	(Huang et al. 2017)
Fluorescent nanosensor array based on SWNTs	PC12 neuroprogenitor cells	High K^+ ion containing solution	NR	(Kruss et al. 2017)
Membrane based sensor	PC 12 cells	NR	LOD = 2.71 ± 0.16 nM LR = 3.1 pM – 17 µM	(Alatraktchi et al. 2014)
CMOS IC incorporating a 10×10 array of potentiostats fabricated at MOSIS through the ON Semiconductor C5F/N process	Bovine adrenal chromaffin cells	KCl, NaCl, $MgCl_2$, $CaCl_2$, and glucose in HEPES/NaOH buffer	LOD = 0.35 µM LR = 3.5 – 105 µM Sensitivity = 1.20 pA/µM	(Kim et al. 2013)
CMOS chips using photolithography after processing using Pt and Al	Bovine adrenal chromaffin cells	Calcium ionophore ionomycin	NR	(Ayers et al. 2010)
CFMEA	PC 12 cells	High K^+ ion containing	NR	(Zhang et al. 2010)

solution				

Table 4: *In vivo* and *ex vivo* electrochemical detection of MNTs.

Sensing probe fabrication	System	Detection method	Analytical Performance	Reference
(A) Detection of MNTs in the *in vivo* model				
4.1 Detection of Dopamine				
Electrodeposition of PEDOT and GO onto the CFE surface results in sensing probe with increased sensitivity and lower LOD. Increase in sensitivity was attributed to increase in electrode surface area due to PEDOT/GO coating and improved adsorption of DA's oxidation product (DA-o-quinone).	Dorsal striatum	FSCV	LOD = < 0.1 µM LR = 0.5 – 10 µM	(Taylor et al. 2017)
Carbon nanotube yarn microelectrodes were treated with laser to improve the sensitivity of electrode by retaining its temoral resolution. Laser treatment increases the surface area and oxygen containing functional	Ventral tegmental area and Nucleus accumbens	FSCV	LOD =13 ± 2 nM	(Yang et al. 2016a)

groups on the electrode surface, which provides more adsorption sites for DA and hence enhanced sensitivity.				
Nafion and PEDOT containing composite polymer was electropolymerized on CFME with goal of creating mechanically stable, robust, and controllable electrode coating that increases the selectivity and sensitivity of *in vivo* electrochemical measurements. Composite coating was deposited on CFME by applying a triangle waveform from +1.5 V to −0.8 V and back in a dilute solution of EDOT and Nafion in acetonitrile	Prefrontal cortex and Nucleus accumbens	FSCV	LOD = 4 ± 1 nM (PEDOT:Nafion, 200 μM EDOT) LOD = 6 ± 1 nM (PEDOT:Nafion, 400 μM EDOT)	(Vreeland et al., 2015)
FSCV compatible CFME arrays were microfabriated. Microfabrication	Caudate putamen	FSCV	NR	(Zachek et al. 2010)

technique allowed better reproducibility and ease of batch fabrication of electrode arrays.				

4.2 Detection of Norepinephrine and Dopamine

Glass-encased CF and Ag/AgCl reference electrodes were prepared as previously described. T-650 untreated carbon fibers with 7 μm in nominal diameter were used and cut to an exposed length of 80−100 μm	Rostral and caudal Nucleus accumbens shell	FSCV	NR	(Park et al., 2017)
Glass-sealed CFME with exposed tip length of 75−100 μm and diameter 7 μm was used. A triangular scan of −0.4 to +1.3 V at 400V/s was applied to the electrode.	Nucleus accumbens and Ventral bed nucleus of the stria terminalis	FSCV	NR	(Park et al., 2015)
Glass-encased, cylindrical CFME and Ag/AgCl reference electrodes were prepared.	Nucleus accumbens and ventral bed nucleus of the stria terminalis	FSCV	NR	(Park et al., 2011)

T-650 carbon fibers with an exposed length of 75–100 μm were used.				
Glass-encased, cylindrical CFME and Ag/AgCl reference electrodes were prepared. T-650 carbon fibers with an exposed length of 75–100 μm were used.	Nucleus accumbens shell	FSCV	NR	(Park et al. 2010)

4.3 Detection of Dopamine, Norepinephrine and Serotonin

NR	*in vivo* microdialysis samples	UHPLC-ECD	NR	(Van Schoors et al. 2016)
NR	Brain Microdialysis samples	UHPLC-ECD	LOD = 58 pM (DA), 83 pM (NE), and 60 pM (5-HT) LR = 100 – 1500 pM	(Van Schoors et al. 2015)
NR	Brain microdialysis sample	Capillary UHPLC-ECD	LOD = 0.75 fmol (NE), 0.75 fmol (DA), and 1.5 fmol (5-HT) LR = 5×10^{-10} – 3×10^{-8} mol/L	(Ferry et al. 2014)
NR	Hippocampal	UHPLC-ECD	LOD = 16 fmol	(Parrot et

	microdialysates		(NE), 16 fmol (DA), and 20 fmol (5-HT) LR = 10^{-9} – 10^{-6} mol/L	al. 2011)
(B) Detection of MNTs in *ex vivo* model				
NR	Caudate putamen slices	Capillary electrophoresis with FSCV	LOD = 5 ± 3 nM (DA), 10 ± 3 nM (5-HT), and 50 ± 20 nM (Ad)	(Fang et al., 2013)
Planar CNT-MEA chips were fabricated by electroplating the ITO microelectrode surfaces to measure release of DA and electrophysiological response.	Striatal and hippocampal slices	ChA	LOD = 1 nM LR = 1 nM – 100 µM	(Suzuki et al., 2013)
CFME	Striatum slices	FSCV	NR	(Maina et al., 2012)

Highlights

- Fundamentals of MNTs and their involvement in various clinical conditions are discussed.
- Detection of MNTs in clinically relevant matrices and models such as; *in vitro, in vivo,* and *ex vivo* are included.
- Emphasis on challenges faced with electrochemical sensing techniques and shortcomings in current research works are also discussed.