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An Update of the Classical and Novel Methods Used for Measuring Fast Neurotransmitters During Normal and Brain Altered Function

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Abstract: To understand better the cerebral functions, several methods have been developed to study the brain activity, they could be related with morphological, electrophysiological, molecular and neurochemical techniques. Monitoring neurotransmitter concentration is a key role to know better how the brain works during normal or pathological conditions, as well as for studying the changes in neurotransmitter concentration with the use of several drugs that could affect or reestablish the normal brain activity. Immediate response of the brain to environmental conditions is related with the release of the fast acting neurotransmission by glutamate (Glu), γ-aminobutyric acid (GABA) and acetylcholine (ACh) through the opening of ligand-operated ion channels. Neurotransmitter release is mainly determined by the classical microdialysis technique, this is generally coupled to high performance liquid chromatography (HPLC). Detection of neurotransmitters can be done by fluorescence, optical density, electrochemistry or other detection systems more sophisticated. Although the microdialysis method is the golden technique to monitor the brain neurotransmitters, it has a poor temporal resolution. Recently, with the use of biosensor the drawback of temporal resolution has been improved considerably, however other inconveniences have merged, such as stability, reproducibility and the lack of reliable biosensors mainly for GABA. The aim of this review is to show the important advances in the different ways to measure neurotransmitter concentrations; both with the use of classic techniques as well as with the novel methods and alternant approaches to improve the temporal resolution.

Keywords: Acetylcholine, GABA, Glutamate, measurement.

INTRODUCTION

Several methods have been used to study the brain functions, these consider several aspects: like behavior, morphology, as well as electrophysiological and biochemical activity. It is important to study the brain with reliable methods and techniques, to understand better the brain activity during normal and pathological conditions. The results of these studies are important to design new strategies, drug treatments and to know the basic mechanisms of drug action. Some techniques to study the brain can be carried out in real time, and with precise spatial resolution, like electroencephalography (EEG), magnetic resonance imaging (MRI), proton nuclear magnetic resonance and biosensors, which could be used to study behavioral and electro-physiological activity. However, the biochemical studies possess low temporal resolution due to the time needed for the sample collection from brain tissue, cerebral spinal fluid or microdyalisates.

The brain microdialysis technique is the preferred method to monitor several neurotransmitters and changes in these during brain activity, which can be related to a particular behavior or brain-altered function. This procedure has gotten a big impact in biochemical studies since Delgado *et al*., introduced it in 1972 [1]. This technique is usually coupled to HPLC methods. The type of neurotransmitter to be studied determines the separation method and the detection system that could include: optical density, fluorescence, electrochemical, luminescence, and mass spectroscopy (MS). Despite the fact microdialysis procedure can be considered as a golden technique to monitor neurotransmitters in brain, it has some disadvantages, like the poor temporal resolution which depends on the speed of sample collection (generally, it is carried out at 2 μl/min) and the minimum volume of sample required for the HPLC analysis is about 5-15 min of time collection. Another disadvantage is the insertion of the probe itself that produces a local injury. With long periods of monitoring, a glial scar could emerge as a consequence of damage, resulting in a poor recovery or underestimation of the neurotransmitter to analyze. With recent advances in HPLC systems, bigger sensitivity can be reached, the volume needed has been reduced considerably and the temporal resolution has been improved using variants of a chromato-graphic method like capillary electrophoresis (CE) [2]. However, handling small volumes could be an important issue, since a great number of samples need to be processed individually before the separation procedure [3]. Another disadvantage is the time needed for the separation and the analysis process, besides it is important to determine the

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dead time from the tip of a microdialysis probe to the collection zone if a good correlation between behavior and EEG activity is attempted, since these events occur in real time [4]. The use of biosensors has provided a useful alternative to avoid the separation process and study the rapid events that can be correlated with a particular neurophysiological activity. These are mainly electrochemical, using oxidases which produce hydrogen peroxide (H_2O_2). Biosensors have allowed the monitoring of changes in Glu and ACh with good temporal and spatial resolution, allowing behavior and EEG activity to be studied simultaneously [5-7]. The use of biosensors opens the possibility to obtain sufficient temporal resolution to correlate changes in a particular neurotransmitter with a specific behavior or EEG activity. At the beginning they could only monitor a single neurotransmitter at once and in the case of ACh they do not distinguish choline (Ch) from ACh. Although the use of multiplexed electrodes can detect more than one neurotransmitter, using different enzymatic coatings [8]. Moreover, additional biosensors for other important neurotransmitters, like GABA are being developed. Nowadays, biosensors are available in a very small size and they have incorporated more advanced technologies. An example for a nanometrical scale biosensors are the carbon fiber (CF) biosensors, CF is a 7 μm graphite monofilament. CF has several mechanical and electrical properties and is an excellent electrode for electrophysiological and electrochemical measurements and has been used since 1979. It has been used for electrochemical detection of catecholamines, ACh, Ch, glucose and Glu [9]. A recent alternative to monitor neurotransmitters with high temporal resolution involves biosensors to design enzymatic reactions to generate a "derivate" from a neurotransmitter as a substrate that could be measured by luminesce [10, 11], electrochemistry [5] and fluorescence [12]. The use of enzymatic reactors avoids the drawback of fixed enzymes in a biosensor, giving a more active enzyme and a better control of the enzymatic reactions as well as the amount of substrate, cofactors and other conditions like pH and temperature. In the light of the above, the objective of this review is to show the recent advances in the classical and new methods to measure neurotransmitter during normal or pathological conditions and their relationship with the use of different pharmacological tools.

Glu Measurement

Glu is the main excitatory neurotransmitter in the central nervous system (CNS) and an important energy and nitrogen source in eukaryotic and mammalian cells [13]. It also plays an important role in several brain functions like learning and memory [14]. It has been found that long lasting increases in extracellular Glu concentrations have excitotoxic properties [15]. Glu alterations have been related with essentially all forms of behavior, including consciousness, sensory perception, motor control, mood [16], seizures [17] and processes like cognitive, formation of memory, learning, biomaterial sensitivity, and synaptic plasticity of the CNS [13, 14, 18]. Furthermore, alterations in Glu levels have been shown to be linked to several neurodegenerative disorders such as Alzheimer's [19], Parkinson's [20], Huntington's disease [21], epilepsy [22], amyotrophic lateral sclerosis [23], mood disorders [24], ischemic stroke [25] and schizophrenia [26]. Studies about the role of Glu and other neurotransmitters could explain the onset and progression of several neurological disorders [8]. Thus measuring this neurotransmitter *in vitro* and *in vivo* is fundamental to understand in a better way its role in normal as well as in pathological conditions. The methods used more frequently are chromatographic, coupled to the following detection systems; spectrophotometric [27], fluorometric [28, 29] and electrochemical [30]. Despite the fact of the existence of several techniques there is a necessity for a reliable method to monitor this neurotransmitter with real time resolution, which could help to design better-personalized medicine, biomedical research and its biotechnological applications [31].

Microdialysis is a useful method to measure Glu in the extracellular space *in vivo* conditions. Samples collected are mainly evaluated by HPLC [32, 33]. This method offers some advantages, like the sample collection that can be carried out in freely moving or anesthetized animals and, information of the compound of interest and its metabolites can be obtained simultaneously. Also the microdialysis probe can be used to administer testing drugs locally to explore their basic mechanisms of action related with a particular neurotransmitter. Nevertheless, there are some important disadvantages, such as the speed at which the information is obtained from microdialysis procedure, as it is considerably slower in relation to other dynamic processes in brain and is carried out between 5-15 min [15]; although some attempts have been done to improve the time resolution [3, 34]. Also the size of the probe can produce an important tissue insult and glial scar; which can be seen 1.4 mm apart from the implanted zone and the data collected could represent pathological conditions instead physiological [35]. Despite of these disadvantages, Glu measurement with this technique has been widely used, and Glu concentrations have been determined in the range of 1.0 to 5.0 μ M in different brain regions without considering probe recovery [36-38]. Chemical stimulation by some drugs administrated with reverse microdialysis induces an increase in extracellular Glu, like 4-aminopyridine (4-AP), which blocks potassium channels, increasing its time aperture [17]. Infusion of the inhibitor of the high affinity Glu transporter, DL-treo-β-benzyloxyaspartate (DL-TBOA), produces an increase of 300% in Glu in nucleus accumbens of young rats [39]. The dialysates were analyzed by HPLC with fluorimetric detection. Also, the local administration of the NMDA and GABA antagonist in the frontal cerebral cortex induced an increase in Glu concentration by 278% with respect to baseline concentrations detected by HPLC in neostriatum [40]. Increases in Glu by electrical stimulation, have also been detected in dialysates, these activate glutamatergic pathways when the nerve endings are stimulated in bone marrow, sciatic nerve and raphe magnus nucleus [41, 42]. Also, the application of electric pulses for 2 minutes increased Glu concentrations from 200 to 300% in samples for 30 second in the nucleus accumbens, returning to baseline within 60 second by the administration of metabotropic agonist trans-1-aminocyclopentane-1,3 dicarboxylate (ACPD) [43]. Certain types of stimulations may affect behavioral changes that reflect Glu increases. In a study of moderate stress a sustained increase in Glu concentrations of 200 to

450% above baseline in 10 to 20 min measured in prefrontal cortex, hippocampus, nucleus accumbens and striatum was reported [44].

The use of biosensors has attracted the attention of many researchers to measure several compounds and now is a popular technique widely used; this due to their simplicity and the relative high sensitivity. Electrochemical biosensors are built with high technology in order to reduce their size and to avoid tissue damage. Several strategies for enzyme immobilization over the electrodes have appeared and used [45]. Although, there are still several obstacles to be solved like the stability, reproducibility, response and a high potential demanded over the working electrode. Most of the biosensors designed to measure Glu *in vivo* are based on the use of glutamate oxidase (GluOx) to generate H_2O_2, which is electrochemically detectable [46]. GluOx is an enzyme that catalyzes the oxidative deamination of Glu in the presence of molecular oxygen (O_2) to produce α ketoglutarate, ammonia (NH_3) and H_2O_2 [47]. Other electroactive species that could interfere with these sensors can be excluded by the use of polymers with selective permeability, giving a more specific signal [48]. Different approaches related with the use of biosensors based on electrochemical sensing of Glu have been developed, like immobilization of GluOx with carbon nanotubes (CNTs) and polymers over platinum (Pt). These have been used to build biosensors with improved response (7 second) and limit of detection ~0.3 μM [49]. Electrochemical microsensors were used to evaluate the extracellular Glu concentration *in vivo* after blocking the Glu transporters EAAT-3 and EAAC1, these studies showed that this method is able to detect discrete changes in this neurotransmitter [31].

Electrochemical methods using microelectrodes allow direct real-time assessment of physiological levels of Glu *in vitro* and *in vivo* with high spatial resolution [50]. These have led to the development of different biosensors that couple microelectrodes with cerium oxide and titanium nanoparticles deposited on a surface of platinum microelectrodes and GluOx, which catalyzes Glu, generating α-ketoglutarate and H_2O_2, which is electrochemically active. The LOD was 0.493 μM with a sensitivity of 793 pA / mM, with 2 and 5 s of time resolution [46]. Also with a flexible microsensor, based on a polymer substrate, having an array of microelectrodes allows online monitoring in specific regions. Pt microelectrodes with electrochemical biosensor for Glu allow detection with high sensitivity of 2.16 nA/mm2/μM [31]. The advantage of ultra microelectrodes, nanoelectrodes or smaller electrodes is the benefit obtained from the enhanced mass transport which takes place. As electrodes decrease in size, radial (3-dimensional) diffusion becomes dominant and results in faster mass transport. This high rate of mass transport (diffusion) at small electrodes enables measurement of kinetics by steady-state experiments rather than by transient techniques [51].

Recently Glu electrochemical detection was improved by the development of nano graphene plates. Graphene exhibits excellent electron transfer promoting ability for some enzymes and excellent catalytic behavior toward small biomolecules such as H_2O_2 and NADH, which makes graphene extremely attractive for enzyme-based biosensors [52]. The incorporation of Pt nanoparticles catalyzes the electrooxidation of H_2O_2 to oxidize Glu by GluOx on a layer of poly-phenylenediamine and showed a greater selectivity, displaying a competitive performance [53]. Graphene quantum dots have been successfully used as a fluorescence chemical sensor, concentrations of Glu at a nanoscale have been efficiently detected in Tris-HCl (pH = 9) buffer solution at a wavelength of 430 nm. The fluorescence intensity of the quantum dot gradually improves with increase in the concentration of Glu and any change in fluorescence intensity is directly proportional to the concentration of Glu. Under optimal conditions, the linear range for detection of glutamate was 0.16 μM to 10 μM with a detection limit of 52 nM. The sensor showed high selectivity for Glu compared with other amino acids [54].

GABA Measurements

GABA has been established as the main inhibitory neurotransmitter in the CNS [55]. GABA is homogenous and widely distributed across the CNS and it has an important role in brain physiology. Precise and accurate measurement of GABA in biological fluids is important to the study of neuropharmacology, since changes in its concentration are of relevance in many neurological pathologies such as; epilepsy, seizures, schizophrenia, anxiety and bipolar disorders [56-61]. Also the GABAergic system is one of the principle targets of general anesthetics [62]. Several methods of analysis have been developed based on the available instruments, the sensitivity required and the species or tissues to be analyzed. The first methods used were paper [63] and column chromatography [64-67]. The presence of GABA *in vivo* can be determined by three ways. The first requires a sample collection carried out through microdialysis procedure [68], push-pull [69] or low-flow push-pull [70]. The second option consists of *in vivo* and *in situ* quantification *via* microsensor or microelectrodes implantation; and the third option are non-invasive imaging techniques *in vivo*, which allows the detection of metabolites in human [71, 72], like the functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) [73].

The direct measurement of GABA in the brain, faces several significant challenges since is neither fluorescent nor electroactive and it is difficult to detect by enzymatic reactions because not an oxidase nor hydrogenase have been found. GABA detection *via* fluorescence and electrochemical methods requires derivatization using different reagents with some fluorogenic compounds [65, 74-76]. However, some derivatizant reagent can cause interference in the chromatography. Another challenge for the direct measurement of GABA is the few reports on GABA biosensors. This is probably due to poor sensitivity and an insufficient detection limit for determining GABA in physiological fluids [77].

The most common technique to measure GABA in microdialysates is HPLC coupled with fluorescence [65, 78-81], electrochemical detection [77], MS [82] and liquid chromatography/tandem MS [83, 84]. Nonetheless, it is necessary a prolonged collection time (20 min) to obtain

enough sample to be measured, resulting in poor temporal resolution [76, 85]. GABA measurements in dialysates require ultrasensitive methods, since it is present in very low concentration (5-50 nM) in normal physiological conditions [78]. Since GABA lacks of fluorescent or electroactive properties, it has to be derivatized to generate a compound that has these characteristics. Several reagents have been tested with this purpose, including: 1-fluoro-2,4-dinitrophenyl-5-alaninamide (FDA) [86], o-phthalaldehyde (OPA) [64, 65, 74, 87], naphthalene-2 3-dicarboxaldehyde (NDA) [67], phenylisothiocyanate (PITC) [88], 9-fluorenylmethyl chloroformate (FMOC), 5-dimethylamino-1 naphthalene-sulfonyl-chloride (DANSYL-Cl) and 4-(Dimethylamino)azobenzene-4´-sulfonyl chloride (DABSYL-Cl) [89]. These processes have a good limit of detection, in the range of 0.03 pmol for electrochemical detection [90] and 0.5 to 1 pmol for fluorescence and have been improved to 100 fmol with microbore columns [78], besides it has excellent selectivity and recovery [91]. Nevertheless electrochemical detection with OPA affects the working electrode and reduces considerably its lifespan and increases the background noise [78]. However, the sensitivity of these methods would be insufficient to quantify GABA *in vivo* microdialysates. After obtaining samples, a prepurification process is not required; for this reason there is no risk of enzymatic degradation. Disadvantages of microdialysis technique should be considered for its applicability, since a stressing stereotaxic surgery is necessary and, as mentioned before, glial scar could result as a consequence of damage. Besides, GABA release is not sensitive to tetrodotoxin (TTX) and calcium [15] although it responds to several pharmacological and behavior stimulation. In this way, the GABA content in microdialysates could give information about the neurotransmission mediated by GABA [92]. In different way, the GABA contained in microdialysates is not related with neuronal activity and has been considered as a non-synaptic signaling molecule [93].

The different HPLC techniques previously mentioned have been used to measure the changes in the concentration of GABA on diverse physical and drug stimuli: the fluctuations in GABA concentration due to high potassium infusion has been measured in microdialysates samples through a HPLC system coupled with fluorescence [94] or MS detection [95]. Also, HPLC-fluorescence detection has been used to examine the effect of lithium, valproic acid [96], sevoflurane [80], lamotrigine [97], and kinurenic acid [98] on GABA concentration, while the effect of menin protein on extracellular GABA concentration was analyzed by electrochemical detection [99].

Capillary Electrophoresis

GABA and other amino acid neurotransmitters are generally analyzed by HPLC and fluorescence or electrochemical detection and, as it was mentioned above, these methods require large volume of sample. CE does not require such volumes [100], instead, low volumes are necessary and the temporal resolution is highly improved [2], especially when the fluorescence is induced with a laser beam. The technique is named capillary electrophoresis-laser induced fluorescence detection (CE-LIFD). For precolumn derivatization, NDA [101] and 4-cloro-7-nitro-2,1,3-benzoxadiazol (NBD-Cl) are commonly used [102]. Additionally, several derivatizing compounds have been tried to get reliable signal detection of GABA and other amino acids, such as fluorescein isotiocyanate (FITC), 5-carboxy- fluorescein succinimidil ester, 6-oxy-(N-succinimidil acetate)-9-(2-methoxycarbonyl) fluorescein, 3-(2-furoyl)-quinoline-2-carboxaldehyde, and 3-(4-carboxy benzoyl)-2-quinoline carboxaldehyde [103]. Some advantages of CE is the low volume required, also other neurotransmitters besides GABA can be measured simultaneously, like Glu, aspartic acid and some drugs like vigabatrin [101] and low limit of detection (LOD) (0.016 μM) has been achieved. A particular disadvantage with respect to GABA analysis by CE is the temperature used for derivatization; in some cases 50^{o}C is necessary; which implies and additional treatment process for samples, leading to a poor temporal resolution [102]. CE-LIFD now is a reliable technique, absolutely validated bringing new data to the interaction of GABA with several drugs [101].

GABA Sensor

Neurotransmitter sensors have been used to pursuit a better spatial and temporal resolution, which in some experiments is 0.1 second with spatial resolution of some microns [104-107].

The sensing of GABA by acoustic means [108] and immune [109], amperometric [110], and fluorescent [111] assays has been used in recent years.

Immunosensing technique for acoustic wave based on mass changes caused by surface binding reactions, is a tool for real-time measurement. Compared to other similar standard methods, like ELISA and radioimmunoassay, mass-sensitive sensor does not require labeling of antigens or antibodies. Another technique of free sensing is surface plasmon resonance (SPR) [112]. It is sensitive to variations in the refractive index at the sensing surface [113, 114]. However, a disadvantage of using this method is that sensors based on optical fibers and SPR require relatively expensive optical analysis system. Furthermore acoustic sensors have several attractive advantages, such as simplicity, convenience, real-time responses and the ability to provide multiple interface information between the medium and the sensor. The sensitivity achieved with this methodology allows sensing GABA concentrations up to 38 μM [108], which is a value above the physiological range and this is of interest for many pharmacological aspects.

The amperometric sensing is based on the catalytic activity of the enzymes GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSDH) known as GABAse; and horseradish peroxidase (HRP) [115, 116]. The product of the GABAse (reaction 1 and 2) is nicotinamide adenine dinucleotide phosphate (NADPH), which is then oxidized by the HRP (3) [117, 118]. Enzymatic reactions can be used separately or together. Therefore, the analytic signal is always given by the measurement of oxygen consumption in reaction 3; it allows the measurement of NADPH formed (in reaction 2), and then, the concentration of GABA in reaction 1.

$$\text{GABA} + \alpha\text{-ketoglutarate} \underset{\leftarrow}{\overset{\rightarrow}{\text{GABA-T}}} \text{succinic semialdehyde} + \text{glutamate} \quad (1)$$

$$\text{Succinic semialdehyde} + \text{NADP} \underset{\leftarrow}{\overset{\rightarrow}{\text{SSDH}}} \text{NADPH} + \text{Succinate} \quad (2)$$

$$2\text{NADPH} + \text{O}_2 + 2\text{H HRP2} \underset{\leftarrow}{\overset{\rightarrow}{\text{H}_2\text{0}}} + 2\text{NADP} \quad (3)$$

The steady state conditions for the concentration of GABA in the CNS are determined by a continuous balance between GABA, formed by decarboxylation of glutamic acid, catalyzed by the enzyme glutamate decarboxylase (GAD) and GABA interaction with α -ketoglutarate to form glutamic acid together with succinic semialdehyde again. Therefore, the simultaneous measurement of both GABA and glutamic acid could be a valuable tool for both assessment of various pathologies that occur in the CNS, and new active pharmaceutical agents in GABAse and on GABA receptors [110].

The immunosensing known as quartz crystal microbalance (QCM) involves sensitive quartz chemical mass transducers widely used as detection devices for studying the affinity of antibodies and antigen interactions fluid-solid interface [119]. Such measurements are simple and easy to use; and require no potentially hazardous materials [120]. These also provide a direct measurement of the reactions of the antigen-antibody interaction at the sensor surface; which is represented by a change in the mass of glass due to the formation of an immune complex on the surface. This produces a proportional change in the resonant frequency according to the Sauerbrey equation [121]. When operating in a liquid environment, the response of the quartz resonator and the viscosity also depends on the density of the liquid. The purpose of this sensor is to detect GABA without the need for an enzyme or a fluorescent marker. For sensing low molecular weight antigens as GABA with a mass sensitive approach direct binding does not produce a sufficient frequency to be detected. Therefore GABA is fixed on a QCM gold surface *via* a layer of the polysaccharide Dextran. A predetermined amount of anti-GABA is mixed with free GABA and GABA immobilized on the sensor surface. GABA molecules bound and free, compete for binding sites on the antibodies. The molecular interactions of GABA molecules immobilized on the surface with anti-GABA antibody can be detected by the change in the resonance frequency corresponding to the amount of antibody bound to the immobilized GABA sensor surface. The decrease in resonant frequency of the sensor is then inversely related to free GABA concentration in the sample [109].

The fluorescent sensing is another alternative available to quantify GABA. For this purpose the GABAse reactions (1) and (2) are used, which produces NADPH. The NADPH is a fluorescent molecule when it is excited upon it with a laser at a wavelength of 340 nm emits fluorescence at 450 nm [122], this fact allows for indirect quantification of the concentration of GABA as production of NADPH, it is stoichiometric and reflects GABA levels in the sample [123]. The sensitivity of this method has allowed the quantification of GABA in the range 500 μM to 10 mM [122-124].

Additionally, NADPH can be used as a donor molecule and can be oxidized through a dehydrogenase like a diaphorase, also resazurin is reduced to resorufin. In this case, the excitation is produced at 544 nm and the fluorescence detected at 590 nm, the increased sensitivity of detection is about 8 times compared with the GABAse assay [122].

Other techniques that are being developed are GABAse luminescent techniques, based on the Photobacterium luciferase enzyme. Because NADPH is the product of the GABAse reaction, it is possible to shoot a luminescent reaction with Photobacterium luciferase [125]. The reaction involves the oxidoreductase enzyme (4) and the luciferase itself (5). The light emission produced is proportional to the concentration of GABA [126]. The sensitivity of this technique depends on the purity of the enzymes involved, and can detect at micromolar levels of GABA. Using a purified enzyme a nanomolar range of NADPH can be detected as referred by NOVOCIB SAS (Lyon, France), nevertheless there is not published data quantifying GABA at this level.

$$\text{H}^+ + \text{NADH} + \text{FMN} \xrightarrow{\text{oxidoreductase}} \text{NAD}^+ + \text{FMNH}_2 \quad (4)$$

$$\text{FMNH}_2 + \text{aliphatic aldehyde} + \text{O}_2 \xrightarrow{\text{Luciferase}} \text{FMN} + \text{Decanoic acid} + \text{light emission} \quad (5)$$

Magnetic Resonance Spectroscopy

Another method widely used for GABA determination is magnetic resonance spectroscopy (MRS), which allows the detection of metabolites in humans. The principle is based on identifying radiofrequency signals emitted by hydrogen nuclear spins that have chemically specific frequencies, determined by the chemical environment of the hydrogen spins. There are three approaches to resolve GABA signals by MRS: (1) using an editing method, which includes J-difference editing, like the MEGA-PRESS (J-difference edited point-resolved spectroscopy) method [127]. This method is based in two experiments; in the first a frequency selective pulse which affects an specific GABA signal is added; the second experiment lacks of this pulse [72]. The result is reported as the difference of these experiments. (2) Through two-dimensional MRS, this consists in plotting the signals in two frequency axes by doing a series of experiments that differ by one parameter (spin echo or spin delay), in this category are included the dimensional J-editing and correlation spectroscopy-based methods [128]. (3) The final approach is to move the edited methods field strength, from a common 3 Teslas (T), to a higher value (usually 7T) [72, 129]. The GABA detection by these "edited methods" has been validated by correlation with chromatographic measurements [130]. The first unambiguous detection of GABA *in vivo* was made using J-edited technique [131].

Using some of the MRS variants mentioned before, it has been possible to estimate the GABA concentrations in healthy and sick people, as well as in animal models. Thus, the concentrations of brain GABA under the effects of topiramate; a $GABA_A$ receptor agonist, were determined in patients with partial epileptic seizures [132]. Also the effects

of vigabatrin and gabapentin (irreversible inhibitor of GABA-T and GABA analogue, respectively), were studied in the occipital lobe of patients using J-edited techniques, and has been shown to raise the levels of GABA in healthy and epileptic subjects [133-139]. A comparative study of gabapentin, pregabalin and vigabatrin in brain GABA on healthy Long Evans rats was reported, only vigabatrin showed a significant increase on the levels of the neurotransmitter [140]. Similar results were obtained in another research, were the effects of vigabatrin and gabapentin; on rat and human neocortical slices were compared, vigabatrin showed a significant increase in GABA levels in both neocortical slices; nonetheless gabapentin increased the levels of this neurotransmitter only in human neocortical slices [141]. Finally the influence of 5'-pyridoxal phosphate and vigabatrin was measured in healthy patients *via* MRS methods, where only vigabatrin showed an increase in brain GABA levels [142]. As shown, a variety of drugs has been tested with NMR methodologies, allowing a valuable information about the GABA dynamics in real time.

Catecholamines

Catecholamines (CAs) are a group of neurotransmitters derived from tyrosine, throughout enzymatic action of tyrosine hydroxylase to form Dopamine (DA), and later aromatic acid decarboxylase to form noradrenaline. In the brain, DA is considered the more abundant catecholaminergic neurotransmitter, with an important role in attention modulation, voluntary motor control, and a series of aspect related with motivated behavior and reinforcement learning [143]. Also, alterations in its extracellular concentrations have been related with schizophrenia [144] and Parkinson's disease [145]. Interest in brain CAs is focused on DA, which, like others electroactives neurotransmitters, has been measured in dialysate fractions or homogenates by HPLC coupled with electrochemical (amperometric or voltammetry) detection [146, 147]. However, these methodologies have some disadvantages: CAs require relatively high potentials to oxidize, producing interferences by others molecules with similar potentials, importantly ascorbic acid and uric acid [148]; as a result, there are deposition of polymeric films onto the electrode, and decay of electrochemical response [149]. To overcome this effect, different electrode materials and electrode modifications have been tested with good results [150, 151].

A relevant methodology to measurement DA and others CAs *in vivo* is the fast-scan cyclic voltammetry associated with carbon fibers microelectrodes, located directly in a brain nucleus of rodents [152-154] and awake monkeys [155]. In this approach, electrodes show changes in the voltage that are proportional to the amount of neurotransmitter present in the site of sensor, with high temporal resolution [156, 157]. Some modifications have been introduced in the microelectrode construction designs to improve the electrical signal, like zeolites-graphite mixtures [158] and diamond nanostructures [159].

Acetylcholine

ACh was identified first as a neurotransmitter in the neuromuscular junction [160], and later accepted as a putative neurotransmitter in the CNS. The biological importance of ACh lies in its association with certain pathologies such as Alzheimer's, schizophrenia and Parkinson's disease, as well as its relationship with seizures and neuropathology associated with organophosphorus compounds, especially those related to the development of biological weapons [161].

The quantification of ACh is complicated, since it is neither electroactive nor chromogenic. In addition, its cationic nature complicates its retention in the conventional reverse-phase columns for chromatographic separation. In the past decade a variety of methods have been developed for ACh quantification, such as quimioluminiscent [162], electrochemical [163], fluorescent [164], and MS [165], but the search for methodological improvements that enable to increase the LOD and limit of quantification (LOQ) still continues.

HPLC-Enzymatic Reactor-Electrochemical Detection (HPLC-ER-ED)

The most common way to determine the content of ACh in samples of brain dialysates or in tissue homogenates, is a combination of HPLC-ER-ED, in which the separation of ACh is carried out in columns for ion pair chromatography or ion exchange retention mechanisms. After the analytes are separated, these are passed through a post-column arrangement with enzymes immobilized in small "cartridges" available in the market, in which the ACh is hydrolyzed by acetylcholinesterase (AChE) in acetate and Ch. Ch is subsequently oxidized by Choline-Oxidase (ChOx), in betaine and H_2O_2. H_2O_2 is detected electrochemically on a carbon or Pt electrode at a determined applied potential in a potentiostat [166, 167]. Finally the arrangement is completed with a reference (Ag/AgCl) and counter electrodes. This method has been used for the determination of extracellular ACh content in multiple regions of the CNS under different paradigms [168-172] with a LOD of 10 fmoles [171, 172] in dialysate fractions between 12 and 30 μL, representing collection time of 6 to 15 min [170, 171]. With these methodologies it has been possible to establish the involvement of cholinergic neurotransmission in several cerebral functions, including different types of memory [171, 173], learning [174], attention [175], and homeostatic aspects; such as body temperature and blood pressure [176], as well as the association of this neurotransmitter with Alzheimer's, Parkinson's, Hungtinton disease and schizophrenia [177]. Although the method was introduced almost 30 years ago [178], it is still valid. However, it has some major drawbacks. A relatively large amount of sample is required to obtain a reliable quantification (> 10 μl). Which means large amount of time for the sample collection to reach the LOD described in the methodology (in the nM range), thus preventing a direct relationship between the neurochemical event of release and its quantification. In addition, the quantification of ACh presents two aspects to solve. The first is the rapid inactivation of ACh by the enzymatic action of AChE, maintaining the basal extracellular concentrations of ACh at very low level. This requires the use of inhibitors to enrich the concentration of ACh in the samples of dialysate, among which the most used, is neostigmine [179-181]. The

second problem is the presence of electroactive elements with similar oxidative potential to ACh, mainly the ascorbic acid (AA). These molecules could interfere with the electrochemical determination of ACh and other neurotransmitters (DA, serotonin) [182, 183].

HPLC Coupled with MS

With the idea of increasing the sensitivity for the analysis of ACh, it was developed a methodology that couples the HPLC with MS [184], in which the ACh molecules, previously separated in a chromatographic system, are introduced in a generating "electrospray interface" at high voltage and temperature; the resulting electrospray (ES) contains ions generated by the breakdown of the original molecule (daughter ions), and enters to the mass spectrometer. This approach provides a sensitive and selective analysis that is unique for individual analytes [185]. In the case of ACh, this has a quaternary amine that carries a positive charge in acidic conditions. This prevents the ionization of the molecule improving its sensitivity. In this way, ACh molecule becomes fragmented, producing the so-called molecular ion m/z 87, which is quantified in the systems of MS. This methodology uses the retention time and the molecular mass to establish the identity of compounds.

The advantage of MS is the possibility to get a direct measure of ACh, without using inhibitors of AChE, and without derivatizing the compound of interest through an enzymatic reactor. However, in this methodology the criteria of selection of HPLC columns and mobile phases are important issues for the detection. Nevertheless, due to poor retention of polar molecules in reversed phase columns, it is necessary to use the ion pair chromatography agents, which significantly interfere with the process of generation of ES. In addition, the relatively high concentrations of organic solvent decrease the efficiency of molecular ions production. For this reason several works have been published proposing various modifications on the column material and the buffers used. From the first attempts to match the typical methodology for ACh detection by HPLC through microbore C18 columns, using octane-sulfonic acid as ion-pairing agent by the production of ES [186], a lot of proposals were quickly developed, which improved the LOD and LOQ for ACh. Hows *et al*. [187] present a HPLC-MS analytical system, in which microdialysis samples (fractions of 24 µL) are separated by cation-exchange chromatography, with mobile phase of ammonium acetate, ammonium formate and acetonitrile. The reported LOD was 1 fmol on column, and allows the quantification of ACh without AChE inhibitors. The same methodology was used for the quantification of ACh, iso-ACh and Ch in the hippocampus of animals in free movement, where the formation of molecular ions from ACh was optimized by reducing the voltage of both production of ES (from 3.5 to 0.9 kV) and the detection of molecular ions (from 30 to 18 kV). Under these conditions, the intensity of ACh-derived ions was not altered with the change of organic composition in the mobile phase [185]. In addition, the use of β-methyl-ACh as internal standard in samples with artificial cerebral spinal fluid (aCSF) significantly improved the quantification, achieving a LOD of 0.01 nM (0.2 fmol on column) and a LOQ in solution of aCSF of 0.05 nM (1 fmol on column) to ACh. Carrozo *et al.,* [188] optimized the separation at low voltages, with the regulation of the acetonitrile concentration entering the ES system, by use a mixing tee post-column system, located after the six-valve port and before ES inlet, in order to improve sensitivity preserving the optimized chromatographic conditions. By this way, the sensitivity of the method increases since acetonitrile improves ionization of ACh with no effect on chromatographic separation. Since the mixing tee is inserted after six-valve port, the inorganic salts of the Ringer's solution were wasted before getting in contact with acetonitrile preventing precipitation in ES. LOD was 0.01 nM (0.05 fmol on colum) for ACh measured in Ringer's solution and the LOQ at signal-to-noise ratio of 10, were 0.05 mM (0.25 fmol on colum) to ACh.

Hydrophilic interaction chromatography (HILIC) has been used since the hydrophilic/polar nature of the ACh. Using polar stationary phases (also known as "aqueous normal phase columns") in combination with organic aqueous mobile phases, it is possible to generate an aqueous layer over the polar stationary phase, which allows the distribution of the analyte between the two phases, according to their polarity. Stationary phases of silica [189], amide [190], polyhydroxyethyl aspartamide [191, 192], or diol [193] have been used for ACh. HILIC systems have become popular for the quantification of ACh, since they represent a good strategy to avoid interference due to ion pair chromatography agents and mobile phases with high concentrations of salts.

Using HILIC mobile phase of 2% (v/v) of acetonitrile and 0.05% (v/v) of trifluoroacetic acid as ion-pairing agent, combined with the atmospheric pressure photoionization (APPI) method rather than ES for the production of derivatives of ion m/z 146, a LOD of 0.15 nM (1.5 fmol on-column) was obtained [194]. Even, miniaturization has been achieved using a microchip "nebulizer" [195], which has been used to quantify ACh in some paradigms of behavior [196]. However, the method of detection by APPI so far has not been validated by other groups.

The use of ACh analogues as internal standards in HILIC system has been reported, showing a significant improvement in the sensitivity of detection of ACh. The incorporation of ACh-d9 bromide as isotope-labeled internal standard and collision-induced dissociation to determine the resulting daughter ions, allowed to obtain LOQ of 0.1 nM (0.2 fmol on-colum) for ACh in dialysates of rat frontal cortex [197]. ACh tetradeuterated (ACh-d4), used as a calibration standard and internal standard, has been used under the "analyte replacement" strategy (introduced by Li and Cohen, in 2003) [198] to eliminate the interference of iso-ACh, the main ACh isomer, which is also an endogenous substance with the same ionic transition to ACh, and it is difficult analyte to separate in HPLC-MS/MS systems. This approach was validated to quantify ACh in SAMP8 mice, used as a model for Alzheimer's disease [199].

An alternative for chromatographic separation is the separation by CE also coupled to MS. This allows a quick and efficient separation of charged compounds in small volumes of sample. ACh has been quantified by CE coupled

with MS at used cell extracts, with a LOD of 5 nM/6 nL, introduced into the capillary *via* hydrodynamic injections [200] in microdialysate fractions, with a LOD of 0.15 nM in 15 μL of dialysate [201], and even there are reports of detections of ACh online using CE, where a LOD of 40 pM was reported [202]. The separation is based on differences in mobility of ions within a capillary tube of silica, with a length between 30-100 cm and an inner diameter less than 100 μm. One end of the capillary tube is placed at the anode and the outlet is located in the cathode. The capillary is subject to procedure of deprotonation of the silanol groups (which gives a negative charge to the inside of the tube) followed by perfusion with a buffer electrolyte to form a double layer in which hydrated positive ions migrate towards the cathode under the influence of an electric field. This creates a natural pumping mechanism inside the capillary, called electro-osmotic flow, which increases the pH. The electrophoretic mobility, which depends on the charge-size relationship, is faster for positive ions, followed by the neutral and finally for negative [166].

The coupling between HPLC and MS has mean a breakthrough for the LOD and LOQ, though it does not resolve the issue of time quantification of ACh, despite the high sensitivities reported, the decrease in the volume of sample and the effectiveness in the separation of the analytes, is still requiring the collection of samples and their subsequent analysis.

Micro-Biosensors

Here we have grouped various types and designs, which involve the inclusion of sensors directly in the brain tissue, in the areas where the determination of neurotransmitters is tried. In general, biosensors contain a biological component (an enzyme, antibody, nucleic acid, membrane receptors, cells) to identify a molecule of interest, producing in the process a chemical signal, which will be captured by a transducer component, whose expression will be optical or electrochemical [203, 204]. Both the biological component and the transducer are a single unit of small size (commonly a Pt wire or carbon fiber), allowing insert it *in vivo* in a discrete region of the brain with minimal injury compared with microdialysis probe [205]. For the quantification of ACh and other neurotransmitters, biosensors are designed with oxidases to oxidize the primary analyte, with the concomitant production of H_2O_2, which is detected amperometrically at potential between 500-700 mV, in relation to a reference of Ag/AgCl electrode [206]. It is important to mention that biosensors are constructed in a multienzyme format; with ACh and ChOx, given the need to produce consecutive reactions to generate H_2O_2. Because the presence of electroactive molecules, such as AA or CAs, generates nonspecific signals, the main challenge for miniaturization of biosensors is to achieve the immobilization of enzymes on a matrix suitable to maintain the activity and biological stability, and also to form a part of a barrier for molecules that are not of interest. With this purpose, systems for enzyme immobilization with crosslinkable polymers as styryl pyrydinium-polivynil alcohol or tetrathiafulvalene-tetracyanoquinodimethane crystals have been developed, reaching LOD between 0.1-0.3 μM to ACh *in vitro* [207, 208]. Another biosensor reported was constructed with poly m-(1,3)-phenylenediamine electro-polymerized on Pt-iridium (Pt-Ir) wire as a template for immobilization of multienzyme layer containing ChOx, AChE, and ascorbic acid oxidase (AAO) immobilized with bovine serum albumin (BSA) and cross-linked with glutaraldeyhyde [209] or base on N-acetylaniline (used as to selective film to reject interferences) electropolymerized onto the Pt electrode with a second layer of zinc oxide sol-gel as a matrix to immobilize a ChOx/AChE/BSA solution [210]. Typically, these micro-biosensors have a LOD of 0.5-0.7 μM for ACh, but there is no evidence of its usage for *in vivo* experiments.

Given the limited real use of micro-biosensors *in vivo*, a biosensor for intracerebral measurement of ACh in awake animals was implemented [211] in which the method reported by Hu *et al.,* [212] was used with several modifications. These biosensors showed a linear response at 20 μM - 80 μM of ACh when tested *in vitro* with a time resolution of 20 second. Also, it was insensitive to AA and norepinephrine. The extracellular concentration of ACh was monitored during induced seizure activity, making evident a significant increase in ACh related with strong seizure behavior activity. The biosensor retains its activity beyond one month when these were store at 4°C [211].

Others micro-biosensors have been developed with CNTs as transducer element. CNTs are 1-2 nm tubules of graphite carbon, that form aggregates among them, providing a large edge/basal plane ratio, enhanced electronic properties, and rapid electrode kinetics [213-215], which improve the electrontransfer reactions of enzymatically generated species, such as H_2O_2 [216]. The CNTs can form a negatively charged surface over which can be deposited an electrostatically and self-layer-by-layer assembly of AChE/polymer, and have become useful elements to estimate the presence of organophosphorus pesticides used in agriculture, medicine, industry and chemical warfare agents [217, 218]. Although the use of microsensors to measure ACh directly into the brain is not a reality, as reflected by the lack of publications about *in vivo* applications, new combinations of CNTs with biological materials are emerging: in a recent paper, Kim *et al*., [219] reported the construction of a lipid membrane sensor for ACh based on CNTs containing M1 type ACh receptors, on a silicon chip. In this device, the binding of ACh onto the receptor was detected by measuring the electrical current change in the underlying CNTs, enabling the real-time detection of 100 pM of ACh concentration. According to this and other reports, this kind of device has a strong potential to be used in a small-size needle-like configurations for *in vivo* applications [219]. Also, the combination of CNTs/sol-gel surface with self-assembly gold nanoparticles-AChE matrices on Pt wire has been proved to the ACh amperometric detection, reaching a LOD of 1 μM *in vitro* [214].

Others Biosensors

In the last 5-6 years, a number of novel biosensors have been developed, which eliminates the requirement of enzyme confinement to a surface and takes some characteristics from biological molecules and processes. Although the sensitivity

of most of them have low range, they are not adequate for monitoring the ACh concentration in the brain. In the future it is possible that some of these ideas could be developed with greater sensitivity to achieve the necessary nanomolar range. In this sense, Schena and Johnsson [220] introduced the semisynthetic ACh-SNIFIT protein, as a fluorescent probe for the direct quantification of ACh within the extracellular matrix. The sensor is constructed with ACh binding to CLIP/SNAP tags: CLIP is labeled with a fluorescence resonance energy transfer (FRET) donor and SNAP with a FRET acceptor. The molecular assembly has the capacity of anchoring to the outer cell membrane through a growth factor receptor transmembrane domain in a cell line derived from embryonic kidney cells. The interaction of ACh with the AChE causes an opening of the assembly, to decrease in the FRET and the emission of a fluorescent signal. The response of this probe was tested by perfusing cells with concentrations of ACh between 1-10 mM, with good response [220].

In a recent paper, the capacity of cysteine to form aggregates with gold nanoparticles was exploited to implement the detection of H_2O_2 generated by the AChE/ ChOx biocatalytic cascade. In this case, the aggregation process leading to a color changes from the red to violet corresponding to the aggregated nanoparticles. The H_2O_2 produced in the catalysis of ACh inhibits these aggregations, and the change in color can be visualized with a spectrophotometer. Results of this paper show linear response between 20 and 200 μM of ACh, the probe was tested only in calibration mode [221]. Also, immobilization of TiO_2 nanoparticles and BiOI nanoflakes onto Indium tin oxide micro-slices has been used as surface to attach anti-AChE antibody/A-protein complex. AChE was after fixed by its immunoreaction with its antibody. Theoretically, the enzymatic reaction between bound AChE and free ACh will produce thiocholine, which acts as electron donor [222]. This array produces a photoelectrochemical signal proportional to the amount of ACh added.

Buiculescu *et al*., [223] developed quantum dots (QD) semiconductors, these have several characteristics of interest, such as small size, high photoluminescent capacity and; narrow, symmetric and size-tunable emission of the spectra covering the blue-red region. Also, the capacity of QD for being conjugated with antibodies, proteins or receptors is important. The construct QD/AChE is conjugated on poly-L-lysine-templated silica matrix. The product analyzed is AA, which is generated in the enzymatic reaction and lowers the pH of the surrounding environment and as a result, a decrease in the photoluminescence is observed. This decrease in photoluminescence is proportional to the AA generated. The LOD obtained in this work was 1 μM [223].

Another method using QD was proposed by Wei *et al*., [224], based on the fact that the fluorescence of QD has linear relation with the production of H_2O_2 in the concentration range of 0.1-40 μM and in presence of Fe^{2+}, by Fenton reaction. Once the enzymatic reaction produces H_2O_2; QD and Fe^{2+} can be introduced into the solution, and the turn-off in fluorescence intensity can be determined. The LOD reported in this work was 0.1 μM.

Finally, interesting attempts have been made to construct molecular reporters by molecular engineering, in a methodology named cell-based neurotransmitter fluorescence engineered reporters (CNiFERs) that allows monitoring *in situ* neurotransmitter receptor activation, using a modified cell line to express the fluorescent protein TN-XXL combined with the quantification of the FRET [225]. Nguyen *et al.,* [226] developed a system based on the Ca^{2+} overload by activation of muscarinic M1 intracellular receptors, *via* the Gq/inositol triphosphate second messenger pathway. Intracellular Ca^{2+} bind to TN-XXL and induces a conformational change that enhances the FRET between two separated protein domains, and produces a fluorescent signal. The CNiFERs was stereotaxically implanted in the brain neocortex of adult rats, and its functionality was assessed by direct deposition of ACh near the implantation site. Besides, to determine if CNiFERs can sense ACh physiological burst, the nucleus basalis magnocellularis was stimulated, which is known to send cholinergic projections into the neocortex, obtaining a positive response in the fluorescence associated to CNiFERs.

CONCLUSION

Measuring the fast neurotransmitters in CNS has a relevant interest in neuroscience field; this is due to the role of these compounds during normal of pathophysiological activity. Alterations in their homeostasis could mean the appearance of a neurological disease. Although, these neurotransmitters are not commonly measured for clinical purpose, they are normally determined in laboratories research to evaluate the effects on many possible therapeutic drugs in order to determine their mechanisms of action, as well as the alteration due to a pathological state. Since many years ago, microdialysis procedure has been a very popular technique despite of its poor temporal resolution; thousands of papers have been published around this method, which means that this technique is still valid. The use of biosensors is positioning as another alternative method and although several advantages come with them, like the improved spatial and temporal resolution and a lesser damage induced in the brain, they can only determine the concentration of one neurotransmitter at a time and perhaps there will not be biosensors for all the neurotransmitters. NMRS methods have a great temporal resolution and are non-invasive, which permits the study in humans. Other method is the immunosensing by acoustic wave, this does not require the use of hazardous materials or an enzymatic reaction, and gives a good temporal resolution. With the appearance of optical methods for measuring compounds in a non-invasive way like: functional near infrared spectroscopy (fNIRS) which determine the difference in the absorption spectra of des-oxy-hemoglobin and oxy-hemoglobin allowing to quantify the relative change in hemoglobin concentration and also, the Raman spectroscopy has been used to try to measure glucose concentration in human patients without the necessity of a catheter to get a sample. Perhaps in the future, the methods reviewed here could be adapted to measure not only the fast neurotransmitters but also several compounds of biological interest in real time and with enough sensitivity to associate

their changes with behavior, normal of pathophysiological states. Although a lot of effort must be put to reach this goal.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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LIST OF ABBREVIATIONS

4-AP	=	4-aminopyridine
AA	=	Acid Ascorbic
AAO	=	Ascorbic acid oxidase
ACh	=	Acetylcholine
ACh-d4	=	ACh tetradeuterated
AChE	=	Acetylcholinesterase
ACPD	=	Trans-1-aminocyclopentane-1,3 dicarboxylate
aCSF	=	Artificial cerebral spinal fluid
APPI	=	Atmospheric Pressure photoionization
BSA	=	Bovine serum albumin
CE	=	Capillary electrophoresis
CE-LED-IF	=	CE-LED-Induced Fluorescence
CE-LIFD	=	Capillary Electrophoresis-Laser Induced Fluorescence Detection
CF	=	Carbon Fiber
Ch	=	Choline
ChOx	=	Choline-Oxidase
CNiFERs	=	Cell-based Neurotransmitter Fluorescence Engineered Reporters
CNS	=	Central Nervous System
CNTs	=	Carbon nanotubes
CP	=	Conducting Polymer
DABSYL-Cl	=	4-(Dimethylamino)azobenzene-4´-sulfonyl chloride
DANSYL-Cl	=	5-dimethylamino-1 naphthalene-sulfonyl-chloride
DL-TBOA	=	DL-treo-β-benzyloxyaspartate
EEG	=	Electroencephalography
ES	=	Electrospray
FDA	=	1-fluoro-2,4-dinitrophenyl-5-alaninamide
FITC	=	Fuorescein isotiocianato
FMOC	=	9-fluorenylmethyl chloroformate
fMRI	=	Functional Magnetic Resonance Imaging
fNIRS	=	Functional near infrared spectroscopy
FRET	=	Fluorescence Resonance Energy Transfer
GA	=	Glutaraldehyde
GABA	=	Gamma-Aminobutyric acid
GABA-T	=	GABA transaminase
GAD	=	Glutamate decarboxylase
GC-FID	=	Gas Cromatography- Flame ionization detector
Glu	=	Glutamate
GluOx	=	Glutamate oxidase
H_2O_2	=	Hydrogen peroxide
HILIC	=	Hydrophilic interaction chromatography
HPLC	=	High Performance Liquid Chromatography
HPLC-ED	=	HPLC- Electrochemical Detection
HPLC-ER-ED	=	HPLC-Enzymatic Reactor – Electrochemical Detection
HPLC-FLD	=	HPLC-Fluorescence Detection
HRP	=	Horseradish peroxidase
LC/MS/MS	=	Liquid Chromatography/Tandem Mass Spectroscopy
LC-ESI-MS	=	LC-Electrospray Ionization-MS
LC-FLD	=	Liquid Chromatography-Fluorescence Detection
LC-MS	=	Liquid Chromatography-Mass Spectroscopy
LOD	=	Limit of Detection
LOQ	=	Limit of quantification
min	=	Minutes
MRS	=	Magnetic Resonance Spectroscopy
MS	=	Mass Spectroscopy
NADPH	=	Nicotinamide adenine dinucleotide phosphate
NAE	=	Nanowire arrays
NBD-Cl	=	4-cloro-7-nitro-2,1,3-benzoxadiazol
NDA	=	Naphthalene-2 3-dicarboxaldehyde
NH_3	=	Ammonia
O_2	=	Molecular oxygen
OPA	=	O-phthalaldehyde
PET	=	Positron Emission Tomography

PITC	=	Phenylisothiocyanate	QD	=	Quantum dots
PPD	=	Poly-Ortho-Phenylenediamine	SPR	=	Surface Plasmon Resonance
Pt	=	Platinum	SSDH	=	Succinic semialdehyde dehydrogenase
Pt-Ir	=	Pt-Iridium	T	=	Tesla
QCM	=	Quartz Crystal Microbalance	TTX	=	Tetrodotoxin

APPENDIX

Table 1. Limits of detection and temporal resolution for techniques used for the quantification of GABA, AChand Glu.

Analyte	Technique	Sensibility	Limit of Detection	Temporal Resolution	References
GABA	HPLC		9.7 nM	minutes	[227]
	HPLC- ED	0.03 pmol		minutes	[228]
	HPLC-FLD, in a boron columns	0.5-1 pmol		minutes	[78]
	HPLC-FLD, in a microboron column	100 fmol		minutes	[78]
	CE	0.016 μM		minutes	[102]
	CE-LIFD		5.1 nM	minutes	[229]
	LC-FLD		10 nM	minutes	[230]
	LC/MS/MS	5 nM	0.5 nM	minutes	[231]
	HPLC-FLD		0.97 μM	minutes	[232]
	LC-MS		48 nM	minutes	[82]
	Peroxidase based amperometric biosensor	50 μM – 1.2mM	20 μM	minutes	[105]
	Acoustic biosensor	38 μM		seconds	[108]
	GABAse Fluorescence-Coupled Assay	0.78 μM	0.41 μM	minutes	[122]
	Immunosensor using QCM		42 μM	seconds	[109]
	Whole-cell sniffer		100 nM	miliseconds	[233]
	Bioluminescent assay		5 pmol/μL	minutes	[126]
	MRS J-difference edited		1mM	real time	[127]
	MRS MEGA-editing		0.75 μmol/g	real time	[129]
ACh	GC-FID detector		0.02 nmol	minutes	[234]
	HPLC-ED		20 fmol	minutes	[235]
	CF electrode		1 μM	minutes	[236]
	HPLC-ER-ED		10 fmol	minutes	[171]
	HPLC-MS		1 fmol	minutes	[187]
	HPLC-MS using Ringer solution	0.05 mM	0.01 nM	minutes	[188]
	HILIC		0.15 nM	minutes	[199]
	CE-MS		5 nM	minutes	[200]
	Pt-electrode based biosensor		0.5 μM	seconds	[210]
	CNTs/sol-gel gold nanoparticles with AChE		1 μM	seconds	[214]
	CLIP/SNAP ACh sensor	1-10 mM		seconds	[220]
	QD/AChE-Poly-L-Lysine-templated silica matrix		1 μM	seconds	[223]

APPENDIX. contd….

Analyte	Technique	Sensibility	Limit of Detection	Temporal Resolution	References
Glu	CE-LIFD		0.070±0.71 nM	seconds	[237]
	CE-LED-IF		1.2 nM	seconds	[238]
	LC-FLD		3-30 nM	minutes	[230]
	LC-ESI-MS		0.5-5 nM		[231]
	LC-MS		0.65 µM	minutes	[239]
	HPLC-MS		0.1 µM	minutes	[83]
	HPLC-FLD		0.1-20 µM	minutes	[232]
	Electrode Pt site	0.016±0.001 nA/µM	1.82±0.17 µM	second	[240]
	Electrochemical microsensor	0.0034±0.001 nA/ µM	1-3 µM	seconds	[105]
	Electrode CP coated	14.0±0.2 nA/ µM	0.1±0.03 µM	seconds	[241]
	Electrode Pt-Ir	0.1 nA/ µM	2 µM	second	[212]
	Electrode Pt-NAE	194.6±9.2 µAmM-1cm-2	1 µM	seconds	[242]
	Pt-PPD-GluOx-GA		3.0±0.6 µM		[243]
	Biosensor basedon covalent immobilization of GluOx on polypyrrole nanoparticles-polyaniline modified gold electrode		0.1 nM	seconds	[244]
	Biosensors and microelectrodes	793 pA/mM	0.493 mM	seconds	[46]
	Graphene quantum dots		5.2x10-8 M	seconds	[54]
	Self-referencing Electrochemical biosensor	473±57 µA/ µM	0.9±0.3 µM	seconds	[246]
	Microsensors and amperometric monitoring	126±5 nA/ µM	2.1±0.2 µM	second	[8]
	Sensor enzyme free Ni nanowire array electrode	65-96 µA/mM	68-88 µM		[242]

REFERENCES

[1] Delgado, J.M.; DeFeudis, F.V.; Roth, R.H.; Ryugo, D.K.; Mitruka, B.M. Dialytrode for long term intracerebral perfusion in awake monkeys. *Arch. Int. Pharmacodyn. Ther*., **1972**, *198*, 9-21. PubMed ID: 4626478

[2] Parrot, S.; Sauvinet, V.; Riban, V.; Depaulis, A.; Renaud, B.; Denoroy, L. High temporal resolution for *in vivo* monitoring of neurotransmitters in awake epileptic rats using brain microdialysis and capillary electrophoresis with laser-induced fluorescence detection. *J. Neurosci. Methods*., **2004**, *140*, 29-38. http://dx.doi.org/10.1016/j.jneumeth.2004.03.025

[3] Rossell, S.; Gonzalez, L.E.; Hernández L. One-second time resolution brain microdialysis in fully awake rats. Protocol for the collection, separation and sorting of nanoliter dialysate volumes. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **2003**, *784*, 385-393. http://dx.doi.org/10.1016/s1570-0232(02)00826-7

[4] Wilson, C.L.; Maidment, N.T.; Shomer, M.H.; Behnke, E.J.; Ackerson, L.; Fried, I. Comparison of seizure related amino acid release in human epileptic hippocampus versus a chronic, kainate rat model of hippocampal epilepsy. *Epilepsy Res*., **1996**, *26*, 245-54. http://dx.doi.org/10.1016/s0920-1211(96)00057-5

[5] Morales-Villagrán, A.; Medina-Ceja, L.; López-Pérez, S.J. Simultaneous glutamate and EEG activity measurements during seizures in rat hippocampal region with the use of an electrochemical biosensor. *J. Neurosci. Methods,* **2008**, *168*, 48-53. http://dx.doi.org/10.1016/j.jneumeth.2007.09.005

[6] Wassum, K.M.; Tolosa, V.M.; Wang, J.; Walker, E.; Monbouquette, H.G.; Maidment, N.T. Silicon Wafer-Based Platinum Microelectrode Array Biosensor for Near Real-Time Measurement of Glutamate *in Vivo*. *Sensors (Basel)*, **2008**, *8*, 5023-5036. http://dx.doi.org/10.3390/s8085023

[7] Hascup, K.N.; Hascup, E.R.; Stephens, M.L.; Glaser P.E.A.; Yoshitake, T.; Mathe A.A.; Gerhard, G.A.; Kehr, J. Resting Glutamate Levels and Rapid Glutamate Transients in the Prefrontal Cortex of the Flinders Sensitive Line Rat: A Genetic Rodent Model of Depression. *Neuropsychopharmacol.*, **2011**, *36*, 1769-1777. http://dx.doi.org/10.1038/npp.2011.60

[8] Tseng, T.T.C.; Monbouquette, H.G. Implantable microprobe with arrayed microsensors for combined amperometric monitoring of the neurotransmitters, glutamate and dopamine. *J. Electroanal. Chem.*, **2012**, *682*, 141-146. http://dx.doi.org/10.1016/j.jelechem.2012.07.014

[9] Budai, D. Carbon Fiber-based Microelectrodes and Micro-biosensors. In: Intelligent and Biosensors. Somerset V. S., Ed.; InTech, Croatia, 2010; pp. 269-284.

[10] Shinohara, H.; Wang, F. Real-time detection of dopamine released from a nerve model cell by an enzyme-catalyzed luminescence method and its application to drug assessment. *Anal. Sci*., **2007**, *23*, 81-84. http://dx.doi.org/10.2116/analsci.23.81

[11] Zakir-Hossain, S.M.; Shinohara, H.; Wang, F.; Kitano, H. Real-time d etection of L-glutamate released from C6 glioma cells using a modified enzyme-luminescence method. *Anal. Bioanal. Chem*., **2007**, *389*, 1961-1966. http://dx.doi.org/10.1007/s00216-007-1569-4

[12] Morales-Villagrán, A.; Beltrán-Ramírez, R.; López-Pérez, S.J; Palomera-Ávalos, V.; Medina-Ceja, L. A capillary fraction collector coupled to a fluorescence reader: a novel device to continuously quantify glutamate during microdialysis. *Neurochem. Res*., **2012**, *37*, 1457-64. http://dx.doi.org/10.1007/s11064-012-0736-y

[13] Gholizadeh, A.; Shahrokhian, S.; Iraji zad, A.; Mohajerzadeh, S.; Vosoughi, M.; Darbari, S.; Sanaee, Z. Mediator-less highly sensitive voltammetric detection of glutamate using glutamate dehydrogenase/ vertically aligned CNTs grown on silicon substrate. *Biosens. Bioelectron.,* **2012**, *31*, 110-115. http://dx.doi.org/10.1016/j.bios.2011.10.002

[14] Jamain, S.; Betancur, C.; Quach, H.; Philippe, A.; Fellous, M.; Giros, B.; Gillberg, C.; Leboyer, M.; Bourgeron, T. Linkage and association of the glutamate receptor 6 gene with autism. *Mol. Psychiatry*, **2002**, *7*, 302-310. http://dx.doi.org/10.1038/sj.mp.4000979

[15] Van der Zeyden, M.; Oldenziel, W.H.; Rea, K.; Cremers, T.I.; Westerink, B.H. Microdialysis of GABA and glutamate: Analysis, interpretation and comparison with microsensors. *Pharmacol. Biochem. Behav.,* **2008**, *90*, 135-147. http://dx.doi.org/10.1016/j.pbb.2007.09.004

[16] Watkins, J.C.; Evans, R.H. Excitatory Amino Acid Transmitters. *Ann. Rev. Pharmacol. Toxicol.,* **1981**, *21,* 165-204.

[17] Peña, F.; Tapia, R. Seizures and neurodegeneration induced by 4-aminopyridine in rat hippocampus *in vivo*: role of glutamate- and GABA-mediated neurotransmission and of ion channels. *Neuroscience*, **2000**, *101*, 547-561. http://dx.doi.org/10.1016/s0306-4522(00)00400-0

[18] Oldenziel, W.H.; Dijkstra, G.; Cremers, T.I.F.H.; Westerink, B.H.C. *In vivo* monitoring of extracellular glutamate in the brain with a microsensor. *Brain. Res.,* **2006**, *1118,* 34-42. http://dx.doi.org/10.1016/j.brainres.2006.08.015

[19] Fayed, N.; Modrego, P.J.; Rojas-Salinas, G.; Aguilar, K. Brain glutamate levels are decreased in Alzheimer's disease: a magnetic resonance spectroscopy study. *Am. J. Alzheimers. Dis. Other Dem.,* **2011**, *26,* 450-456. http://dx.doi.org/10.1177/1533317511421780

[20] Amara, A.W.; Watts, R.L.; Walker, H.C. The effects of deep brain stimulation on sleep Parkinson´s disease. *Ther. Adv. Neurol. Disord.,* **2011**, *4,* 15-24. http://dx.doi.org/10.1177/1756285610392446

[21] Behrens, P.F.; Franz, P.; Woodman, B.; Lindenberg, K.S.; Landwehrmeyer G.B. Impared glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. *Brain,* **2002**, *125,* 1908-1922. http://dx.doi.org/10.1093/brain/awf180

[22] Buckingham, S.C; Robel, S. Glutamate and tumor-associated epilepsy: glial cell dysfunction in the peritumoral environment. *Neurochem. Int.,* **2013**, *63,* 696-701. http://dx.doi.org/10.1016/j.neuint.2013.01.027

[23] Rao, S.D.; Yin, R.Z.; Weiss, J.H. Disruption of glial glutamate transport by reactive oxygen species produced in motor neurons. *J Neurosci.,* **2003**, *23,* 2627-2633. PubMed ID: 12684448

[24] Sanacora, G.; Rothman, D.L.; Manson, G.; Krystal, J.H. Clinical studies implementing glutamate neurotransmission in mood disorders. *Ann. NY. Acad. Sci.,* **2003**, *1003,* 292-308. http://dx.doi.org/10.1196/annals.1300.018

[25] Baltan, S. Ischemic injury to white matter. *Neuroscientist,* **2009**, *15,* 126-133. http://dx.doi.org/10.1177/1073858408324788

[26] Javitt, D.C. Glutamate as a therapeutic target in psychiatric disorders. *Mol Psychiatry,* **2004**, *9,* 984-997. http://dx.doi.org/10.1038/sj.mp.4001551

[27] Valero, E.; García-Carmona, F.A. Continuous spectrophotometric method based on enxymatic cycling for determining L-glutamate. *Anal.Biochem.,* **1998**, *259,* 265-271. http://dx.doi.org/10.1006/abio.1998.2650

[28] Chapman, J.; Zhou, M. Microplate-based fluorometric methods for the enzymatic determination of L-glutamate: application in measuring L-glutamate in food samples. *Anal. Chim. Acta,* **1999**, *402,* 47-52. http://dx.doi.org/10.1016/s0003-2670(99)00533-4

[29] Namiki, S.; Sakamoto, H.; Iinuma, S.; Iino, M.; Hirose, K. Optical glutamate sensor for spatio temporal analysis of synaptic transmission. *Eur. J. Neurosci.*, **2007**, *8*, 2249-2259. http://dx.doi.org/10.1111/j.1460-9568.2007.05511.x

[30] Medina-Ceja, L.; Morales-Villagrán, A.; Tapia, R. Action 4-aminopyridine on extracellular amino acids in hippocampus and entorhinal cortex: a dual microdialysis and electroencephalographic study in awake rats. *Brain Res. Bull.,* **2000**, *53*, 255-262. http://dx.doi.org/10.1016/s0361-9230(00)00336-1

[31] Weltin, A.; Kieninger, J.; Enderle, B.; Gellner, A.K.; Fritsch, B.; Urban, G.A. Polymer-based, flexible glutamate and lactate microsensors for *in vivo* applications. *Biosens. Bioelectron.*, **2014**, *61*, 192-199. http://dx.doi.org/10.1016/j.bios.2014.05.014

[32] Chefer, V.I.; Thompson, A.C., Zapata, A.; Shippenberg, T.S. Overview of brain microdialysis. *Curr. Protoc.Neurosci.,* **2009**, *Chapter 7: Unit. 1.* DOI: 10.1002/0471142301.ns0701s47

[33] Bicker, J.; Fortuna, A.; Alves, G.; Falcão, A. Liquid chromatographic methods for the quantification of catecholamines and their metabolites in several biological samples-A review. *Anal. Chim. Acta*, **2012**, *768*, 12-34. http://dx.doi.org/10.1016/j.aca.2012.12.030

[34] Rada, P.; Mendialdua, A.; Hernández, L.; Hoebel, B.G. Extracellular glutamate increases in the lateral hypothalamus during meal initiation, and GABA peaks during satiation: microdialysis measurements every 30 s. *Behav. Neurosci.*, **2003**, *117*, 222-227. http://dx.doi.org/10.1037/0735-7044.117.2.222

[35] Clapp-Lilly, K.L.; Roberts, R.C.; Duffy, L.K.; Irons, K.P.; Hu, Y.; Drew, K.L. An ultrastructural analysis of tissue surrounding a microdialysis probe. *J. Neurosci. Methods*, **1999**, *90*, 129-142. http://dx.doi.org/10.1016/s0165-0270(99)00064-3

[36] Miele, M.; Boutelle, M.G.; Fillenz, M. The source of physiologically stimulated glutamate efflux from the striatum of conscious rats. *J. Physiol*, **1996**, *497*, 745-751. http://dx.doi.org/10.1113/jphysiol.1996.sp021805

[37] Shiraishi, M.; Kamiyama, Y.; Huttemeier, P.C.; Benveniste, H. Extracellular glutamate and dopamine measured by microdialysis in the rat striatum during blockade of synaptic transmission in anesthetized and awake rats. *Brain Res*., **1997**, *759*, 221-227. http://dx.doi.org/10.1016/s0006-8993(97)00258-8

[38] [38] Lada, M.W.; Vickroy, T.W.; Kennedy, R.T. Evidence for neuronal origin and metabotropic receptor-mediated regulation of extracellular glutamate and aspartate in rat striatum *in vivo* following electrical stimulation of the prefrontal cortex. *J. Neurochem*., **1998**, *70*, 617-625. http://dx.doi.org/10.1046/j.1471-4159.1998.70020617.x

[39] Xi, Z.X.; Shen, H.; Baker, D.A.; Kalivas, P.W. Inhibition of non-vesicular glutamate release by group III metabotropic glutamate receptors in the nucleus accumbens. *J. Neurochem*., **2003**, *87*, 1204-1212. http://dx.doi.org/10.1046/j.1471-4159.2003.02093.x

[40] Palmer, A.M.; Hutson, P.H.; Lowe, S.L.; Bowen, D. Extracellular concentrations of aspartate and glutamate in rat neostriatum following chemical stimulation of frontal cortex. *Exp. Brain. Res*., **1989**, *75*, 659-663. DOI: 10.1007/BF00249918

[41] Kapoor, K.; Nakahara, D., Blood, R.J.; Chalmers, J.P. Preferential release of neuroactive amino acids from the ventrolateral medulla of the rat *in vivo* as measured by microdialysis. *Neurosci*., **1990**, *37*, 889-900.

[42] Paleckova, V.; Palecek, J.; McAdoo, D.J; Willis, W.D. The non-NMDA antagonist CNQX prevents release of amino acids into the rat spinal cord dorsal horn evoked by sciatic nerve stimulation. *Neurosci. Lett*., **1992**, *148,* 19-22. http://dx.doi.org/10.1016/0304-3940(92)90794-8

[43] Tucci, S.; Contreras, Q.; Paez, X.; Gonzalez, L.; Rada, P.; Hernandez, L. Medial prefrontal transaction enhances social interaction. II: neurochemical studies. *Brain Res*., **2000**, *887*, 259-265. http://dx.doi.org/10.1016/s0006-8993(00)02932-2

[44] Timmerman, W.; Westerink, B.H.C. Brain microdialysis of GABA and glutamate: what does it signify? *Synapse*, **1997**, *27*, 242-261. http://dx.doi.org/10.1002/(sici)1098-2396(199711)27:3<242::aid-syn9>3.0.co;2-d

[45] Bhambi, M.; Sumana, G.; Malhotra, B.D.; Pundir, C.S. An amperomertic uric acid biosensor based on immobilization of uricase onto polyaniline-multiwalled carbon nanotube composite film. *Artif. Cells Blood Substit. Immobil. Biotechnol*., **2010**, *38*, 178-185. DOI: 10.3109/10731191003716344

[46] Özel, R.E.; Ispas, C.; Ganesana, M.; Leiter, J.C.; Andreescu, S. Glutamate oxidase biosensor based on mixed ceria and titania nanoparticles for the detection of glutamate in hypoxic environments. *Biosens. Bioelectron*., **2014**, *52*, 397-402. http://dx.doi.org/10.1016/j.bios.2013.08.054

[47] Kusakabe, H.; Midorikawa, Y.; Fujishima, T.; Kuninaka, A.; Yoshino, H. Purification and properties of a new enzyme, L-

glutamate oxidase, from Streptomyces sp. X-119-6 grown on wheat bran. *Agric. Biol. Chem.,* **1983**, *47,* 1323-1328. http://dx.doi.org/10.1271/bbb1961.47.1323

[48] Gerhardt, G.A.; Oke, A.F.; Nagy, G.; Moghaddam, B.; Adams, R.N. Nafion-coated electrodes with high selectivity for CNS electrochemistry. *Brain Res,* **1984**, *290,* 390-395. http://dx.doi.org/10.1016/0006-8993(84)90963-6

[49] Ammam, M.; Fransaer, J. Highly sensitive and selective glutamate microbiosensor based on cast polyurethane/AC-electrophoresis deposited multiwalled carbon nanotubes and then glutamate oxidase/electrosynthesized polypyrrole/Pt electrode. *Biosens. Bioelectron.,* **2010**, *25,* 1597-1602. http://dx.doi.org/10.1016/j.bios.2009.11.020

[50] Lee, K.H.; Kristic, K.; van Hoff, R.; Hitti, F.L.; Blaha, C.; Harris, B.; Roberts, D.W.; Leiter, J.C. High-frequency stimulation of the subthalamic nucleus increases glutamate in the subthalamic nucleus of rats as demonstrated by *in vivo* enzyme-linked glutamate sensor. *Brain Res.*, **2007**, *1162*, 121-129. http://dx.doi.org/10.1016/j.brainres.2007.06.021

[51] Arrigan, D.W.M. Nanoelectrodes, nanoelectrode arrays and their applications. *Analyst,* **2004**, *129,* 1157-1165. DOI: 10.1039/b415395m

[52] Shao, Y.; Wang, J.; Wu, H.; Liu, J.; Aksay, I. A.; Lin, Y. Graphene Based Electrochemical Sensors and Biosensors: A Review. *Electroanalysis,* **2009**, *22*(10), 1027-1036. http://dx.doi.org/10.1002/elan.200900571

[53] Hu, J.; Wisetsuwannaphum, S.; Foord, J.S. Glutamate biosensors based on diamond and graphene plataforms. *Faraday Discuss*, **2014**, *172*, 457-472. PMID: 25427169

[54] Hosseini, M.; Khabbaz, H.; Dezfoli, A.S.; Ganjai, M.R.; Dadméhr M. Selective recognition of Glutamate based on fluorescence enhancement of graphene quantum dot. *Spectrochim. Acta a Mol. Biomol. Spectrosc.*, **2015**, *136*, 1962-1966. http://dx.doi.org/10.1016/j.saa.2014.10.117

[55] Meldrum, B.S. Epilepsy and gamma-aminobutyric acid-mediated inhibition. *Int. Rev. Neurobiol*., **1975**, *17*, 1-36. http://dx.doi.org/10.1016/s0074-7742(08)60205-6

[56] Ling, W.; Shoptaw, S.; Majewska, D. Baclofen as a cocaine anti-craving medication: a preliminary clinical study. *Neuropsychopharmacol.,* **1998**, *18*, 403-404. DOI: 10.1016/S0893-133X(97)00128-0

[57] Feucht, M.; Fuchs, K.; Pichlbauer, E.; Hornik, K.; Scharfetter, J.; Goessler, R.; Füreder, T.; Cvetkovic, N.; Sieghart, W.; Kasper, S.; Aschauer, H. Possible association between childhood absence epilepsy and the gene encoding GABRB3. *Biol. Psychiatry*, **1999**, *46*, 997-1002. http://dx.doi.org/10.1016/s0006-3223(99)00039-6

[58] Benes, F.M.; Berretta, S. GABAergic interneurons: implications for understanding schizophrenia disorder. *Neuropsychopharmacol.*, **2001**, *25*, 1-27. http://dx.doi.org/10.1016/s0893-133x(01)00225-1

[59] Wallace, R.H.; Marini, C.; Petrou, S.; Harkin, L.A.; Bowser, D.N.; Panchal, R.G.; Williams, D.A.; Sutherland, G.R.; Mulley, J.C.; Scheffer, I.E.; Berkovic, S.F. Mutant GABA (A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat. Genet.* **2001**, *28*, 49-52. http://dx.doi.org/10.1038/ng0501-49

[60] Wong, C.G.T.; Bottiglieri, T.; Snead, O.C. GABA, gamma-hydroxybutyric acid, and neurological disease. *Ann. Neurol.*, **2003**, *54*, S3-12. http://dx.doi.org/10.1002/ana.10696

[61] Shiah, I.S.; Yatham, L.N. GABA function in mood disorders: an update and critical review. *Life Sci*., **1998**, *63*, 1289-1303. http://dx.doi.org/10.1016/s0024-3205(98)00241-0

[62] Mihic, S.J.; Ye, Q.; Wick, M.J.; Koltchine, V.V.; Krasowski, M.D.; Finn S.E.; Mascia, M.P.; Valenzuela, C.F.; Hanson, K.K.; Greenblatt, E.P.; Harris, R.A.; Harrison, N.L. Sites of alcohol and volatile anaesthetic action on GABA (A) and glycine receptors. *Nature*, **1997**, *389*, 385-389. http://dx.doi.org/10.1038/38738

[63] Steward, F. C.; Thompson, J. F.; Dent, C. E. C-aminobutyric acid: A constituent of the potato tuber. *Science*., **1949**, *110*, 439-440.

[64] Naini, A. B.; Vontzalidou, E.; Coˆte´, L.J. Isocratic HPLC assay with electrochemical detection of free c-aminobutyric acid in cerebrospinal fluid. *Clin. Chem*., **1993**, *39*, 247-250.

[65] Rea, K.; Cremers T.I.; Westerink B.H. HPLC conditions are critical for the detection of GABA by microdialysis. *J. Neurochem*., **2005**, *94*, 672-679. http://dx.doi.org/10.1111/j.1471-4159.2005.03218.x

[66] Kagan, I.A.; Coe B.L.; Smith L.L.; Huo C.J.; Dougherty C.T.; Strickland J.R. A validated method for gas chromatographic analysis of gamma-aminobutyric acid in tall fescue herbage. *J. Agric. Food Chem*., **2008**, *56*, 5538-5543. http://dx.doi.org/10.1021/jf8000229

[67] Clarke, G.; Mahoney S.; Malone G.; Dinan T.G. An isocratic high performance liquid chromatography method for the determination of GABA and glutamate in discrete regions of the rodent brain. *J. Neurosci. Meth*., **2007**, *160*, 223-230. http://dx.doi.org/10.1016/j.jneumeth.2006.09.006

[68] Nandi, P.; Lunte, S.M. Recent trends in microdialysis sampling integrated with conventional and micro-analytical systems for monitoring biological events: A review. *Anal. Chim. Acta*, **2009**, *651*, 1−14. http://dx.doi.org/10.1016/j.aca.2009.07.064

[69] Wolf, R.; TScherne, U.; Hinderk, M.E. Suppression of preoptic GABA release caused by push-pull perfusión with sodium valproate. *Naunyn-Schmiedeberg´s Arch. Pharmacol*., **1988**, *338*, 658-663. http://dx.doi.org/10.1007/bf00165631

[70] Slaney, T.R.; Mabrouk, O.S.; Porter-Stransky, K.A.; Aragona, B.J.; Kennedy R.T. Chemical Gradients within Brain Extracellular Space Measured using Low Flow Push−Pull Perfusion Sampling *in Vivo*. *ACS Chem. Neurosci*., **2013**, *4*, 321−329. http://dx.doi.org/10.1021/cn300158p

[71] O'Gorman, R.L.; Michels, L.; Edden, R.A.; Murdoch, J.B.; Martin E. *In Vivo* Detection of GABA and Glutamate With MEGA-PRESS: Reproducibility and Gender Effects. *J. Magn. Reson. Imaging*, **2011**, *33*, 1262-1267. http://dx.doi.org/10.1002/jmri.22520

[72] Puts, N.A.J.; Edden, R.A.E. *In vivo* magnetic resonance spectroscopy of GABA: A methodological review. *Progress in Nuclear Magnetic Resonance Spectroscopy*, **2012**, *60*, 29-41. http://dx.doi.org/10.1016/j.pnmrs.2011.06.001

[73] Robinson, D.L.; Hermans, A.; Seipel A.T.; Wightman R.M. Monitoring rapid chemical communication in the brain. *Chem. Rev*., **2008**, *108*, 2554-84. http://dx.doi.org/10.1021/cr068081q

[74] Kehr, J.; Ungerstedt, U. Fast HPLC estimation of c-aminobutyric acid in microdia- lysis perfusates: Effect of nipecotic and 3-mercaptopropionic acids. *J. Neurochem*., **1988**, *51*, 1308-1310. http://dx.doi.org/10.1111/j.1471-4159.1988.tb03101.x

[75] Westerink B.H.; de Vries J.B. On the origin of extracellular GABA collected by brain microdialysis and assayed by a simplified on-line method. *Naunyn Schmiedebergs Arch. Pharmacol*., **1989**, *339*, 603-607. http://dx.doi.org/10.1007/bf00168650

[76] Rowley, H.L.; Martin, K.F.; Marsden, C.A. Determination of *in vivo* amino acid neurotransmitters by high-performance liquid cromatography with o-phthalaldehyde-sulphite derivatization. *J. Neurosci. Methods*., **1995**, *57*, 93-99. http://dx.doi.org/10.1016/0165-0270(94)00132-z

[77] Sekioka, S.; Kato, D.; Kurita, R.; Hirono, S.; Niwa, O. Improved detection limit for an electrochemical -aminobutyric acid sensor based on stable NADPH detection using an electron cyclotron resonance sputtered carbon film electrode. *Sensors and Actuators B*, **2008**, *129*, 442-449. http://dx.doi.org/10.1016/j.snb.2007.08.040

[78] Kher, J. Determination of g-aminobutyric acid in microdialysis samples by microbore column liquid chromatography and fluorescence detection. *Journal of Chromatography B*., **1998**, *708*, 49-54. http://dx.doi.org/10.1016/s0378-4347(97)00657-9

[79] Bianchi, L.; Della, C.L.; Tipton, K. F. Simultaneous determination of basal and evoked output levels of aspartate, glutamate, taurine and 4-aminobutyric acid during microdialysis and from superfused brain slices. *J. Chromatogr. B Biomed. Sci. Appl.,* **1999**, *723*, 47-59. http://dx.doi.org/10.1016/s0378-4347(98)00519-2

[80] Nishikawa, K.; Kubo, K.; Obata, H.; Yanagawa, Y.; Saito, S. The influence of manipulations to alter ambient GABA concentrations on the hypnotic and immobilizing actions produced by sevoflurane, propofol, and midazolam. *Neuropharmacology***2010**, *61*, 172-180. http://dx.doi.org/10.1016/j.neuropharm.2011.03.025

[81] Ballini, C.; Corte, L.D.; Pazzagli, M.; Colivicchi, M.A.; Pepeu, G.; Tipton, K.F.; Giovannini, M.G. Extracellular levels of brain aspartate, glutamate and GABA during an inhibitory avoidance response in the rat. *J. Neurochem*., **2008**, *106*, 1035-1043. http://dx.doi.org/10.1111/j.1471-4159.2008.05452.x

[82] Ma, D.; Zhang J.; Sugahara, K.; Ageta, T.; Nakayama, K.; Kodama, H. Simultaneous determination of gamma-aminobutyric acid and glutamic acid in the brain of 3-mercaptopropionic acid-treated rats using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *J Chromatogr. B Biomed.*

Sci. Appl., **1999**, *726 (1-2)*, 285-90. http://dx.doi.org/10.1016/s0378-4347(99)00025-0

[83] Song, Y.; Shenwu, M.; Dhossche, D. M.; Liu, Y. M. A capillary liquid chromatographic/ tandem mass spectrometric method for the quantification of gammaaminobutyric acid in human plasma and cerebrospinal fluid. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2005**, *814*, 295-302. http://dx.doi.org/10.1016/j.jchromb.2004.10.046

[84] Eckstein, J. A.; Ammerman, G. M.; Reveles, J. M.; Ackermann, B. L. Analysis of glutamine, glutamate, pyroglutamate, and GABA in cerebrospinal fluid using ion pairing HPLC with positive electrospray LC/MS/MS. *J. Neurosci. Methods,* **2008**, *171*, 190-196. http://dx.doi.org/10.1016/j.jneumeth.2008.02.019

[85] Khan, G.M.; Smolders, I.; Lindekens, H.; Manil, J.; Ebinger, G.; MIchotte, I. Effects of diazepam on extracellular brain neurotransmitters in pilocarcarpine-induced seizures in rats. *Eur. J. Pharmacol*, **1999**, *4*, 153-161. http://dx.doi.org/10.1016/s0014-2999(99)00209-5

[86] Kuneman, D.W.; Braddock J.K.; McChesney L.L. HPLC profile of amino acids in fruit juices as their (1-fluoro-2, 4-dinitrophenyl-) 5-alaninae amide derivatives.*J. Agric. Food Chem*. **1968**, *36*, 6-9. http://dx.doi.org/10.1021/jf00079a002

[87] Piepponen, T.P.; Skujins, A. Rapid and sensitive step gradient assays of glutamate, glycine, taurine and c-aminobutyric acid by high-performance liquid chromatography- fluorescence detection with o-phthalaldehyde-mercaptoethanol derivatization with an emphasis on microdialysis samples. *J. Chromatogr. B Biomed. Sci. Appl.*, **2001**, *757*, 277-283. http://dx.doi.org/10.1016/s0378-4347(01)00156-6

[88] Pyo, Y.H.; Song, S.M. Physicochemical and sensory characteristics of a medicinal soy yogurt containing health-benefit ingredients. *J. Agric. Food Chem*., **2008**, *57*, 170-5. http://dx.doi.org/10.1021/jf8026952

[89] Syu, K.Y.; Lin, C.L.; Huang, H.C.; Lin J.K. Determination of theanine, GABA, and other amino acids in green, oolong, black, and pu-erh teas with dabsylation and high-performance liquid chromatography. *J. Agric. Food Chem*., **2008**, *56*, 7637-7643. http://dx.doi.org/10.1021/jf801795m

[90] Zhang, S.; Takeda, Y.; Hagioka, S.; Takata, K.; Aoe, H.; Nakatsuka, H.; Yokoyama, M.; Morita, K. Measurement of GABA and glutamate *in vivo* levels with high sensitivity and frequency. *Brain Res. Protoc*., **2005**, *14*, 61-66. http://dx.doi.org/10.1016/j.brainresprot.2004.03.005

[91] Renlund, S.; Erlandsson, I.; Hellman, U.; Silberring, J.; Wernstedt, C.; Lindström, L.; Nyberg F. Micropurification and amino acid sequence of casomorphin-8 in milk from a woman with postpartum psychosis. *Peptides*. **1993**, *14*, 1125-1132. http://dx.doi.org/10.1016/0196-9781(93)90165-d

[92] Anderzhanova, E.; Wotjak, C.T. Brain microdialysis and its applications in experimental neurochemistry. *Cell Tissue Res*., **2013**, *354*, 27-39. http://dx.doi.org/10.1007/s00441-013-1709-4

[93] Nyitrai, G.; Kékesi, K.A.; Juhász, G. Extracellular level of GABA and Glu: *In vivo* Microdialysis-HPLC Measurements. *Curr. Top. Med. Chem*., **2006**, *6*, 935-40. http://dx.doi.org/10.2174/156802606777323674

[94] Soukupová, M.; Binaschi, A.; Falcicchia, C.; Zucchini, S.; Roncona, P.; Palma, E.; Magri, E.; Grandi, E.; Simonato, M. Impairment of GABA release in the hippocampus at the time of the first spontaneous seizure in the pilocarpine model of temporal lobe epilepsy. *Exp. Neurol.,* **2014**, *257*, 39-49 http://dx.doi.org/10.1016/j.expneurol.2014.04.014

[95] Buck, K.; Voehringer, P.; Ferger, B. Rapid analysis of GABA and glutamate in microdialysis samples using high performance liquid chromatography and tandem mass spectrometry. *J. Neurosci. Methods*, **2009**, *182*, 78-84. http://dx.doi.org/10.1016/j.jneumeth.2009.05.018

[96] Vargas, C.; Tannhauser, M.; Barros, H. M. T. Dissimilar Effects of Lithium and Valproic Acid on GABA and Glutamine Concentrations in Rat Cerebrospinal Fluid. *Gen. Pharm.*, **1998**, *30(4)*, 601-604. http://dx.doi.org/10.1016/s0306-3623(97)00328-5

[97] Shiah, I.S.; Yatham, L.N.; Gau, Y.C.; Baker, G. B. Effect of lamotrigine on plasma GABA levels in healthy humans. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2003**, *27*, 419-423. http://dx.doi.org/10.1016/s0278-5846(03)00028-9

[98] Beggiato, S.; Tanganelli, S.; Fuxe, K.; Antonelli, T.; Schwarcz, R.; Ferraro, L. Endogenous kynurenic acid regulates extracellular GABA levels in the rat prefrontal cortex. *Neuropharmacology*, **2014**, *82*, 11-18. http://dx.doi.org/10.1016/j.neuropharm.2014.02.019

[99] Shen, X.; Liu, Y.; Xu, S.; Zhao, Q.; Wu, H.; Guo, X.; Shen, R.; Wang, F. Menin regulates spinal glutamate-GABA balance through GAD65 contributing to neuropathic pain. *Pharmacol. Rep.*, **2014**, *66*, 49-55. http://dx.doi.org/10.1016/j.pharep.2013.06.005

[100] Pérez, J.; Colasante, C.; Tucci, S.; Hernández, L.; Rada, P. Effects of feeding on extracellular levels of glutamate in the medial and lateral portion of the globus pallidus of freely moving rats. *Brain Res*., **2000**, *877*, 91-94. http://dx.doi.org/10.1016/s0006-8993(00)02658-5

[101] Benturquia, N.; Parrot, S.; Sauvinet, V.; Renaud, B.; Denoroy, L. Simultaneous determination of vigabatrin and amino acid neurotransmitters in brain microdialysates by capillary electrophoresis with laser-induced fluorescence detection. *J. Chromatogr. BAnalyt. Technol. Biomed. Life Sci*., **2004**, *806*, 237-44. http://dx.doi.org/10.1016/j.jchromb.2004.03.061

[102] Shi, X.; Liang, P.; Song, D.; Yang, W.; Gao, X. Quantification of γ-aminobutyric acid in the heads of houseflies (Musca domestica) and diamondback moths (*Plutella xylostella* (L.)), using capillary electrophoresis with laser-induced fluorescence detection. *J. Sep. Science*, **2012**, *35*, 548-555. http://dx.doi.org/10.1002/jssc.201100802

[103] Szoco, E.; Tabi, T. Analysis of biological samples by capillary electrophoresis with laser induced fluorescence detection. *J. Pharm. Biomed. Anal*., **2010**, *53*, 1180-92. http://dx.doi.org/10.1016/j.jpba.2010.07.045

[104] Kawagoe, K.T.; Garris, P.A.; Wiedemann, D.J.; Wightman, R.M. Regulation of transient dopamine concentration gradients in the microenvironment sorrounding nerve terminals in the rat striatum. *Neuroscience*, **1992**, *51*, 55-64. http://dx.doi.org/10.1016/0306-4522(92)90470-m

[105] Kulagina, N.V.; Shankar, L.; Michael A.C. Monitoring glutamate and ascorbate in the extracellular space of brain tissue with electrochemical microsensors. *Anal. Chem*., **1999**, *71*, 5093-5100. http://dx.doi.org/10.1021/ac990636c

[106] Burmeister, J.J; Gerhardt, G.A. Self-referencing ceramic-based multisite microelectrodes for the detection and elimination of interferences from the measurement of L-glutamate and other analytes. *Anal. Chem*., **2001**, *5*, 1037-1042. http://dx.doi.org/10.1021/ac0010429

[107] Georganopoulou, D.G.; Carley, R.; Jones, D.A.; Boutelle, M.G. Development and comparison of biosensors for *in-vivo* applications. *Faraday Discuss*, **2000**, *116*, 291-303. http://dx.doi.org/10.1039/b003256p

[108] Zhou, A.; Muthuswamy J. Acoustic biosensor for monitoring antibody immobilization and neurotransmitter GABA in real-time. *Sensors and Actuators B*., **2004**, *101*, 8-19. http://dx.doi.org/10.1016/j.snb.2004.02.015

[109] Wang, T.; Muthuswamy, J. Immunosensor for detection of inhibitory neurotransmitter γ-Aminobutyric Acid Using Quartz Crystal Microbalance. *Anal.Chem*., **2008**, *80*, 8576-8582. http://dx.doi.org/10.1021/ac801463a

[110] Mazzei, F.; Botre, F.; Lorenti, G.; Porcelli, F. Peroxidase based amperometric biosensors for the determination of y-aminobutyric acid. *Anal. Chim. Acta.*, **1996**, *328*, 41-46. http://dx.doi.org/10.1016/0003-2670(96)00089-x

[111] Masharina A.; Reymond L.; Maurel D.; Umezawa K.; Johnsson K. A fluorescent sensor for GABA and synthetic GABA(B) receptor ligands. *J. Am. Chem. Soc*., **2012**, *134*, 19026-19034. http://dx.doi.org/10.1021/ja306320s

[112] Malmqvist M. Biospecific interaction analysis using biosensor technology. *Nature,* **1993**, *361*, 186-187. http://dx.doi.org/10.1038/361186a0

[113] Homola, J.; Yee, S.S.; Gauglitz, G. Surface plasmon resonance sensors: review. *Sens. Actuators*, **1999**, *54*, 3-15. http://dx.doi.org/10.1016/s0925-4005(98)00321-9

[114] Kambhampati D.K.; Knoll W. Surface-plasmon optical techniques. *Curr. Opin. Colloid Interface Sci*., **1999**, *4*, 273-280. http://dx.doi.org/10.1016/s1359-0294(99)90008-0

[115] Scott, E.M.; Jakoby, W.B. Soluble gamma-aminobutyric-glutamic transaminase from Pseudomonas fluorescens. *J. Biol. Chem*., **1959**, *234*, 932-936.

[116] Burke, J.R.; Silverman, R.B. Mechanism of Inactivation of γ-Aminobutyric Acid Aminotransferase by 4-Amino-5-hexynoic Acid (γ-Ethynyl GABA). *J. Am. Chem. Soc*., **1991**, *113*, 9329-9340. http://dx.doi.org/10.1021/ja00024a042

[117] Akazawa, T.; Conn E.E. The oxidation of reduced pyridine nucleotides by peroxidase. *J. Biol. Chem.*, **1958**, *232*, 403-415. PubMed ID: 13549429

[118] Moiroux, J.; Elving P.J. Optimization of the analytical oxidation of dihydronicotinamide dinucleotide at carbon and platinum electrodes. *Anal. Chem*., **1979**, *51*, 346-350. http://dx.doi.org/10.1021/ac50039a007

[119] Janshoff A.; Galla H.J.; Steinem C. Piezoelectric Mass-Sensing Devices as Biosensors-An Alternative to Optical Biosensors? *Angew. Chem. Int. Ed. Engl*., **2000**, *39*, 4004-4032. http://dx.doi.org/10.1002/1521-3773(20001117)39:22<4004::aid-anie4004>3.0.co;2-2

[120] Bunde, R.L.; Jarvi, E.J.; Rosentreter, J.J. Piezoelectric quartz crystal biosensors. *Talanta,* **1998**, *6*, 1223-1236. http://dx.doi.org/10.1016/s0039-9140(97)00392-5

[121] Sauerbrey, G. Verwendung von schwinquarzen zur wagung dunner schichten und zur mikrowagung. *Zeitschrift fur Physik,* **1959**, *155*, 206-222. http://dx.doi.org/10.1007/bf01337937

[122] Ippolito, J.E.; Piwnica-Worms, D. A fluorescence-coupled assay for gamma aminobutyric acid (GABA) reveals metabolic stress-induced modulation of GABA content in neuroendocrine cancer. *PLoS One*., **2014**, *9*, e88667. http://dx.doi.org/10.1371/journal.pone.0088667

[123] O'Byrne, C.P.; Feehily, C.; Ham, R.; Karatzas, K.A. A modified rapid enzymatic microtiter plate assay for the quantification of intracellular γ-aminobutyric acid and succinate semialdehyde in bacterial cells. *J. Microbiol. Methods*, **2011**, *84*, 137-139. http://dx.doi.org/10.1016/j.mimet.2010.10.017

[124] Morishima, T.; Uematsu, M.; Furukawa, T.; Yanagawa, Y.; Fukuda, A.; Yoshida, S. GABA imaging in brain slices using immobilized enzyme-linked photoanalysis. *Neurosci. Res*., **2010**, *67*, 347-353. doi:10.1016/j.neures.2010.04.005

[125] Duane, W.C. NAD(P)H:FMNoxidoreductaseofPhoto-bacteriumfischerii: its role in bacterial bioluminescence. Ph.D. Thesis, University ofIllinois, Urbana-Champaign, **1969**.

[126] Israël, M.; Lesbats, B. A bioluminescent gamma-aminobutyrate assay for monitoring its release from inhibitory nerve endings. *J. Neurochem*., **1996**, *67*, 2624-2627. DOI: 10.1046/j.1471-4159.1996.67062624.x

[127] Waddell, K.W.; Avison, M.J.; Joers, J.M.; Gore, J.C. A practical guide to robust detection of GABA in human brain by J-difference spectroscopy at 3 T using a standard volume coil. *Magn. Reson. Imaging*, **2007**, *25*, 1032-1038. doi:10.1016/j.mri.2006.11.026

[128] Banakar, S.; Thomas, M.A.; Deveikis, A.; Watz, J.Q.; Hayes, J.; Keller, M.A. Two-dimensional 1H MR spectroscopy of the brain in human immunodeficiency virus (HIV)-infected children. *J. Magn. Reson. Imaging,* **2008**, *27*, 710-717. DOI: 10.1002/jmri.21251

[129] Terpstra, M.; Ugurbil, K.; Gruetter, R. Direct *in vivo* measurement of human cerebral GABA concentration using MEGA-editing at 7 Tesla. *Magn. Reson. Med.*, **2002**, *47*, 1009-1012 DOI: 10.1002/mrm.10146

[130] Bielicki, G.; Chassain, C.; Renou, J. P.; Farges, M.C.; Vasson, M.P.; Eschalier, A.; Durif, F. Brain GABA editing by localized *in vivo* (1)H magnetic resonance spectroscopy. *NMR Biomed*., **2004**, *17*, 60-68. PMID: 15052553

[131] Rothman, D.L.; Petroff, O.A.; Behar, K.L.; Mattson, R.H. Localized 1H NMR measurements of gamma-aminobutyric acid in human brain *in vivo*. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 5662-5666. PMID: 8516315

[132] Petroff, O. A.; Hyder, F.; Rothman, D. L.; Mattson, R. H. Topiramate rapidly raises brain GABA in epilepsy patients. *Epilepsia*, **2001**, *42(4)*, 543-8. DOI: 10.1046/j.1528-1157.2001.18800.x

[133] Petroff, O.A.; Rothman, D.L.; Behar, K.L.; Mattson R.H. Initial observations on effect of vigabatrin on *in vivo* 1H spectroscopic measurements of gamma aminobutyric acid, glutamate, and glutamine in human brain. *Epilepsia*, **1995**, *36*, 457-464. DOI: 10.1111/j.1528-1157.1995.tb00486.x

[134] Petroff, O.A.; Rothman, D.L.; Behar, K.L.; Mattson R.H. Human brain GABA levels rise after initiation of vigabatrin therapy but fail to rise further with increasing dose. *Neurology*, **1996a**, *46*, 1459-1463. doi: http://dx.doi.org/10.1212/WNL.46.5.1459

[135] Petroff, O.A.; Behar, K.L.; Mattson, R.H.; Rothman, D.L. Human brain gammaaminobutyric acid levels and seizure control following initiation of vigabatrin therapy. *J. Neurochem.*, **1996b**, *67*, 2399-2404. DOI: 10.1046/j.1471-4159.1996.67062399.x

[136] Petroff, O.A.; Mattson, R.H.; Behar, K.L.; Hyder, F.; Rothman, D.L. Vigabatrin increases human brain homocarnosine and improves seizure control. *Ann. Neurol*. **1998**, *44*, 948-952. DOI: 10.1002/ana.410440614

[137] Petroff, O.A.; Hyder, F.; Collins, T.; Mattson, R.H.; Rothman, D.L. Acute effects of vigabatrin on brain GABA and homocarnosine in patients with complex partial seizures. *Epilepsia*, **1999**, *40*, 958-964. DOI: 10.1111/j.1528-1157.1999.tb00803.x

[138] Weber, O. M.; Verhagen, A.; Duc, C. O.; Meier, D.; Leenders, K. L.; Boesiger, P. Effects of vigabatrin intake on brain GABA activity as monitored by spectrally edited magnetic resonance spectroscopy and positron emission tomography. *Magnetic Resonance Imaging*, **1999**, *17(3)*, 417-425. doi:10.1016/S0730-725X(98)00184-2

[139] Petroff, O.A.C.; Hyder, F.; Rothman, D.L.; Mattson, R.H. Effects of Gabapentin on Brain GABA, Homocarnosine, and Pyrrolidinone in Epilepsy Patients. *Epilepsy*, **2000**, *41(6)*, 675-680. DOI: 10.1111/j.1528-1157.2000.tb00227.x

[140] Errante, L. D.; Petroff , O. A. C. Acute effects of gabapentin and pregabalin on rat forebrain cellular GABA, glutamate, and glutamine concentrations. *Seizure*, **2003**, *12*, 300-306. doi:10.1016/S1059-1311(02)00295-9

[141] Errante, L. D.; Williamson, A.; Spencer, D. D.; Petroff, O. A. C. Gabapentin and vigabatrin increase GABA in the human neocortical slice. *Epilepsy Res*., **2002**, *49*, 203-210. doi:10.1016/S0920-1211(02)00034-7

[142] Mueller, S. G.; Weber, O. M.; Boesiger, P.; Wieser, H. G. Influence of pyridoxal 59-phosphate alone and in combination with vigabatrin on brain GABA measured by 1H-NMR-spectroscopy. *Brain Res. Bull.*, **2001**, *55(4)*, 555-560. doi:10.1016/S0361-9230(01)00565-2

[143] Ikemoto, S.; Panksepp, J. The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Res. Rev*., **1999**, *31*, 6-41. doi:10.1016/S0165-0173(99)00023-5

[144] Brisch, R.; Saniotis, A.; Wolf, R.; Bielau, H.; Bernstein, H.G.; Steiner, J.; Bogerts, B.; Braun, K.; Jankowski, Z.; Kumaratilake, J.; Henneberg, M.; Gos, T. The role of dopamine in schizophrenia from a neurobiological and evolutionary perspective: old fashioned, but still in vogue. *Front Psychiatry*, **2014**, *5*, 47. doi: 10.3389/fpsyt.2014.00047

[145] Benazzouz, A.; Mamad, O.; Abedi, P.; Bouali-Benazzouz, R.; Chetrit, J. Involvement of dopamine loss in extrastriatal basal ganglia nuclei in the pathophysiology of Parkinson´s disease. Front. *Aging Neurosci.*, **2014**, *6*, article 87. doi: 10.3389/fnagi.2014.00087

[146] Chen, Y.; Engidawork, E.; Loidl, F.; Dell´Anna, E.; Goiny, M.; Lubec, G.; Andersson, K.; Herrera-Marschitz, M. Short- and long-term effects of perinatal asphyxia on monoamine, amino acid and glycolysis product levels measured in the basal ganglia of the rat. *Dep. Brain Res*., **1997**, *14(4)*, 19-30. doi:10.1016/S0165-3806(97)00131-4

[147] Kennedy, R.T. Emerging trends in *in vivo* neurochemical monitoring by microdialysis. *Curr. Opin. Chem. Biol.*, **2013**, *17*, 860-867. doi:10.1016/j.cbpa.2013.06.012

[148] Ernst, H.; Knoll, M. Electrochemical characterisation of uric acid and ascorbic acid at a platinum electrode. *Anal. Chim. Acta*, **2001**, *449*, 129-134. doi:10.1016/S0003-2670(01)01350-2

[149] Benton, B.J.; Troyer, K.P.; Wightman, R.M. Response times of carbon microelectrodes to dynamic changes in catecholamine concentration. *Anal. Chem.*, 2002, *74*, 539-546. DOI: 10.1021/ac010819a

[150] Zhou, J.; Sheng, M.; Jiang, X.; Wu, G.; Gao, F. Simultaneous determination of dopamine, serotonine and ascorbic acid at a glassy carbon electrode modified with carbon-spheres. *Sensors*, **2013**, *13*, 14029-14040. doi:10.3390/s131014029

[151] Veerakumar, P.; Madhu, R.; Chen, S.M.; Hung, C.T.; Tang, P.H.; Wang, C.B.; Liu, S.B. Porous carbon-modified electrodes as highly

selective and sensitive sensors for detection of dopamine. *Analyst,* **2014**, *139*, 4994-5000. DOI: 10.1039/C4AN01083C

[152] Clark, J.; Sandberg, S.; Wanat, M.; Gan, J.; Horne, E.; Hart, A.; Akes, Ch.; Parker, J.; Willuhn I.; Martinez, V.; Evans, S.; Stella, N.; Phillips, P.E. Chronic microsensors fos longitudinal, subsecond dopamine detection in behaving animals. *Nat. Methods*, **2010**, *7*, 126-129. doi:10.1038/nmeth.1412

[153] Badrinarayan, A.; Wascott, S.; Vander-Weele, C.; Saunders, T.; Couturier, B.; Maren, S.; Aragona, B. Aversive stimuli differentially modulate real-time dopamine transmission dynamics within the nucleus accumbens core and shell. *J. Neurosci.*, **2012**, *32*, 15779-15790. doi: 10.1523/JNEUROSCI.3557-12.2012

[154] Hashemi, P.; Walsh, P.; Guillot, T.; Gras-Najjar, J.; Takmakov, P.; Crews, F.; Wightman, R. Chronically implanted, nafion-coated Ag/AgCl reference electrodes for neurochemical applications. *ACS Chem. Neurosci.*, **2011**, *2*, 658-666. DOI: 10.1021/cn2000684

[155] Schluter, E.W.; Mitz, A. R.; Cheer, J. F.; Averbeck, B.B. Real-time dopamine measurement in awake monkeys. *PLOS One*, **2014**, *9*, e98692. doi: 10.1371/journal.pone.0098692

[156] Keithley R.B.; Takmakov, P.; Bucher, E.S.; Belle, A.M.; Owesson-White, C.A.; Park, J.; Wightman, R.M. Higher sensitivity dopamine measurement with faster-scan cyclic voltammetry. *Ann. Chem*., **2011**, *83*, 3563-3571. DOI: 10.1021/ac200143v

[157] Kile, B-M.; Walsh, P.L.; McElligott, Z.A.; Bucher, E.S.; Guillot, T.S.; Salahpour, A.; Caron, M.G.; Wightman, R.M. Optimizing the temporal resolution of fast-scan voltammetry. *ACS Chem. Neurosci*., **2012**, *3*, 285-292. PMID: 22708011

[158] Ilinoiu, E.C.; Manea, F.; Andrea-Sierra, P.; Pode, R. Simultaneous/ selective detection of dopamine and ascorbic acid at synthetic zeolite-modified/graphite-epoxy composite macro/quasi-micro-electrodes. *Sensors*, **2013**, *13*, 7296-7307. doi:10.3390/s130607296

[159] Arumugam, P.U.; Zeng, H.; Siddiqui, S.; Covey, D.; Carlisle, J.; Garris, P. Characterization of ultrananocrystalline diamod microsensors for *in vivo* dopamine detection. *Appl. Phys. Lett.*, **2013**, *102*, 253107. http://dx.doi.org/10.1063/1.4811785

[160] Dale, H. H.; Feldberg, W.; Vogt, M. Release of acetylcholine at voluntary motor nerve endings. *J. Physiol. (London),* **1936**, *86*, 353-380. DOI: 10.1113/jphysiol.1936.sp003371

[161] Pohanka, M.; Adam, V.; Kizek, R. An acetylcholinesterase-based chronoamperometric biosensor for fast and reliable assay of nerve agents. *Sensors*, **2013**, *13*, 11498-11506. doi:10.3390/s130911498

[162] Ternaux, J.P.; Chamoin, M.C. Enhanced chemiluminescent assays for acetylcholine. *J. Biolumin. Chemilumin*., **1994**, *9*, 65-72. DOI: 10.1002/bio.1170090205

[163] Huang, T.; Yang, L.; Gitzen, J.; Kissinger, P.T.; Vreeke, M.; Heller, A. Detection of basal acetylcholine in rat brain microdialysate. *J. Chromatogr. B. Biomed. Appl.* **1995**, *670*, 323-327. doi:10.1016/ 0378-4347(95)00181-6

[164] MacDonald, R.C. A fluorometric assay for acetylcholine with picomole sensitivity. *J. Neurosci. Methods,* **1989**, *29*, 73-76. doi:10.1016/0165-0270(89)90110-6

[165] Ikarashi, Y.; Itoh, K.; Maruyama, Y. Application of FRIT fast atom bombardment liquid chromatography/mass spectrometry for the determination of acetylcholine levels in rat brain regions. *Biol. Mass Spectrom.,* **1991**, *20*, 21-25. DOI: 10.1002/bms.1200200106

[166] Barkhimer, T.V.; Kirchhoff J.R.; Hudson, R.A.; Messer, W.S.; Viranga-Tillekeratne, L.M. Electrochemical detection of acetylcholine and choline: application to the quantitative nonradiochemical evaluation of choline transport. *Anal. Bioanal. Chem.,* **2008**, *392*, 651-662. DOI 10.1007/s00216-008-2307-2

[167] Zapata, A.; Chefer, V.I.; Shippenberg, T.S.; Denoroy, L. Detection and quantification of neurotransmitters in dialysates. *Curr. Protoc. Neurosci.,* **2009**, CHAPTER: Unit-7.430. Available from: http:// www.ncbi.nlm.nih.gov/pmc/articles/PMC2950641.

[168] Cecchi, M.; Passani, M.B.; Bacciottini, L.; Mannaioni, P.F.; Blandina, P. Cortical acetylcholine release elicited by stimulation of histamine H1 receptors in the nucleus basalis magnocellularis: a dual-probe microdialysis study in the freely moving rat. *Eur. J. Neurosci.,* **2001**, *13*, 68-78. DOI: 10.1111/j.1460-9568.2001.01361.x

[169] O´Donnell, J.C.; McDonough, J.H.; Shih, T.M. *In vivo* microdialysis and electroencephalographic activity in freely moving guinea pigs exposed to organophosphorus nerve agents sarin and VX: analysis of acetylcholine and glutamate. *Arch. Toxicol.,* **2011**, *85*, 1607-1616. DOI 10.1007/s00204-011-0724-z

[170] Puron-Sierra, L.; Miranda, M.I. Histaminergic modulation of cholinergic release from the nucleus basalis magnocellularis into insular cortex during taste aversive memory formation. *PLoS ONE*, **2014**, Available from: http://www.pubmedcentral.nih.gov/ articlerender.fcgi?artid=3953328&tool=pmcentrez&rendertype=abstract. doi:10.1371/journal.pone.0091120

[171] Roland, J.J.; Stewart, A.L.; Janke, K.L.; Gielow, M.R.; Kostek, J.A.; Savage, L.M.; Servatius, R.J.; Pang, K.C.Medial septum-diagonal band of Broca (MSDB) GABAergic regulation of hippocampal acetylcholine efflux is dependent on cognitive demands. *J. Neurosci.,* **2014**, *34*, 506-514. doi: 10.1523/JNEUROSCI.2352-13.2014

[172] Takase, K.; Sakimoto, Y.; Kimura, F.; Mitsushima, D. Developmental trajectory of contextual learning and 24-h acetylcholine release in the hippocampus. *Sci. Rep.*, **2014**, *4*, 3738-3744. doi:10.1038/ srep03738

[173] Pilly, P.K.; Grossberg, S. How reduction of theta rhytm by medial septum inactivation may covary with disruption of entorhinal grid cell responses during to reduced cholinergic transmission. *Front. Neural Circuits,* **2013**, *173*, 1-13.Available from: http://www. pubmedcentral.nih.gov/articlerender.fcgi?artid=3814006&tool=pmcentrez&rendertype=abstract. doi: 10.3389/fncir.2013.00173.

[174] Rokem, A.; Silver, M.A. The benefits of cholinergic enhancement during perceptual learning are long-lasting. *Front. Comput. Neurosci.* **2013**, *7*, 66, 1-7. Available from: http://www.pubmedcentral.nih. gov/articlerender.fcgi?artid=3665931&tool=pmcentrez&. doi: 10.3389/ fncom.2013.00066

[175] Demeter, E.; Sarter, M. Leveraging the cortical cholinergic system to enhance attention. *Neuropharmacology,* **2013**, *64*, 294-304. doi:10.1016/j.neuropharm.2012.06.060

[176] Wessler, I.; Kirkpatrick, C.J.; Racke, K. Non-neuronal acetylcholine, a locally acting molecule, widely distributed in biological systems: expression and function in humans.*Pharmacol. Ther.,* **1998**, *77*, 59-79. doi:10.1016/S0163-7258(97)00085-5

[177] Scarr, E.; Gibbons, A.S.; Neo, J.; Udawela, M.; Dean, B. Cholinergic connectivity: it's implications for psychiatric disorders. *Front. Cell. Neurosci.*, **2013**, *7*, article 55, 1-26. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3642390&tool=pmcentrez&rendertype=abstract. doi: 10.3389/fncel.2013.00055

[178] Damsma, G.; Westerink, B.H.; Horn, A.S. A simple, sensitive, and economic assay for choline and acetylcholine using HPLC, an enzyme reactor, and an electrochemical detector. *J. Neurochem.,* **1985**, *45*, 1649-1652. DOI: 10.1111/j.1471-4159.1985.tb07238.x

[179] Messamore, E.; Warpman, U.; Ogane, N.; Giacobini, E. Cholinesterase inhibitor effects on extracellular acetylcholine in rat cortex. *Neuropharmacology*, **1993**, *32*, 745-750. doi:10.1016/0028-3908(93)90182-3

[180] Joosen, M.J.A.; van Helden, H.P.M. Correlations between acetylcholinesterase inhibition, acetylcholine levels and EEG changes during perfusion with neostigmine and N6-cyclopentyladenosine in rat brain. *Eur. J. Pharmacol.,* **2007**, *555*, 122-128. doi:10.1016/j. ejphar.2006.10.006

[181] Ray, A.; Liu, J.; Karanth, S.; Gao, Y.; Brimijoin, S.; Pope, C. Cholinesterase inhibition and acetylcholine accumulation following intracerebral administration of paraoxon in rats. *Toxicol. Appl. Pharmacol.,* **2009**, *236*, 341-347. doi:10.1016/j.taap.2009.02.022

[182] Medeiros, R. a.; Benchick, a.; Rocha-Filho, R. C.; Fatibello-Filho, O.; Saidani, B.; Debiemme-Chouvy, C.;Deslouis, C. Simultaneous detection of ascorbic acid and dopamine with electrochemically pretreated carbon nitride electrodes: Comparison with boron-doped diamond electrodes. *Electrochem. commun.*, **2012**, *24*, 61-64. doi:10.1016/j.elecom.2012.08.011

[183] Wang, Y.; Xiao, Y. Glassy carbon electrode modified with poly(dibromofluorescein) for the selective determination of dopamine and uric acid in the presence of ascorbic acid. *Microchim. Acta* **2012**, *178*, 123-130. DOI 10.1007/s00604-012-0821-6

[184] Zhu, Y.; Wong, P. S.; Cregor, M.; Gitzen, J. F.; Coury, L. A.; Kissinger, P. T. *In vivo* microdialysis and reverse phase ion pair liquid chromatography/tandem mass spectrometry for the determination and identification of acetylcholine and related compounds in rat brain. *Rapid Commun. Mass Spectrom.,* **2000**, *14*, 1695-700. DOI: 10. 1002/1097-0231(20000930)14:18<1695::AID-RCM79>3.0.CO;2-5

[185] Zhang, M.-Y.; Hughes, Z. A.; Kerns, E. H., Lin, Q.; Beyer, C. E. Development of a liquid chromatography/tandem mass spectrometry method for the quantitation of acetylcholine and related neurotransmitters in brain microdialysis samples. *J. Pharm. Biomed. Anal.*, **2007***, 44*, 586-593. doi:10.1016/j.jpba.2007.02.024

[186] Liberato, D.J.; Weintraub, S.T.; Thermospray liquid chromatography/ mass spectrometry studies on mechanisms of nucleic acid alkylationby some deuterated carcinogens. *Chem. Res. Toxicol*., **1989**, *2* 307-311. DOI: 10.1021/tx00011a007

[187] Hows, M. E. P.; Organ, A. J.; Murray, S.; Dawson, L. A.; Foxton, R.; Heidbreder, C.; Hughes, Z. A.; Lacroix, L.; Shah, A. J. High-performance liquid chromatography/tandem mass spectrometry assay for the rapid high sensitivity measurement of basal acetylcholine from microdialysates. *J. Neurosci. Methods***2002**, *121*, 33-39. doi:10.1016/S0165-0270(02)00228-5

[188] Carrozzo, M. M.; Cannazza, G.; Pinetti, D.; Di Viesti, V.; Battisti, U.; Braghiroli, D.; Parenti, C.; Baraldi, M. Quantitative analysis of acetylcholine in rat brain microdialysates by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J. Neurosci. Methods,* **2010**, *194*, 87-93. doi:10.1016/j.jneumeth. 2010.09.016

[189] Grumbach, E. S.; Diane, M.; Jeffrey, R.; Alden, B.; Iraneta, P. C. Hydrophilic Interaction Chromatography Using Silica Columns for the Retention of Polar Analytes and Enhanced ESI-MS Sensitivity. *LC-GC North America,* **2004**, *10*, 1010-1023. DOI: 10.1021/jp0535689

[190] Guo, D.-J.; Xiao, S.-J.; Xia, B.; Wei, S.; Pei, J.; Pan, Y.; You, X.-Z.; Gu, Z. Z.; Lu, Z. Reaction of porous silicon with both end-functionalized organic compounds bearing alpha-bromo and omega-carboxy groups for immobilization of biomolecules. *J. Phys. Chem. B.,* **2005**, *109*, 20620-20628 doi:10.1016/j.jpba.2008. 02.022

[191] Wang, Y.; Wang, T.; Shi, X.; Wan, D.; Zhang, P.; He, X.; Gao.P.; Yang, S.; Gu, J.; Xu, G. Analysis of acetylcholine, choline and butyrobetaine in human liver tissues by hydrophilic interaction liquid chromatography-tandem mass spectrometry. *J. Pharm. Biomed. Anal.,* **2008**, *47*, 870-875. DOI: 10.1002/rcm.3251

[192] Zhang, X.; Rauch, A.; Lee, H.; Xiao, H.; Rainer, G.; Logothethis, N.K. Capillary hydrophilic interaction chromatography/mass spectrometry for simultaneous determination of multiple neurotransmitters in primate cerebral cortex. *Rapid Commun. Mass Spectrom.,* **2007**, *21*, 3621-3628. DOI: 10.1002/rcm.3251

[193] Uutela, P.; Reinila, R.; Piepponen, P.; Ketola, R.A.; Kostiainen, R. Analysis of acetylcholine and choline in microdialysis samples by liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.*, **2005**, *19*, 2950-2056. DOI: 10.1002/rcm.2160

[194] Keski-Rahkonen, P.; Lehtonen, M.; Ihalainen, J.; Sarajärvi, T.; Auriola, S. Quantitative determination of acetylcholine in microdialysis samples using liquid chromatography/atmospheric pressure spray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 2933-2943. DOI: 10.1002/rcm.3162

[195] Keski-Rahkonen, P.; Haapala, M.; Saarela, V.; Franssila, S.; Kotiaho, T.; Kostiainen, R.; Auriola, S. Atmospheric pressure thermospray ionization using a heated microchip nebulizer. *Rapid. Commun. Mass Spectrom.,* **2009**, *23*, 3313-3322. DOI: 10.1002/ rcm.4252

[196] Ihalainen, J.; Sarajärvi, T.; Rasmusson, D.; Kemppainen, S.; Keski-Rahkonen, P.; Lehtonen, M.; Banerjee, P. K.; Semba, K.; Tanila, H. Effects of memantine and donepezil on cortical and hippocampal acetylcholine levels and object recognition memory in rats. *Neuropharmacology,* **2011**, *61*, 891-899 doi:10.1016/j. neuropharm.2011.06.008

[197] Liu, L.; Huang, J.; Li, K.; Hu, X.; Sun, C. Analysis of acetylcholine from extracellular fluid in brain by *in vivo* microdialysis and LC-ESI-MS/MS with the stable isotope-labeled internal standard. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.,* **2011**, *879*, 56-60. doi:10.1016/j.jchromb.2010.11.009

[198] Li, W.; Cohen, L. H. Quantitation of endogenous analytes in biofluid without a true blank matrix. *Anal. Chem.,* **2003**, *75*, 5854-9 DOI: 10.1021/ac034505u DOI: 10.1021/ac034505u

[199] Peng, L.; Jiang, T.; Rong, Z.; Liu, T.; Wang, H.; Shao, B.; Ma, J.; Yang, L.; Kang, L.; Shen, Y.; Li, H.; Qi, H.; Chen, H. Surrogate based accurate quantification of endogenous acetylcholine in murine brain by hydrophilic interaction liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.,* **2011**, *879*, 3927-3931. doi:10.1016/j.jchromb. 2011.09.020

[200] Lapainis, T.; Rubakhin, S.S.; Sweedler, J. V. Capillary Electrophoresis with Electrospray Ionization Mass. *Anal. Chem.,* **2009**, *15*, 5858-5864.

[201] Ihalainen J.; Sarajarvi, T.; Kemppainen, S.; Keski-Rahkonen, P.; Lehtonen, M.; Tanila, H. A novel delayed non-match to sample object recognition task that allows simultaneous *in vivo* microdialysis. *J. Neurosci. Methods,* **2010**, *189*, 210-215. doi:10.1016/j.jneumeth.2010.04.006

[202] Shackman, H. M.; Shou, M.; Cellar, N. A.; Watson, C. J.; Kennedy, R. T. Microdialysis coupled on-line to capillary liquid chromatography with tandem mass spectrometry for monitoring acetylcholine *in vivo*. *J. Neurosci. Methods,* **2007**, *159*, 86-92. doi:10.1016/j.jneumeth.2006.06.020

[203] Wilson, G. S.; Gifford, R. Biosensors for real-time *in vivo* measurements. *Biosens. Bioelectron*, **2005**, *20*, 2388-23403. doi:10.1016/j.bios.2004.12.003

[204] Thevenot, D. R.; Tóth, K.; Durst, R.A.; Wilson G.S. Electrochemical Biosensors: Recommended Definitions and Classification. *Pure and Applied Chemistry*, **2009**, *71*, 2333-2348. DOI:10.1081/AL-100103209

[205] Khan, A.S.; Michael, A.C. Invasive consequences of using micro-electrodes and microdialysis probes in the brain. *Tr. A.C.,* **2003***, 22*, 503-508. doi:10.1016/S0165-9936(03)00908-7

[206] Dale, N.; Hatz, S.; Tian, F.; Llaudet, E. Listening to the brain: microelectrode biosensors for neurochemicals. *Trends Biotechnol.* **2005**, *23*, 420-428. doi:10.1016/j.tibtech.2005.05.010

[207] Navera, E.N.; Sode, K.; Tamiya, E.; Karube, I. Development of acetylcholine sensor using carbon fiber (amperometric determination). *Biosens. Bioelectron,* **1991**, *6*, 675-680. doi:10. 1016/0956-5663(91)87021-3

[208] Kawagoe, J.L.; Niehaus, D.E.; Wightman, R.M. Enzyme-modified organic conducting salt microelectrode. *Anal. Chem.,* **1991**, *63*, 2961-2965. DOI: 10.1021/ac00024a029

[209] Mitchell, K.M. Acetylcholine and choline amperometric enzyme sensors characterized *in vitro* and *in vivo*. *Anal. Chem.,* **2004**, *76*, 1098-1106. DOI: 10.1021/ac034757v

[210] Yang, M.; Yang, Y.; Yang, Y.; Shen, G.; Yu, R. Microbiosensor for acetylcholine and choline based on electropolymerization/sol-gel derived composite membrane. *Anal. Chim. Acta,* **2005**, *530*, 205-211. doi:10.1016/j.aca.2004.09.010

[211] Morales-Villagrán, A.; López-Pérez, S.; Ortega-Ibarra, J. Electrochemical Biosensors to Monitor Extracellular Glutamate and Acetylcholine Concentration in Brain Tissue.In: *Biosensors for Health, Environment and Biosecurity*, Pier Andrea Serra, Ed.; InTech, Croatia, **2011**; pp. 445-452. DOI: 10.5772/16909

[212] Hu, Y.; Mitchell, K.M.; Albahadily, F.N.; Michaelis, E.K.; Wilson, G.S. Direct measurement of glutamate release in the brain using a dual enzyme-based electrochemical sensor. *Brain Res.,* **1994**, *659*, 117-125. doi:10.1016/0006-8993(94)90870-2

[213] Firdoz, S.; Ma, F.; Yue, X.; Dai, Z.; Kumar, A.; Jiang, B. A novel amperometric biosensor based on single walled carbon nanotubes with acetylcholine esterase for the detection of carbaryl pesticide in water. *Talanta,* **2010**, *83*, 269-273. doi:10.1016/j.talanta.2010. 09.028

[214] Hou, S.; Ou, Z.; Chen, Q.; Wu, B. Amperometric acetylcholine biosensor based on self-assembly of gold nanoparticles and acetylcholinesterase on the sol-gel/multi-walled carbon nanotubes/ choline oxidase composite-modified platinum electrode. *Biosens. Bioelectron,* **2012**, *33,* 44-49. doi:10.1016/j.bios.2011.12.014

[215] Iijima, S. Helical microtubules of graphitic carbon. *Nature,* **1991**, *354*, 56-58. doi:10.1038/354056a0

[216] Hu, C.; Hu, S. Carbon Nanotube-Based Electrochemical Sensors: Principles and Applications in Biomedical Systems. *J. Sensors,* **2009**, 1-40. http://dx.doi.org/10.1155/2009/187615

[217] Liu, G.; Lin, Y. Biosensor based on self-assembling acetylcholinesterase on carbon nanotubes for flow injection/ amperometric detection of organophosphate pesticides and nerve agents. *Anal. Chem.,* **2006**, *78*, 835-843. DOI: 10.1021/ac051559q

[218] Periasamy, A. P.; Umasankar, Y.; Chen, S.-M. Nanomaterials acetylcholinesterase enzyme matrices for organophosphorus pesticides electrochemical sensors: a review. *Sensors (Basel),* **2009**, *9*, 4034-5405. doi:10.3390/s90604034

[219] Kim, B.; Song, H. S.; Jin, H. J.; Park, E. J.; Lee, S. H.; Lee, B. Y.; Park, T. H.; Hong, S. Highly selective and sensitive detection of neurotransmitters using receptor-modified single-walled carbon nanotube sensors. *Nanotechnology,* **2013**, *24*, 285501. doi:10.1088/0957-4484/24/28/285501

[220] Schena, A.; Johnsson, K. Sensing acetylcholine and anticholinesterase compounds. *Angew. Chem. Int. Ed. Engl.,* **2014**, *53*, 1302-1305. DOI: 10.1002/ange.201307754

[221] Wang, F.; Liu, X.; Lu, C.-H.; Willner, I. Cysteine-mediated aggregation of Au nanoparticles: the development of a H2O2 sensor and oxidase-based biosensors. *ACS Nano.,* **2013**, *7*, 7278-7286. DOI: 10.1021/nn402810x

[222] Zhao, W.; Shan, S.; Wan, L.; Xu, J.; Chen, H. Acetylcholine Esterase Antibodies on BiOI Nano fl akes/TiO 2 Nanoparticles Electrode: A Case of Application for General Photoelectrochemical Enzymatic Analysis. *Anal. Chem.,* **2013**, *17*, 11686-11690 DOI: 10.1021/ac403691a

[223] Buiculescu, R.; Hatzimarinaki, M.; Chaniotakis, N. A. Biosilicated CdSe / ZnS quantum dots as photoluminescent transducers for acetylcholinesterase-based biosensors. *Anal. Bioanal. Chem*., **2010**, *398(7-8)*, 3015-3021. DOI 10.1007/s00216-010-4253-z

[224] Wei, J., Ren, J.; Liu, J.; Meng, X.; Ren, X. Biosensors and Bioelectronics An eco-friendly, simple, and sensitive fl uorescence biosensor for the detection of choline and acetylcholine based on C-dots and the Fenton reaction. *Biosens. Bioelectron.,* **2014**, *52*, 304-309. doi:10.1016/j.bios.2013.09.006

[225] Direnberger, S.; Mues, M.; Micale, V.; Wotjak, C.T.; Dietzel, S.; Schubert, M.; Scharr, A.; Hassan, S.; Wahl-Schott, C.; Biel, M.; Krishnamoorthy, G.; Griesbeck, O. Biocompatibility of a genetically encoded calcium indicator in a transgenic mouse model. *Nat. Commun.,* **2012**, *3,* 1031-1035. doi:10.1038/ncomms2035

[226] Nguyen, Q.-T.; Schroeder, L. F., Mank, M.; Muller, A.; Taylor, P.; Griesbeck, O.; Kleinfeld, D. An *in vivo* biosensor for neurotransmitter release and in situ receptor activity. *Nat. Neurosci.,* **2010**, *13*, 127-32. doi:10.1038/nn.2469

[227] Zacharis, C.K.; Theodoridis, G.A.; Voulgaropoulos, A.N. On-line coupling of sequential injection with liquid chromatography for the automated derivatization and determination of gamma-aminobutyric acid in human biological fluids. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **2004**, *808(2)*, 169-75. doi:10.1016/j.jchromb.2004.05.003

[228] Zhang H., Wu S.F., Yao H.Y. Determination of c-aminobutyric acid in rice germ by HPLC with automatic pre-column derivation and UV detection. *Food Fermen. Indus*. **2003**, *29*, 50-52.

[229] Klinker, C.C.; Bowser, M.T. 4-fluoro-7-nitro-2, 1, 3-benzoxadiazole as a fluorogenic labeling reagent for the *in vivo* analysis of amino acid neurotransmitters using online microdialysis-capillary electrophoresis. *Anal. Chem.*, **2007**, *79* (22), 8747-54. DOI: 10.1021/ac071433o

[230] Devall, A.J.; Blake, R.; Langman, N.; Smith, C.G.S.; Richards, D.A.; Whitehead K.J. Monolithic column-based reversed-phase liquid chromatography separation for amino acid assay in microdialysates and cerebral spinal fluid. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **2007**, *848(2)*, 323-8. doi:10.1016/j.jchromb.2006.10.049

[231] Uutela, P.; Ketola, R.A.; Piepponen, P.; Kostiainen, R. Comparison of different amino acid derivatives and analysis of rat brain microdialysates by liquid chromatography tandem mass spectrometry. *Anal.Chim. Acta,* **2009**, *633(2)*, 223-31. doi:10.1016/j.aca.2008.11.055

[232] Silva, D.M.D.; Ferraz, V.P.; Ribeiro, A.M. Improved high-performance liquid chromatographic method for GABA and glutamate determination in regions of the rodent brain. *J. Neurosci. Methods*, **2009**, *177(2)*, 289-93. doi:10.1016/j.jneumeth.2008.10.011

[233] Christensen, R.K.; Petersen, A.V.; Schmitt, N.; Perrier, J.-F.Fast detection of extrasynaptic GABA with a whole-cell sniffer. *Cell Neurosci.*, **2014**, *8*, 133. doi: 10.3389/fncel.2014.00133

[234] Jenden, D.J.; Booth, R.A.; Roch, M. Simultaneous microestimation of choline and acetylcholine by gas chromatography. *Anal. Chem*., **1972**, *44(13)*, 1879-81. DOI: 10.1021/ac60319a056

[235] Greaney, M. D.; Marshall, D.L.; Bailey, B.A.; Acworth, I. N. Improved method for the routine analysis of acetylcholine release *in vivo*: quantitation in the presence and absence of esterase inhibitor. *J. Chromatogr*. **1993**, *622(2)*, 125-35. doi:10.1016/0378-4347(93)80258-6

[236] Schuvailo, O. N.; Dzyadevych, S. V.; El'skaya, A. V.; Gautier-Sauvigne, S.; Csoregi, E.; Cespuglio, R.; Soldatkin, A. P. Carbon fibre-based microbiosensors for *in vivo* measurements of acetylcholine and choline. *Biosens. Bioelectron.*, **2005**, *21(1)*, 87-94. doi:10.1016/j.bios.2004.09.017

[237] Braun, K. L.; Hapuarachchi, S.; Fernández F. M.; Aspinwall C. A. High-sensitive detection of biological amines using fast Hadamard transform CE coupled with photolytic optical gating. *Electrophoresis*, **2007**, *28(17),* 3115-3121. DOI: 10.1002/elps.200700087

[238] Hapuarachchi, S.; Aspinwall, C.A. Design, characterization, and utilization of a fast fluorescence derivatization reaction utilizing o-phthaldialdehyde coupled with fluorescent thiols. *Electrophoresis*, **2007**, *28(7)*, 1100-1106. DOI: 10.1002/elps.200600567

[239] Song, Y.; Feng, Y.; LeBlanc, M. H.; Zhao, S.; Liu Y. M. Assay of Trace D-Amino Acids in Neural Tissue Samples by Capillary Liquid Chromatography/Tandem Mass Spectrometry. *Anal. Chem.*, **2006**, *78(23),* 8121-8128. DOI: 10.1021/ac061183w

[240] Day, B. K.; Pomerleau, F.; Burmeister, J. J.; Huetl, P.; Gerhardt, G. A. Microelectrode array studies of basal and potassium-evoked reléase of L-glutamate in the anesthetized rat brain. *J. Neurosci. Methods*, **2006**, *96*, 1626-1635. DOI: 10.1111/j.1471-4159.2006.03673.x

[241] Rahman, A.; Kwon, N. H.; Won, M. S.; Choe, E. S.; Shim Y. B. Functionalized Conducting Polymer as an Enzyme-Immobilizing Substrate: An Amperometric Glutamate Microbiosensor for *in vivo* Measurements. *Anal. Chem.*, **2005**, *77(15)*, 4854-4860. DOI: 10.1021/ac050558v

[242] Jamal, M.; Xu, J.; Razeeb, K.M. Disposable biosensor base don immobilisation of glutamate oxidase on Pt nanoparticles modified Au nanowire array electrode. *Biosens. and Bioelectron.*, **2010**, *26(4)*, 1420-1424. doi:10.1016/j.bios.2010.07.071

[243] Hamadan, K. S.; Zain, Z.M.; Halim, M.I.A.; Abdullah, J. M.; O'Neil, R.D. Development of a Brain Glutamate Microbiosensor. *World Acad. Sci. Eng. Technol.*, **2013**, *7(6)*, 1485-1489.

[244] Batra, B.; Kumari, S.; Pundier, C. S. Construction of glutamate biosensor base don covalente immobilization of glutamate oxidase on polypyrrole nanoparticles/polyaniline modified gold electrode. *Enzyme Microb. Technol.*, **2014**, *57*, 69-77. doi:10.1016/j.enzmictec.2014.02.001

[245] McLamore, E.S.; Mohanty, S.; Shi, J.; Claussen, J.; Jedlicka, S.S.; Rickus J.L.; Porterfield, D.M. A self-referencing glutamate Biosensor for measuring real time neuronal glutamate flux. *J. Neurosci. Methods*, **2010**, *189*, 14-22. doi:10.1016/j.jneumeth.2010.03.001

[246] Baker, D.A.; Xi, Z.; Shen, H.; Swanson, C.J.; Kalicas, P.W. The origin and neuronal function of *in vivo* nonsynaptic glutamate. *J. Neurosci.*, **2002**, *22(20)*, 9134-41. PMID: 12388621

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