Anal Bioanal Chem (2016) 408:2727–2741
DOI 10.1007/s00216-015-9160-x

REVIEW

Nanosensors for neurotransmitters

Elena Polo[1] · **Sebastian Kruss**[1]

Received: 21 August 2015 / Revised: 19 October 2015 / Accepted: 28 October 2015 / Published online: 19 November 2015

Abstract Neurotransmitters are an important class of messenger molecules. They govern chemical communication between cells for example in the brain. The spatiotemporal propagation of these chemical signals is a crucial part of communication between cells. Thus, the spatial aspect of neurotransmitter release is equally important as the mere time-resolved measurement of these substances. In conclusion, without tools that provide the necessary spatiotemporal resolution, chemical signaling via neurotransmitters cannot be studied in greater detail. In this review article we provide a critical overview about sensors/probes that are able to monitor neurotransmitters. Our focus are sensing concepts that provide or could in the future provide the spatiotemporal resolution that is necessary to 'image' dynamic changes of neurotransmitter concentrations around cells. These requirements set the bar for the type of sensors we discuss. The sensor must be small enough (if possible on the nanoscale) to provide the envisioned spatial resolution and it should allow parallel (spatial) detection. In this article we discuss both optical and electrochemical concepts that meet these criteria. We cover techniques that are based on fluorescent building blocks such as nanomaterials, proteins and organic dyes. Additionally, we review electrochemical array techniques and assess limitations and possible future directions.

Published in the topical collection featuring *Young Investigators in Analytical and Bioanalytical Science* with guest editors S. Daunert, A. Baeumner, S. Deo, J. Ruiz Encinar, and L. Zhang.

✉ Sebastian Kruss
skruss@uni-goettingen.de

[1] Institute of Physical Chemistry, Göttingen University, 37077 Göttingen, Germany

Keywords Nanosensors · Fluorescent probes · Neurotransmitters · Chemical imaging · Electrochemistry

Introduction

Neurotransmitters are molecules that are used by cells to exchange information. Their most prominent role is chemical neurotransmission in the brain. Therefore, their detection is necessary to understand how the brain works but also to cure and treat diseases [1]. Interestingly, other cells are also able to produce and detect neurotransmitters but this process is much less explored [2]. The typical picture that we have of neurotransmitter release is shown in Fig. 1a. Neurons have different types of protrusions and form 'kissing points' (synapses) between each other. Neurotransmitters are stored in vesicles and when an electrical potential arrives at the synapse, a complex cascade is started [3]. The vesicles fuse with the cell membrane and release their content into the extracellular space, which is usually the nanometer scale synaptic cleft [4]. The released neurotransmitters are freely diffusing and binding to receptors of a second cell on the other side of the synaptic cleft, which triggers further electrical or chemical processes. This picture of neurotransmitter release is oversimplified because it reduces the role of neurotransmitters to simply bridging one side of the synaptic cleft to the other side of the synaptic cleft. Obviously, such a view raises the question why detection of neurotransmitters should provide any more information than measuring the electrical processes that lead to release of neurotransmitters. In reality, neurotransmitters are not confined to the synaptic cleft, but spill over and diffuse to hundreds or thousands adjacent neurons (non-synaptic or volume transmission) [5, 6]. Additionally, more than one neurotransmitter can be released at a time (co-release) [7]. It is also known that neurons can switch between different

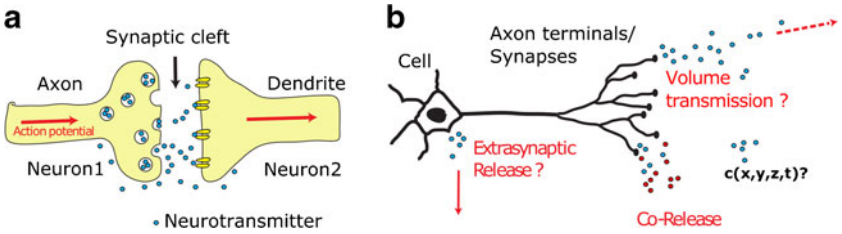

Fig. 1 Neurotransmitter release and analytical challenges. (**a**) Schematic of a synapse that connects two neurons. (**b**) Modes of neurotransmitter release beyond the simplified synapse picture (other cells/synapses are not shown for clarity)

neurotransmitters (switching) [8]. Altogether only tools that directly monitor chemicals can assess these aspects of chemical communication [9–11].

Analytical chemistry provides many tools to detect the presence of neurotransmitters. Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) are noninvasive and work in vivo but they lack the temporal, spatial, or chemical resolution that is necessary to study neurotransmitters around single cells [12, 13]. Classic analytical techniques such as mass spectroscopy provide exquisite sensitivity and can identify and distinguish different neurotransmitters such as γ-aminobutyric acid (GABA) even inside single cells [14]. However, most approaches measure rather static concentrations and not temporal fluctuations of analyte concentrations in space. These fast location-dependent fluctuations contain a large portion of the information that finally governs biology. Therefore, sampling on a similar length and time scale is desirable.

Very good examples to illustrate how a tool from analytical chemistry influences a whole field are fluorescent calcium-sensitive probes. By using them it is now possible to image the activity (calcium-dynamics) of neural circuits in freely moving animals [15, 16].

Similarly, sensors for neurotransmitters could have tremendous impact on the life sciences. However, the development of neurotransmitter sensors is still in its infancy, but the goal is clear: chemical imaging of neurotransmitters around cells with high spatial and temporal resolution.

In this review article, we focus on sensing concepts that allow the detection of neurotransmitters around cells with high spatiotemporal resolution. Neurotransmitter detection in bulk samples is an important but different topic and will not be discussed here and we refer to other reviews [17]. Our criterion is that the approach is or could in the future enable chemical imaging of dynamic neurotransmitter changes in networks of cells. For example, we will not discuss concepts that are based on the aggregation of nanoparticles because such a sensing scheme would be difficult to implement for spatiotemporal detection. We will discuss electrochemical concepts with multiple electrodes even though they are not nanoscale but further miniaturization seems to be only a matter of time. Our criteria limit the number of published reports mainly to optical methods that have been demonstrated in vitro.

Under the section heading "Requirements and design criteria for spatiotemporal neurotransmitter sensors" in this review article, we discuss the length, time, and concentration scales that are relevant for spatiotemporal neurotransmitter detection (imaging) around cells. This chapter can serve as an empiric guideline for the design of novel sensors. Owing to the limited number of reports that met our criteria yet, we will organize the article along different techniques and not different neurotransmitters. Some of the sensing concepts that are discussed here are real 'sensors,' whereas for others the term 'probes' would be more appropriate. In order to simplify the text for the reader, we will nevertheless name all of them sensors from now on.

Under the heading "Optical sensing concepts" we review optical techniques that are based on fluorescent building blocks. Under the heading "Electrochemical sensing concepts" we present electrochemical concepts that allow parallel (spatial) detection. Finally, we compare the different approaches and discuss limitations and future directions in the Conclusions.

Requirements and design criteria for spatiotemporal neurotransmitter sensors

A central question for analytical techniques is the requirements for measuring biologically relevant concentrations. In this section, we will discuss time, length, and concentration scales for the neurotransmitter dopamine because it is better studied than other neurotransmitters. Even though these numbers are specific for certain cell lines and for dopamine, they provide an idea of the scales that are involved.

Static neurotransmitter concentrations in brain tissue have been measured but dynamic concentration changes remain a challenge. However, it is possible to derive some numbers. The probably best-understood model system for exocytosis is the pheochromocytoma cells (PC12) [18]. They release catecholamines including dopamine upon stimulation, which has been studied by electrochemical techniques. Dopamine containing vesicles have a size of≈150 nm (determined by electron microscopy). The number of molecules in isolated vesicles is≈230 000 (380 zmol) [19]. If we assume that the vesicles are spheres, the concentration of dopamine inside those

vesicles is≈0.2 M. However, by analyzing amperometry data it was found that only a fraction of the dopamine content is released (85 000 molecules, 140 zmol) [19]. In vivo cyclovoltammetry measurements of dopamine release from the nucleus accumbens in rats showed concentrations of 250 nM (extracellular space) [20]. Using reasonable size assumptions, concentrations of 1.6 mM in the synaptic cleft and 25 mM in the vesicles were estimated [20]. However, those numbers approximate real concentrations because the electrodes were larger than the biological structures and the distance between probe and release sites is difficult to control.

In this context, it is not useful to talk only about absolute concentrations and limits of detection because the time and spatial resolution is equally important. Figure 2 shows the simulated results for dopamine release from a point source [5]. The parameters including the diffusion constant for dopamine are for brain tissue at 37 °C. In general, diffusion from a point source in open space can be described by $c(r,t) \sim \frac{e^{\frac{-r^2}{4Dt}}}{(Dt)^{\frac{3}{2}}}$ where c is the concentration at distance r and after time t [5]. D is the diffusion constant. Figure 2a shows the time-dependent profile in a certain distance (black curves). From these plots it is obvious that the necessary sensitivity of a sensor depends on the location but also on the acquisition speed. Cells can also uptake dopamine and therefore the presence of transporters changes local concentrations (red curves).

Figure 2b shows 'plumes' where dopamine reaches a certain maximum concentration. The simulations that are presented in Fig. 2 are based on the parameters known for single vesicle events. If more vesicles are involved and parameters change (e.g., more neurotransmitters per vesicle) the spatiotemporal concentration profiles change but could be approximated by superposition of single release events.

Nevertheless, we can derive a few specifications for neurotransmitter sensors that are able to catch spatiotemporal dynamics: For example, a fast (ms) nanoscaled sensor with nM-sensitivity/dynamic range would be useful to observe the release but not the concentration gradient around the cell.

Moreover, μM-mM concentrations of neurotransmitters can be expected but only for short time periods (mM≈1 ms, μM≈100 ms) and depending on the distance to the source (μM for distances<10 μm and time periods≈100 ms). The binding kinetics of the neurotransmitter to the sensor should be fast (ms) to ensure that the sensor 'sees' the analyte before it has diffused away. Finally, if we want to capture the profile, we have to place multiple sensors around the cell and the sensors have to be small (<1000 nm). Such sensors would enable spatiotemporal imaging of neurotransmitter dynamics.

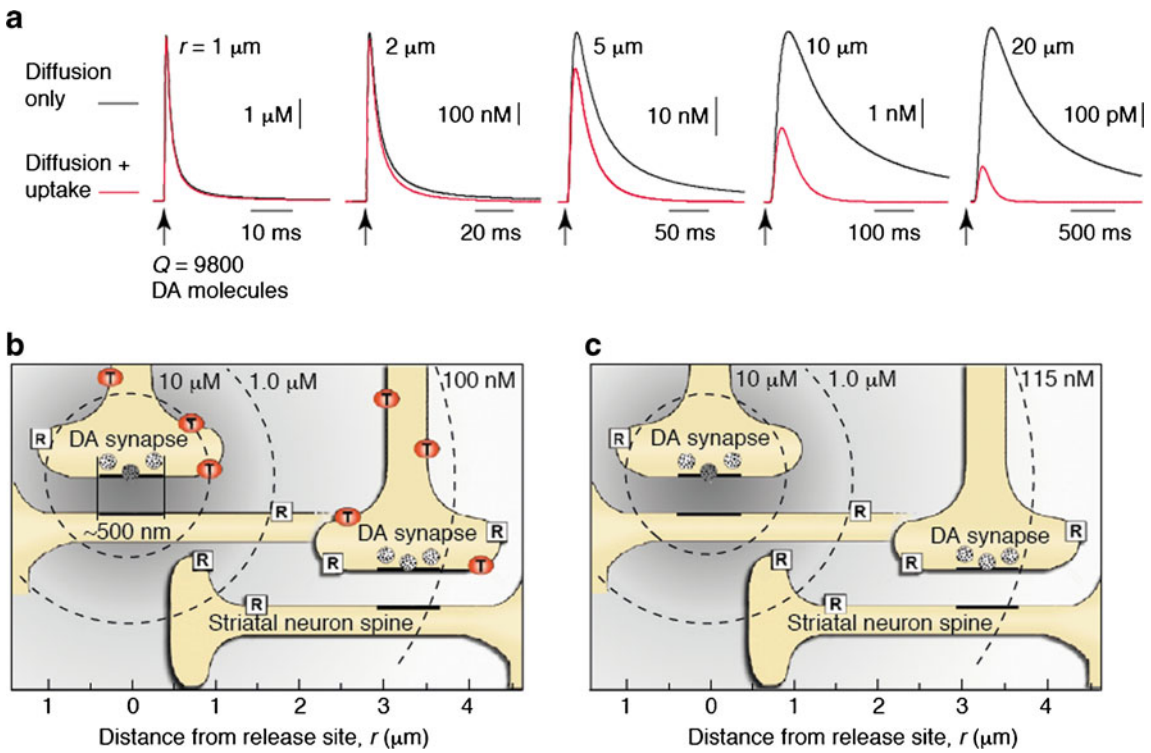

Fig. 2 Concentration, length and time scales of neurotransmitter release. (**a**) Simulated concentrations of dopamine (Q=9800 molecules) released from a point source in different radial distances. Black: without uptake by dopamine transporters. Red: with uptake. (**b**)Typical scenario of a dopamine releasing synapse with adjacent neurons and dopamine receptors (R) and transporters (T). The dashed arcs define the regions in which the specified (maximum) concentrations are reached. (**c**) Same plot as in b but without uptake by dopamine transporters. Reproduced and modified with permission from [5]

Optical sensing concepts

In this review article, our aim is to discuss concepts that are compatible with the idea of spatiotemporal imaging of neurotransmitters around cells. Therefore, optical sensors hold very much promise and here we discuss different building blocks, their limitations, and possible future directions. Fluorescence provides several key advantages in imaging and we organized this section according to the active/fluorescent building block in the sensor.

Fluorescent nanomaterials

Nanomaterials are valuable building blocks for a variety of applications and devices ranging from basic research, biosensors, biomedicine to energy applications [21–29]. In the context of biosensing, they are valuable because they provide rich optoelectronic properties and their size matches relevant biological length scales.

Optical properties of nanoparticles are often superior compared with classic fluorophores. Therefore, they are used more and more as building blocks for sensors [30, 31]. The underlying idea is always the same: the nanomaterial provides superior fluorescence characteristics and the environment has to be designed in such a way that fluorescence changes in the presence of the analyte.

An interesting class of fluorescent nanomaterials is carbon nanotubes. Single walled carbon nanotubes (SWCNTs) are rolled-up cylinders of graphene. Semiconducting SWCNTs have a bandgap and fluorescence in the near-infrared (nIR) region of the electromagnetic spectrum [32]. This region is beneficial for biological applications because it falls into the tissue-transparency window. Moreover, the ultra-low photobleaching and absence of intrinsic blinking make these materials ideal building blocks for biosensors [33]. In the past few years, fluorescent SWCNT-based sensors for different analytes such as glycans, proteins, and reactive oxygen species have been developed [33–36].

Recently, Kruss et al. reported on a concept to detect neurotransmitters by using polymer-functionalized SWCNTs [37]. The basic idea is that the SWCNT's fluorescence is very sensitive to the chemical environment. However, this sensitivity is not very useful if it is not selective. Therefore, the polymer serves two functions. First, it is necessary to noncovalently solubilize the (hydrophobic) SWCNTs in aqueous solution. Second, it enables molecular recognition and plays the role of a transducer between the SWCNT's optical properties and binding of the analyte (Fig. 3a).

The authors found that certain polymers and especially certain DNA-sequences create an organic phase around the SWCNTs that enables the detection of neurotransmitters, most notably catecholamines such as dopamine. In the presence of the neurotransmitter dopamine the nIR-fluorescence of the SWCNTs increased (Fig. 3b). Single $(GT)_{15}$-ssDNA wrapped SWCNTs even increased their fluorescence by a factor >400 %.

These sensors are reversible as shown by flow chamber experiments on the single nanotube level (Fig. 3d). Interestingly, the selectivity profile shows that the sensor cannot distinguish between dopamine, epinephrine, and norepinephrine (Fig. 3c). Dopamine and its homologues are redox active, which could explain why homologues interfere but it cannot explain why substances of similar redox potential (homovanillic acid, uric acid) do actually not interfere and the response of other dopamine homologues is smaller (DOPAC, L-DOPA).

This biosensor concept paves a clear route for the future because it indicates that variation of the polymer sequence (e.g., DNA) or composition yields sensors with different selectivity and sensitivity. The molecular recognition of the polymer phase is surprising and not yet completely understood. The authors showed in single-molecule experiments that the DNA-conformation on the nanotube changes in the presence of dopamine, which suggests that dopamine interactions move quenching moieties of the DNA away from the surface of the SWCNT. As a consequence, the quantum yield and, therefore, fluorescence intensity increased.

Another theory for molecular recognition on SWCNTs assumes that adsorption of the analyte changes the fluorescence. The DNA-sequence wraps with a specific pitch and that controls which analytes do adsorb on the nanotube surface and which do not [38]. Nevertheless, this concept cannot directly explain how signal transduction (turn-on) and selectivity of this dopamine sensor works.

The nIR-fluorescence of SWCNTs is a very attractive property but needs also special equipment. Thus fluorescent building blocks in the visible region are valuable, too.

One of the first examples for a fluorescent nanoparticle capable of dopamine detection is from the lab of Willner et al. [39]. Cd-Se-ZnS quantum dots (QD) were functionalized with a boronic acid moiety. Boronic acids are known to bind molecules with two adjacent hydroxy-groups and therefore they bind the catechol-part of dopamine. An organic fluorophore was conjugated to dopamine so that it binds to the boronic acid groups on the QD. Fluorescence of the QD is decreased through Förster resonance energy transfer (FRET) until a free dopamine molecule replaces this compound. Boronic acids also bind sugars and therefore this concept is in general useful to detect species with two adjacent hydroxy groups. A drawback of this method is that when the dopamine-fluorophore conjugate is replaced, the sensor loses its sensitivity. Therefore it would be difficult to operate such a sensor reversibly in a biological setting.

Another example for a Cd-Se-ZnS-based sensor uses adenosine capping to impart selectivity [40]. In the presence of

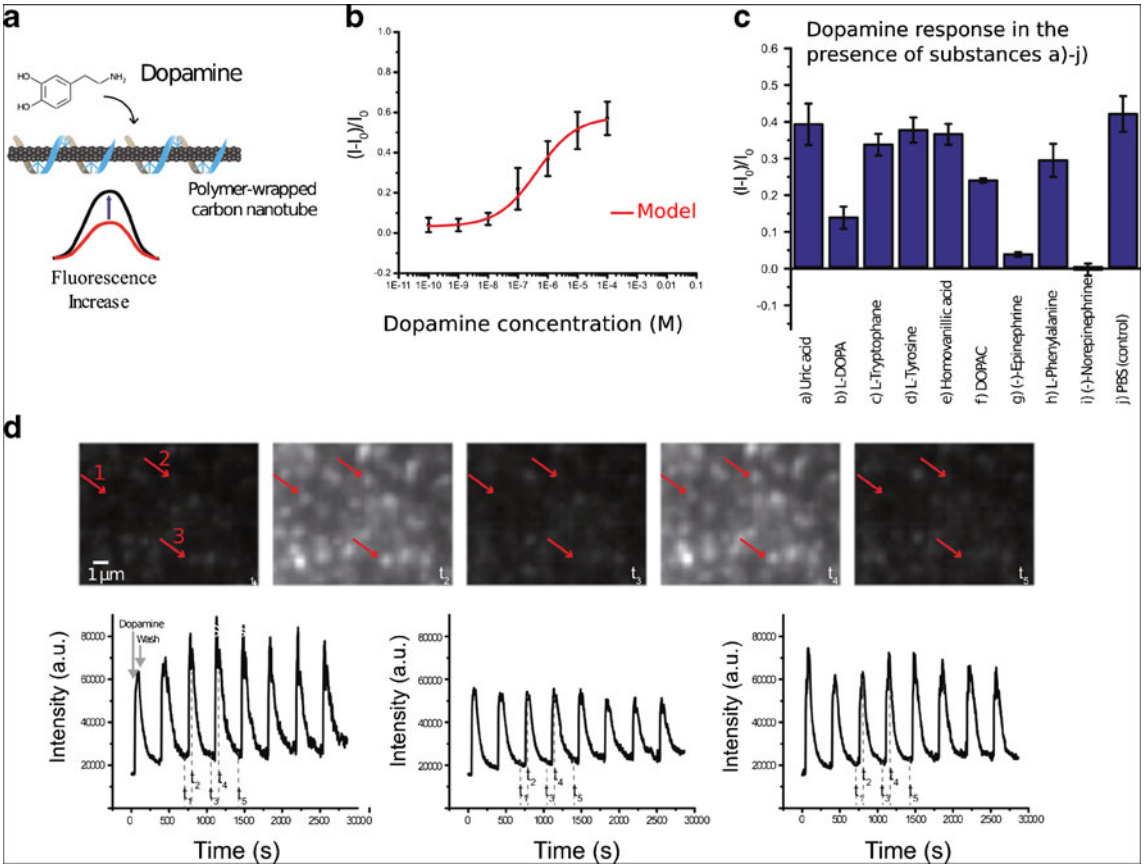

Fig. 3 Dopamine sensors based on fluorescent carbon nanotubes. (**a**) Near infrared (nIR) fluorescent SWCNTs serve as central building block. The polymer (e.g. DNA) is responsible for molecular recognition and signal transduction. In the presence of dopamine, the polymer/SWCNT complex increases its fluorescence. (**b**) Normalized fluorescence change of a $(GT)_{15}$-ssDNA-SWCNT complex as a function of dopamine concentration. Limit of detection=11 nM. (**c**) Dopamine response (100 μM) of the sensor in the equimolar presence of homologues. (**d**) Single nanotube sensors imaged in the nIR on a surface. The red arrows indicate single nanosensors (resolution-limited spots). The surface was mounted in a flow chamber and periodically exposed to dopamine (100 μM) and buffer. The traces show that the sensor is periodically lightening up and reversibly reporting about the local dopamine concentration. Adapted with permission from [37]

dopamine, this sensor gets quenched (LOD=30 nM, in a pure buffer solution). The authors hypothesized that oxidized dopamine (dopamine-quinone) binds to adenosine. Fluorescence is then quenched by an electron transfer from QD to quinone as evidenced by a lower fluorescence life time. Interestingly, this sensor showed a good selectivity towards ascorbic acid and uric acid, which are common interferents in redox-based sensing schemes.

Another carbon nanomaterial that has been explored for bioanalytical applications is graphene oxide. Graphene oxide nanosheets fluoresce at 660 nm and are quenched by dopamine, which can be used for sensing applications [41]. The authors hypothesized that dopamine binds to graphene oxide via π–π stacking and photo-induced charge transfer is responsible for quenching. This effect was used to determine dopamine concentrations in urine samples. The finding that bare graphene oxide responds to dopamine is important. However, it is likely that further engineering of molecular recognition is necessary to distinguish between dopamine and other catecholamines or other molecules with extended π-systems.

Engineering of selectivity is central for sensor design. Yu et al. showed how fluorescent mesoporous silica nanoparticles and cyclodextrines form a tandem system for dopamine detection [42]. The basic idea is that the cyclodextrines cover the porous nanoparticle and serve as a selective diffusion barrier because only molecules of the right size and charge distribution can pass the cyclodextrine pore. If dopamine is present and enters, it increases the silica nanoparticle's fluorescence emission at 440 nm. The sensor showed an interesting selectivity profile. Epinephrine and ascorbic acid did not interfere but norepinephrine and histidine showed similar responses as dopamine. This result suggests that optimization of the cyclodextrine barrier could lead to even better selectivity.

Zhang et al. synthesized fluorescent silicon nanoparticles (SiNPs) to detect dopamine [43]. They used amino functionalized SiNPs and when exposed to dopamine, fluorescence was dramatically quenched. The approach showed high sensitivity (LOD=0.3 nM, without interfering molecules) and a certain selectivity as evidenced by a four times smaller response to norepinephrine. The mechanism was described as

a FRET-based mechanism. However, upon addition of dopamine, the samples needed >3 h until constant fluorescence was reached. Dopamine is a redox-active compound and therefore the long reaction time of the nanoparticles could indicate that the results are biased by dopamine polymerization. So far the detection time of 3 h is not suitable for detection of fast neurotransmitter dynamics.

All examples that were discussed so far are sensors for catecholamines, and there are a few more examples in literature such as pollypyrol/graphene oxide quantum dots hybrids [44] or nitrilotriacetic acid functionalized graphene oxide [45].

Catecholamine neurotransmitters such as dopamine are very important and they are involved in reward control, learning, but also in diseases such as Parkinson's disease [46, 47]. Their chemistry is special because they are redox active and polymerize [48, 49]. This property can bias results because simultaneous polymerization of catecholamines can mask the real sensor response. Therefore it is necessary to rule out artifacts by performing control experiments. Imaging a single sensor and exposing it to catecholamines for time periods less than the typical time scale of polymerization is a good way to prove this (see Fig. 3c). Another way is to show reversibility. The redox activity is also relevant for the mechanism of recognition. For electrochemical sensors oxidation is the clear mechanism, but for the mentioned fluorescent sensors the situation is less clear because it is difficult to distinguish between molecular recognition of a redox active compound and oxidation of a redox active compound.

A key challenge of all mentioned approaches is engineering of selectivity. Therefore it is a common approach in the biosensors field to use biological recognition units to impart selectivity. Cash and Clark used enzyme-coated phosphorescent nanoparticles to detect histamine even in vivo [50]. The enzyme catalyzes the reaction of oxygen with histamine (Fig. 4a). Thus, the oxygen concentration is locally reduced in the presence of histamine. At the same time, the polymer-nanoparticle is oxygen-sensitive because it is loaded with a phosphorescent platinum complex that is quenched by oxygen. In consequence histamine decreases the local oxygen concentration, which finally increases the phosphorescence (Fig. 4b). This phosphorescence signal was used to demonstrate that histamine injections into mice could be monitored (Fig. 4c). This approach is very powerful because it combines advantages of artificial nanosensors and the selectivity of a natural enzyme.

In summary, fluorescent nanomaterials provide usually superior optical properties. Most examples, however, do not yet provide the selectivity that is desired for biological applications. Nevertheless, in the future, precise engineering of molecular recognition could tremendously increase selectivity. Another challenge is to increase the time resolution of the imaging approaches.

Engineered proteins

Neurotransmitters convey information and therefore nature has developed tools (receptors) to bind and detect neurotransmitters. Thus, a valid strategy is to use and integrate neurotransmitter receptors into sensors. The high selectivity of molecular recognition is a key advantage of this concept. Usually these sensors end up on the surface of cells and therefore they can only report about neurotransmitter concentrations close to the cell surface. In this section we will discuss three complementary approaches: (1) all-protein- based sensors; (2) hybrid protein-fluorophore sensors; and (3) cell-based sensors.

Genetically encoded fluorescent sensors hold very much promise for neurotransmitter detection [51]. They benefit from high selectivity of naturally occurring proteins and genetic targeting.

One of the first examples was a genetically encoded fluorescent sensor for glutamate [52]. It consists of a bacterial glutamate-binding protein (ybeJ) and two fluorescent proteins (YFP and CFP). Förster resonance energy transfer (FRET) between these two fluorophores depends on the conformation of the glutamate binding protein. Therefore, changes of the FRET signal can be used to detect glutamate. The sensor mutant with the highest affinity provided a K_d of 630 nM. The authors called this sensor fluorescent indicator protein for glutamate (FLIPE) and also attached a periplasmic binding sequence that directs the sensor to the membrane of the transfected cells.

When hippocampal neurons were electrically stimulated, the sensors on the surface of the cells responded and indicated a glutamate concentration of≈300 nM. This approach is one of the first that enabled imaging of neurotransmitters instead of imaging related processes such as vesicle fusion.

One limitation of this method is that the conformational change of the binding protein is rather small, which limits its sensitivity and signal to noise ratio. Another drawback is the broad absorption and emission spectrum of the fluorophores and a lower photostability of the fluorescent proteins compared with advanced organic dyes or fluorescent nanomaterials. Similar examples have been reported for an improved sensor (SuperGluSnFR) [53] and an intensity-based sensor (iGluSnFR) [54]. For a complete overview on this topic, we refer to the review by Tian et al. [51].

The before-mentioned example relies on a conformational change upon binding of the neurotransmitter. Additionally, the conformational change has to impact the FRET signal. Not all proteins will provide these two properties. It is also very difficult to foresee which modifications of the sequence will lead to higher sensitivity etc. Therefore, rational design of such sensors is difficult.

Johnsson et al. presented a semisynthetic approach to overcome certain limitations [55]. They designed a metabolite-binding protein conjugated to a fluorophore A and a SNAP-

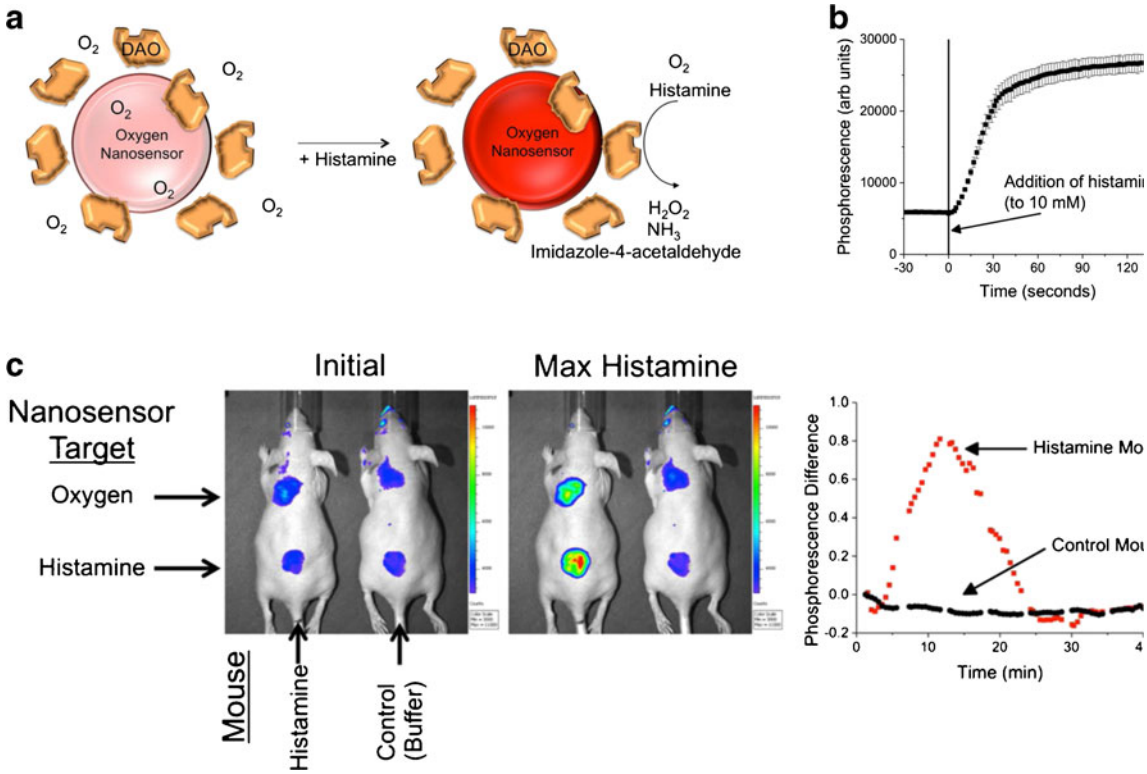

Fig. 4 Enzyme conjugated fluorescent polymer nanoparticles for histamine detection. (**a**) Schematic of the concept. The enzyme diamine oxidase (DAO) consumes local oxygen when oxidizing histamine. The enzyme is conjugated to a phosphorescent polymer nanoparticle that is quenched by oxygen. (**b**) Sensor response to histamine. (**c**) In vivo experiments demonstrating the ability of intradermal sensors to continuously monitor histamine levels. As histamine levels increase (via injection), the sensor's phosphorescence drastically increases (left mouse) compared with the control (right mouse). Reproduced with permission from [50]

tag (see Fig. 5a). The SNAP-tag is used to attach a tethered synthetic ligand, which also contains a second fluorophore B. The synthetic ligand competes with the analyte for the binding pocket of the protein. The FRET-signal between the two fluorescent parts A and B changes if the analyte binds. Fluorophore A can be either an organic fluorophore or a fluorescent protein. This concept is very modular because it uses well-defined building blocks. Engineering this FRET signal appears to be easier because it does not rely on a complex conformational change but the binding of a ligand to a defined binding pocket on a protein. It was used to detect sulfonamides. Later it was shown that the protein part of the sensor can be genetically encoded and presented on the surface of live cells [57]. This concept was named SNIFIT (SNAP-tag based indicator proteins with a fluorescent intramolecular tether). For this purpose the concept was generalized by incorporating a CLIP-tag for artificial fluorophores. The protein part can be genetically encoded, but to receive a functional sensor, the donor fluorophore and the ligand with an acceptor fluorophore have to be conjugated via the SNAP-tag and CLIP-tag.

This strategy has been used to create sensors for glutamate [58], γ-aminobutyric acid (GABA) [56], and acetylcholine [59]. The concept of these sensors is similar and here we will only discuss the GABA sensor in greater detail. In this case, the binding protein was the $GABA_B$ receptor, the donor fluorophore DY-547 (conjugated via CLIP-tag), and the acceptor fluorophore Cy5 (part of the tethered ligand and conjugated via SNAP-tag). The ligand's structure was based on the known GABA-antagonist CGP 51783 ((3-[[4-chlorophenyl)methyl]amino-propyl]-(P-diethoxymethyl)-phos-phinic acid). In the presence of GABA, the ligand is displaced and the FRET signal changes (Fig. 5b). In this work, the building blocks of the SNIFIT sensor were varied to increase the sensor's performance. For example, the FRET-ratio was typically around 1.5 but depended on the nature of the two fluorophores. Further variation of these parameters likely yields even better sensors. $GABA_B$ is a G-protein coupled receptor (GPCR). When GABA binds, the GPCR gets activated; this finally triggers the release of calcium from the endoplasmatic reticulum. The GABA-receptor part of this SNIFIT was still functional and calcium-sensitive dyes were used to quantify how much GABA is needed for a certain calcium response (EC_{50}=56 nM). In order to demonstrate the use of this concept in biological system, HEK 293 cells were transfected and the FRET-response was used to calibrate

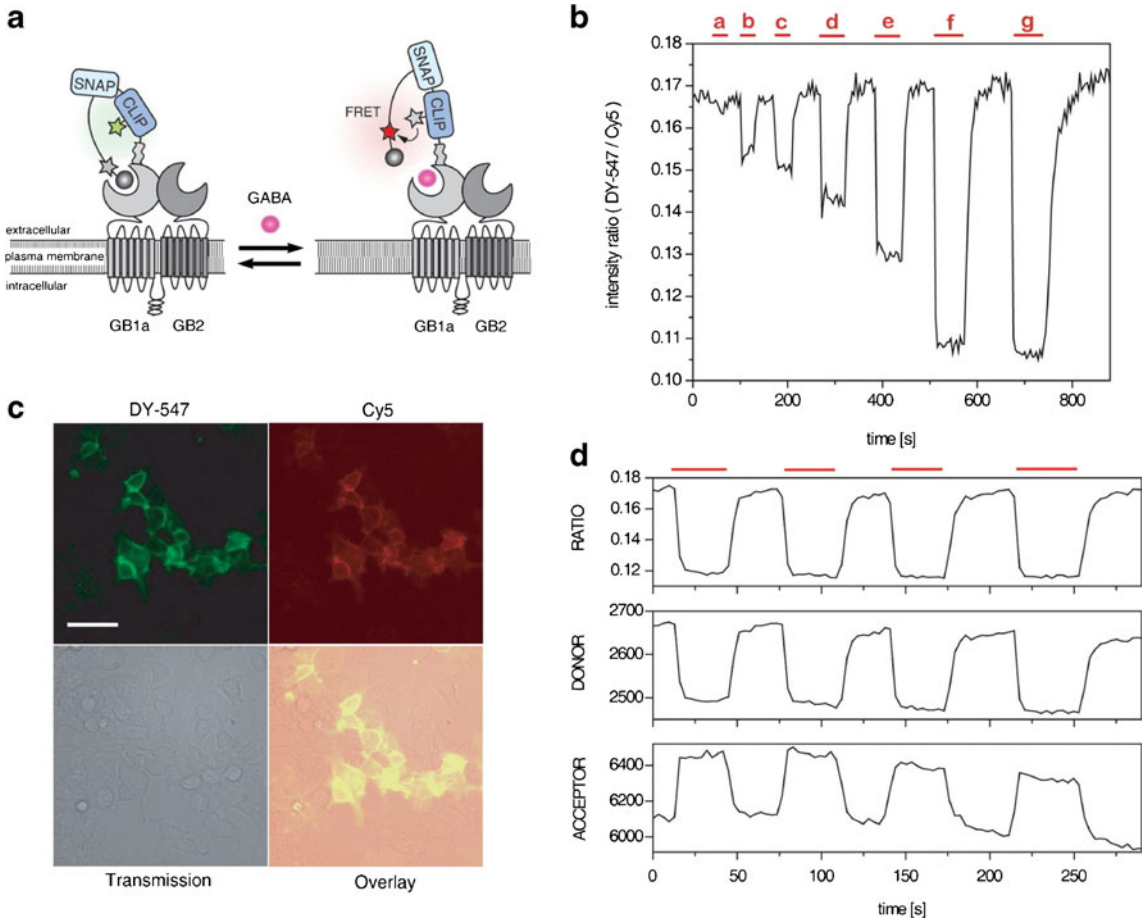

Fig. 5 Semisynthetic protein-based sensors for GABA. (**a**) Schematic of a semisynthetic (SNIFIT) sensor. It contains a fusion protein with a SNAP-tag, CLIP-tag, and a receptor protein (RP) for the analyte of interest (molecule containing a fluorophore is marked as red star, ligand as gray ball, CLIP-tag labeled with a second fluorophore as green star). GABA-SNIFIT detects GABA through displacement of the intramolecular antagonist and a resulting change in FRET efficiency. (**b**) Perfusion of GABA-SNIFIT with increasing GABA concentrations on the surface of HEK 293. Cells were perfused with GABA (a=1 μM, b=10 μM, c=20 μM, d=40 μM, e=100 μM, f=1 mM, g=10 mM). Shown is the ratio of donor (DY-547) and acceptor (Cy5) emission. (**c**) Microscope image of donor channel (DY-547), FRET channel (Cy5), and transmission channel, scale bar 50 μm. (**d**) Time course of the intensity ratio of donor emission versus acceptor emission (top), of the donor channel (middle), and the acceptor channel (bottom) upon addition and removal of 1 mM GABA. The red bar indicates the time span of GABA perfusion. Reproduced with permission from [56]

the system (Fig. 5 c, d). For this purpose, the cells were perfused with different concentrations of GABA and the FRET signal from the cell surface was analyzed (K_D=100 μM).

So far this approach was not used to detect physiological GABA-release from cells. Some of the characteristics of the discussed sensor are not yet suitable to detect biological neurotransmitter signals. Opening and closing kinetics of the sensor are in the order of seconds, which is not the time scale that is requested for many biological experiments (milliseconds). However, the perfusion experiments may have convolved the kinetics and the real kinetics might be faster. Another issue is the sensitivity of the sensors. The acetylcholine-SNIFIT could detect acetylcholine in the 1–100 mM range with sensor kinetics in the seconds' range [59]. Therefore, fast synaptic release events would be difficult to capture. Even though sensor characteristics are not yet in the desired range for certain neurobiological applications, it is important to stress that there is currently no other technology available that can image neurotransmitter release on this scale. Therefore, further improvements in terms of time resolution and sensitivity could make these sensors very useful for biology.

A central topic for all discussed approaches is optimization of the sensor. Even if there is a rational design strategy, building blocks, sequences, etc. have to be varied to find the best candidate.

Takikawa et al. addressed this challenge by using a high-throughput screening approach to identify hybrid protein-fluorophore sensors for glutamate [60, 61]. The sensor consists of a mutant glutamate-binding protein (GlutBP), which was derived from the glutamate receptor GluA2 subunit. They used a cysteine residue in the protein to attach a fluorophore. Therefore, this approach combines the selectivity of a natural

recognition unit and the beneficial photophysical properties of an artificial fluorophore. The sensor concept is based on the idea that glutamate binding causes conformational changes that affect the fluorescence of the sensor. Obviously the location of the fluorophore plays a central role and, therefore, they used cysteine-scanning mutagenesis to create different GlutBP variants and screened the response to glutamate. They identified 28 candidates with fluorescence responses >20 %. The best candidate (eEOS) showed a fluorescence enhancement of 2400 % upon addition of glutamate. The increase of fluorescence is due to an increase of the fluorescence quantum yield from 0.045 to 0.82. However, the affinity for glutamate was around two orders of magnitude lower (EC_{50}=66 μM) than for the original GlutBP. The authors attached this sensor to the surface of neurons, electrically stimulated them, and imaged glutamate release. The signal to noise ration of this approach appears to be very high, which enabled imaging of glutamate release from cells. For certain biological applications it is a drawback that the sensors were not expressed on the cells. Another general advantage of FRET approaches such as SNIFIT compared with this intensity-based method is that a ratiometric signal is more robust to changes in the optical path etc.

The previous examples show that even though there is tremendous progress, most optical sensors for neurotransmitters still lack the sensitivity and time resolution that neuroscience requests. Genetically encoded fluorescent calcium sensors are more advanced in their development and are heavily used in biological research.

The group of Kleinfeld made use of these existing sensors and developed cell-based neurotransmitter fluorescent engineered reporters (CNiFERs) [62]. In this sensing scheme, a cell is transfected with a neurotransmitter receptor that triggers a rise of the cytosolic calcium concentration. This signal is then detected by the calcium sensors. Thus, the whole cell serves as a sensor. The first realization of this concept was demonstrated in transfected HEK cells with a M1 muscarinic receptor to detect acetylcholine. These cells were deposited in the frontal cortex of rats and used to quantify the impact of neuropharmaceuticals on acetylcholine signaling.

In this section the authors discussed protein-based neurotransmitter sensors ranging from pure proteins, semisynthetic proteins, to whole cells. The major advantage of all these concepts is the high selectivity that is accomplished by borrowing technology from nature. Another key advantage, which is often underestimated by analytical chemists, is the possibility to transfect and genetically target specific cells. Drawbacks are signal to noise ratios and in some cases time resolution. Moreover, these sensors are in general limited to the cell membrane and do not provide information about the extracellular space. Some approaches try to combine the advantages from two worlds by combining proteins and photophysically more sophisticated artificial fluorophores. In the future, using fluorescent nanomaterials as building blocks that specifically attach to genetically encoded proteins might be another promising route.

Small organic fluorophores

Organic fluorophores are valuable building blocks for biosensors because they are small, cheap, possess beneficial photophysical properties, and are available with different chemical structures. Recently, a very good overview that focused on chemical probes for neurotransmitters was published [63]. Here, we will highlight a few examples that have the potential to image neurotransmitter release.

In this context it is important to distinguish between direct detection of neurotransmitters via probes/sensors and fluorescent labeling of structures that are related to neurotransmitter release. For example, fluorescent staining of neurotransmitter-containing vesicles has been used to visualize the process of exocytosis [64–67]. Even though this process is correlated with neurotransmitter release, it cannot replace a method that directly detects the neurotransmitter.

Another example for an indirect (nonlabel-free) method is false fluorescent neurotransmitters (FFNs) [68]. FFNs are fluorescent molecules that have a similar structure as neurotransmitters. The concept was demonstrated with dopamine FFNs. The basic idea is that the FFN is up-taken into the cell and later into vesicles via monoamine transporters. When cells are stimulated to do exocytosis, the FFNs are released and imaging vesicles provides valuable information about this process. The concept was demonstrated for single cells but also in brain slices.

The scope of this review article is to discuss concepts that enable spatiotemporal detection of neurotransmitters in the extracellular space. However, most examples of neurotransmitter sensitive fluorophores are designed for detection in bulk liquid samples or rely on processes that are not compatible with biological experiments [63]. If at all, they are designed to work inside cells. Nevertheless, here we review a few promising concepts because we anticipate that they could be easily used outside cells for spatiotemporal imaging.

An early example for the detection of catecholamines including dopamine was presented by Secor and Glass [69]. The sensor molecule consisted of a coumarin aldehyde conjugated to a boronic acid derivate. The idea of this design was that the amine group of the catecholamines reacts with the aldehyde forming an iminium ion. Additionally, the catechol moiety is supposed to react with the boronic acid to form a boronate ester. The fluorescence of the coumarin building block (505 nm emission) changed in the presence of catecholamines (K_D≈300 μM for dopamine). The sensor showed similar responses for catecholamines (dopamine, epinephrine, and norepinephrine) but much smaller responses for amines (lysine, glutamic acid) or sugars.

Later on, the original catecholamine sensor design was changed [70]. It turned out that the catechol group strongly quenched the sensor. Therefore, the new sensor (NeuroSensor 521, NS521) was designed only with the aldehyde group to associate with the analyte's amines (Fig. 6a). The boronic acid group was exchanged to a p-methoxyphenyl group. The resulting sensor increased its fluorescence (521 nm emission) in the presence of catecholamines but had a lower affinity ($K_D \approx 10$ mM for dopamine) compared with the previous sensor. This new sensor was used to detect catecholamines in vesicles of chromaffin cells (Fig. 6b). Since the concentration of catecholamines in secretory vesicles is high, the lower affinity did not hamper the experiments. The concept of this sensor was extended to a three-input system that responds to glutamate, Zn^{2+}, and pH [71, 72]. This is a very compelling approach because it could be used to image co-release of different compounds, which is a longstanding challenge in neuroscience.

The neurotransmitter sensitive fluorophores that were presented here are not sensors in a classic sense. So far they are rather used as labels/reporters (as shown in Fig. 6b). Nevertheless, the design implicates that the reaction to an iminium ion is reversible. If these sensors are immobilized outside the cell on a substrate or onto the cell membrane and imaged, they could measure extracellular neurotransmitter dynamics. However, so far the sensitivities are not suitable for concentrations outside neurotransmitter-containing vesicles. Another drawback might be photobleaching. In the future, one could combine molecular recognition concepts from these sensors and the photophysical properties of certain fluorescent nanomaterials to get closer to biological applications.

Electrochemical sensing concepts

Electrochemical sensing concepts for neurotransmitters are currently the gold standard, and several excellent reviews have been published on this topic [73–76]. The advantage of electrochemical concepts is a very good time resolution and that they can be assembled and integrated in various designs [77]. Obviously, analytes have to be redox-active, which limits the number of neurotransmitters that can be directly detected. Electrochemical methods have already provided biological insights and were employed in vivo [73]. Most of this work was done with single electrodes and is therefore not within the scope of this article.

Nanomaterials have been heavily used to increase the performance of electrochemical approaches, for example by coating electrodes with carbon nanotubes [78]. This approach is successful to improve sensitivity and selectivity, but it does not necessarily increase the spatial resolution. Here, we are discussing concepts that aim to improve spatial resolution by using multiple 'small' electrodes.

Lindau et al. used electrochemical detector (ECD) arrays to overcome this disadvantage [79]. They performed electrochemical imaging of fusion pore openings with time resolutions in the millisecond range. Their ECD arrays consisted of four platinum microelectrodes pointing at the cell (3 μm wide and 150 nm thick). Chromaffin cells were positioned on top of the array and chemically stimulated to release catecholamines (Fig. 7a). It was possible to detect individual exocytosis events of single vesicles. The time-resolved oxidation current reflected the time course of neurotransmitter arrival of at the electrode surface (Fig. 7c). The different shapes of the curves and the time delay show that this method can provide additional information that is lost with only one electrode. Consequently, the signals from the four electrodes were used in combination with diffusion simulations to estimate the most likely exocytosis position on the cell (red cross in Fig. 7b). To verify that this location is reasonable, a fluorescent dye loaded into the vesicles was imaged during the whole process. The images (Fig. 7b) indicate that the processes related to exocytosis and the spatiotemporal current profiles are correlated.

This work clearly demonstrates the potential of spatiotemporal imaging techniques even if the image has only four points. A drawback is that cells must be placed on the ECD array using a patch pipette, which might affect the cell.

Increasing the number of electrodes per area can serve two goals. First, it is useful to increase the number of data points from different cells. Yakushenko et al. demonstrated that arrays are useful to get statistically meaningful data from different cells [81]. Second, higher densities of electrodes can be used for spatiotemporal imaging of a single cell. Obviously both developments go hand in hand.

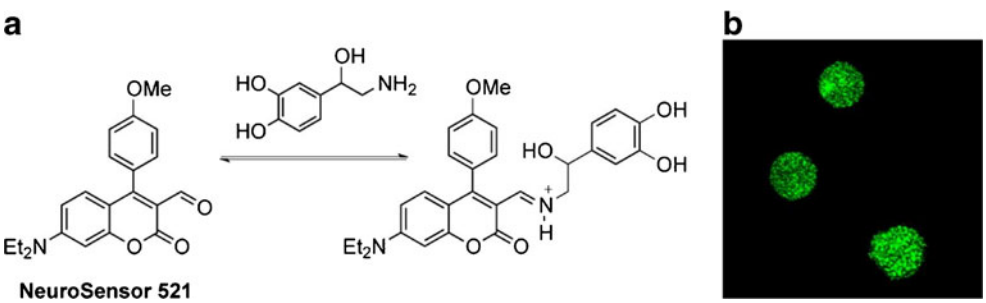

Fig. 6 Organic fluorophores as reporters for neurotransmitters. (**a**) Schematic of catecholamine sensor design. (**b**) Fluorescence images of chromaffin cells that contain vesicles with norepinephrine incubated with NS521. Reproduced with permission from [70]

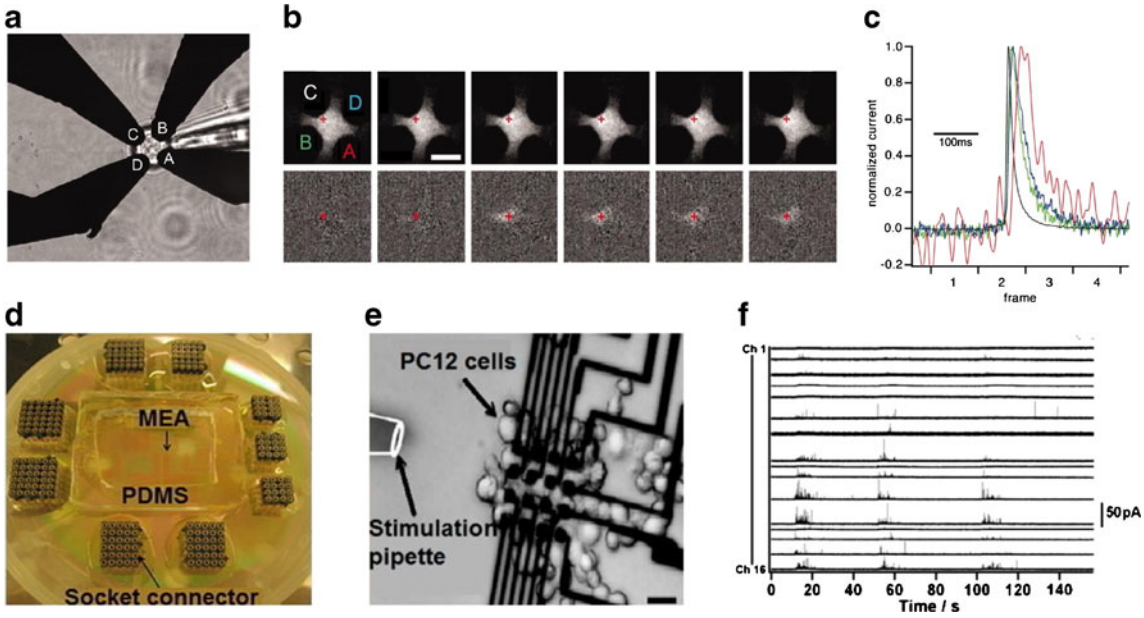

Fig. 7 Electrochemical dopamine detection with electrode arrays. (**a**) Light microscopy image of a chromaffin cell placed on top of an electrochemical detector (ECD) array with four electrodes (A–D). (**b**) Spatiotemporal correlation of secretion of catecholamines and release of the fluorescent vesicle marker acridine orange. Original fluorescence images (upper row) and difference images (lower row) are shown (scale bar is 5 μm). The red cross indicates the simulated position of the exocytosis event. (**c**) Currents from the four electrodes reveal spatiotemporal differences (e.g., a time delay). (**d**) Photo of another microelectrode array (MEA) device integrated in a PDMS chamber. (**e**) Microscopy image of the electrochemical imaging setup. It shows the 16-electrode array covered by a cluster of PC12 cells and the stimulation pipet is positioned on the left. (**f**) Amperometric traces from different electrodes of the array after cell stimulation. Adapted with permission from [79] (a–c). Adapted with permission from [80] (d–f)

The group of Ewing used microelectrode arrays (MEAs) to detect dopamine release from PC12 cells [80]. Electrodes were individually addressable and integrated into a biocompatible PDMS chamber (Fig. 7d). Owing to a thin coating of collagen IV, it was possible to culture PC12 cells directly on the top of the MEAs device. Exocytotic events and release of dopamine were recorded with a spatial resolution of 2 μm. The MEAs were used to analyze the release of dopamine from clusters of cells (Fig. 7e). Figure 7f shows traces from different electrodes that were in contact with different cells of the MEA. The difference in the amperometric signals again demonstrates that the spatiotemporal domain of neurotransmitter release contains additional information.

This 16-electrode MEA was used to show that pharmacologic treatments change dopamine release. The results are also consistent with single cell and single electrode experiments. This work shows that increasing the number of electrodes provides valuable additional information about exocytosis events. Diffusion of molecules could be affected by the electrodes and therefore data analysis is more complicated. Additional calculations might be necessary to reconstruct the original concentration profile. This approach provides information about small clusters of cells but lacks subcellular resolution. In another piece of work, the number of electrodes was increased (n=36) to achieve subcellular resolution [82]. Moreover, the cultivation conditions were modified to let single cells adhere on the MEA. Consequencely, the approach was used to collect amperometric data with subcellular resolution.

The mentioned examples are amperometric approaches, and there are further reports known in literature [83–85]. Fast scan cyclic voltammetry provides much better chemical resolution and has also been implemented on-chips but there are certain limitations attributable to cross-talk between individual electrodes [86, 87].

The size of all discussed electrodes was on the order of micrometers. Decreasing the size of electrodes is an obvious goal and there are several reports about (single) nanoscale electrodes for dopamine detection [88–91]. Using them in arrays would further increase the spatial resolution of multi-electrode setups.

In summary, electrochemical methods to detect redox-active neurotransmitters benefit from the high time-resolution. The challenge is the engineering of arrays to interrogate both cell networks and subcellular processes. In the future, electrodes could be further miniaturized to reach the nanoscale and the number of electrodes per area could be increased.

Conclusions

We reviewed different and complementary methods that are compatible with the idea of spatiotemporal neurotransmitter

Table 1 Summary of different sensing concepts

Concept/ building block	Concept/example	Advantages	Disadvantages	Ref.
Fluorescent nanomaterials				
Fluorescent carbon nanotubes (SWCNTs)	Semiconducting single walled carbon nanotubes (SWCNTs) serve as a nIR fluorescent scaffold. A polymer phase around the nanotube is created to enable molecular recognition of the neurotransmitter. Selectivity and sensitivity depend on the polymer sequence (e.g., DNA-sequence) and can be tuned.	- nIR fluorescence: deeper tissue penetration, less background fluorescence. - Photophysical properties such as ultra-low. photobleaching and blinking. - Molecular recognition concept without natural recognition units.	- Special nIR detectors necessary. - Typically lower quantum yield.	[37]
Quantum dots (QDs)	Quantum dots (Cd-Se-ZnS, graphene oxide,…) serve as fluorescent building block. The surface is functionalized to mediate interactions between analyte and QD. The mechanism is probably related to electron transfer from redox-active neurotransmitters (e.g., dopamine) to the QD.	- Photophysical properties such as high quantum yield, tunable absorption, and emission spectra, ultra-low photobleaching.	- Maybe limited to redox active neurotransmitters (all examples are for catecholamines).	[39–45]
Enzyme conjugated nanoparticles	A luminescent nanoparticle sensor for a small reactive compound (e.g., oxygen) is conjugated to an enzyme. The enzyme (e.g., diamine oxidase) catalyzes the reaction of a neurotransmitter (e.g., histamine) to a product. In this reaction, another small compound (e.g., oxygen) is either consumed or produced. Thus, the luminescence change indirectly indicates presence of the neurotransmitter.	- Combines high selectivity of an enzyme and advantages of artificial fluorophores. - Simplifies a difficult molecular recognition task (neurotransmitter) to recognition of small reactive species.	- Indirect detection. - Robustness if other molecules interfere with the enzyme.	[50]
Engineered proteins				
All-protein-based	Two fluorescent GFP variants are fused with a neurotransmitter binding protein. When the neurotransmitter binds, the protein's conformation is changed, which finally affects FRET efficiency between the two fluorophores.	- High selectivity of the natural binding protein/receptor for analyte - Genetic targeting.	- Confined to cell surface. - Signal to noise ratio. - Photophysical properties of the fluorophore.	[52–54]
Semisynthetic protein-fluorophore conjugates	A neurotransmitter binding protein is conjugated to an artificial fluorophore. One concept (SNIFIT) uses a binding protein fused with a CLIP- and SNAP-tag. They are used to conjugate a fluorophore-ligand construct and a second fluorophore. The ligand competes with the analyte for the binding pocket of the protein and the fluorescence signal (FRET) changes when the ligand is displaced by the analyte.	- Selectivity of the natural binding protein/receptor for analyte. - Modular building blocks and rational design. - Genetic targeting (some). - Artificial fluorophores with beneficial photophysical properties.	- Response kinetics (some). - Sensitivity (some). -Confined to cell membrane.	[55–61]
Engineered cells	Cells are transfected with a neurotransmitter receptor that triggers a rise of the cytosolic calcium concentration when a neurotransmitter binds to it. This signal is then detected by calcium sensors.	- Calcium sensors are more advanced in their development. - Genetic targeting.	- Limited to the cell membrane. - Indirect detection of calcium and not neurotransmitters themselves. - Size.	[62]
Small organic fluorophores	A small organic fluorophore is modified to enable covalent binding of an analyte. Fluorescence changes when the analyte is bound. Examples include aldehyde groups that react with amine groups and boronic acids that bind the two hydroxy groups of catecholamines such as dopamine.	- Organic fluorophores are small, cheap, and versatile. - Size.	- Photobleaching. - Immobilization necessary for extracellular experiments.	[69–72]
Electrochemical microelectrode arrays	Redox-active neurotransmitters such as dopamine are oxidized at the surface of an electrode. The current reflects the time course of neurotransmitter arrival of at the electrode surface.	- High temporal resolution. - Single electrodes are routinely used in vivo and in vitro.	-Low spatial resolution. - Size of the electrodes. -Analytes have to be redox-active.	[79–83, 85]

detection. The different concepts show great promise but need further improvements to become tools for cell and neurobiology (see Table 1).

The major challenge for all optical concepts remains the desired time resolution and selectivity. On the other side, spatial resolution and parallel detection can be easily achieved in optical concepts once the mentioned challenges are solved.

Electrochemical sensors, especially amperometric sensors, provide a superior time resolution. For them the challenge is rather to provide the spatial resolution. The design of appropriate arrays is not trivial. Furthermore, there might be cross talk between individual electrodes if they are getting smaller and smaller.

All mentioned approaches would benefit from multiplexing and also more versatile geometric arrangements of sensors. Multiplexing is important because ultimately one would like to detect and distinguish all chemicals that get out of cells. A sensor that monitors the whole spectrum of neurotransmitters or other released compounds would be a perfect tool to investigate the underlying biology.

Another challenge is the arrangement of sensors on or near cells or in arrays. One way to do this is to place many sensors in vitro on cell culture substrates. However, in vivo or in tissue samples it is necessary to arrange sensors in three dimensions to monitor the whole process.

In summary, nanosensors for neurotransmitters are developing quickly and the field receives inputs from nanotechnology, protein engineering, molecular recognition, and electrochemistry. The increasing performance of these sensors will in the future enable biological studies and a full understanding of how cells use chemicals to communicate and exchange information.

Acknowledgments The authors thank the Fonds der chemischen Industrie (FCI) for financial support (Liebig fellowship for S.K.).

Compliance with ethical standard

Conflicts of interest The authors declare that they have no conflict of interest.

References

1. Eccles JC (1982) The synapse: from electrical to chemical transmission. Annu Rev Neurosci 5:325–339
2. Bergquist J, Tarkowski A, Ekman R, Ewing A (1994) Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. Proc Natl Acad Sci U S A 91(26):12912–12916
3. Hyman SE (2005) Neurotransmitters. Curr Biol 15(5):R154–158
4. Gundelfinger ED, Kessels MM, Qualmann B (2003) Temporal and spatial coordination of exocytosis and endocytosis. Nat Rev Mol Cell Biol 4(2):127–139
5. Cragg SJ, Rice ME (2004) DAncing past the DAT at a DA synapse. Trends Neurosci 27(5):270–277
6. Olah S, Fule M, Komlosi G, Varga C, Baldi R, Barzo P, Tamas G (2009) Regulation of cortical microcircuits by unitary GABA-mediated volume transmission. Nature 461(7268):1278–1281
7. Hnasko TS, Edwards RH (2012) Neurotransmitter corelease: mechanism and physiological role. Annu Rev Physiol 74:225–243
8. Dulcis D, Jamshidi P, Leutgeb S, Spitzer NC (2013) Neurotransmitter switching in the adult brain regulates behavior. Science 340(6131):449–453
9. Devor A, Bandettini PA, Boas DA, Bower JM, Buxton RB, Cohen LB, Dale AM, Einevoll GT, Fox PT, Franceschini MA, Friston KJ, Fujimoto JG, Geyer MA, Greenberg JH, Halgren E, Hamalainen MS, Helmchen F, Hyman BT, Jasanoff A, Jernigan TL, Judd LL, Kim SG, Kleinfeld D, Kopell NJ, Kutas M, Kwong KK, Larkum ME, Lo EH, Magistretti PJ, Mandeville JB, Masliah E, Mitra PP, Mobley WC, Moskowitz MA, Nimmerjahn A, Reynolds JH, Rosen BR, Salzberg BM, Schaffer CB, Silva GA, So PT, Spitzer NC, Tootell RB, Van Essen DC, Vanduffel W, Vinogradov SA, Wald LL, Wang LV, Weber B, Yodh AG (2013) The challenge of connecting the dots in the B.R.a.I.N. Neuron 80(2):270–274
10. Andrews AM (2015) The future of monitoring molecules. ACS Chem Neurosci 6(1):1–2
11. Wightman RM (2015) Monitoring molecules: insights and progress. ACS Chem Neurosci 6(1):5–7
12. Lee T, Cai LX, Lelyveld VS, Hai A, Jasanoff A (2014) Molecular-level functional magnetic resonance imaging of dopaminergic signaling. Science 344(6183):533–535
13. Meyer-Lindenberg A (2010) From maps to mechanisms through neuroimaging of schizophrenia. Nature 468(7321):194–202
14. Aerts JT, Louis KR, Crandall SR, Govindaiah G, Cox CL, Sweedler JV (2014) Patch clamp electrophysiology and capillary electrophoresis-mass spectrometry metabolomics for single cell characterization. Anal Chem 86(6):3203–3208
15. Deisseroth K, Schnitzer MJ (2013) Engineering approaches to illuminating brain structure and dynamics. Neuron 80(3):568–577
16. Wilt BA, Burns LD, Wei Ho ET, Ghosh KK, Mukamel EA, Schnitzer MJ (2009) Advances in light microscopy for neuroscience. Annu Rev Neurosci 32:435–506
17. Perry M, Li Q, Kennedy RT (2009) Review of recent advances in analytical techniques for the determination of neurotransmitters. Analy Chim Acta 653(1):1–22
18. Westerink RH, Ewing AG (2008) The PC12 cell as model for neurosecretion. Acta Physiol (Oxf) 192(2):273–285
19. Omiatek DM, Dong Y, Heien ML, Ewing AG (2010) Only a fraction of quantal content is released during exocytosis as revealed by electrochemical cytometry of secretory vesicles. ACS Chem Neurosci 1(3):234–245
20. Garris PA, Ciolkowski EL, Pastore P, Wightman RM (1994) Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. J Neurosci 14(10):6084–6093
21. Kruss S, Wolfram T, Martin R, Neubauer S, Kessler H, Spatz JP (2010) Stimulation of cell adhesion at nanostructured teflon interfaces. Adv Mater 22(48):5499–5506
22. Kruss S, Erpenbeck L, Schon MP, Spatz JP (2012) Circular, nanostructured, and biofunctionalized hydrogel microchannels for dynamic cell adhesion studies. Lab Chip 12(18):3285–3289
23. Kruss S, Erpenbeck L, Amschler K, Mundinger TA, Boehm H, Helms HJ, Friede T, Andrews RK, Schon MP, Spatz JP (2013) Adhesion maturation of neutrophils on nanoscopically presented platelet glycoprotein ibalpha. ACS Nano 7(11):9984–9996
24. Carregal-Romero S, Caballero-Diaz E, Beqa L, Abdelmonem AM, Ochs M, Huhn D, Suau BS, Valcarcel M, Parak WJ (2013) Multiplexed sensing and imaging with colloidal nano- and microparticles. Annu Rev Anal Chem (Palo Alto, CA) 6(1):53–81

25. Amschler K, Erpenbeck L, Kruss S, Schon MP (2014) Nanoscale integrin ligand patterns determine melanoma cell behavior. ACS Nano 8(9):9113–9125
26. Wang QH, Bellisario DO, Drahushuk LW, Jain RM, Kruss S, Landry MP, Mahajan SG, Shimizu SFE, Ulissi ZW, Strano MS (2014) Low dimensional carbon materials for applications in mass and energy transport. Chem Mater 26(1):172–183
27. Kruss S, Srot V, van Aken PA, Spatz JP (2012) Au-Ag hybrid nanoparticle patterns of tunable size and density on glass and polymeric supports. Langmuir 28(2):1562–1568
28. Balasubramanian K, Kern K (2014) 25th Anniversary article: label-free electrical biodetection using carbon nanostructures. Adv Mater 26(8):1154–1175
29. Balasubramanian K, Burghard M (2006) Biosensors based on carbon nanotubes. Anal Bioanal Chem 385(3):452–468
30. Wolfbeis OS (2015) An overview of nanoparticles commonly used in fluorescent bioimaging. Chem Soc Rev 44(14):4743–4768
31. Borisov SM, Wolfbeis OS (2008) Optical biosensors. Chem Rev 108(2):423–461
32. O'Connell MJ, Bachilo SM, Huffman CB, Moore VC, Strano MS, Haroz EH, Rialon KL, Boul PJ, Noon WH, Kittrell C, Ma J, Hauge RH, Weisman RB, Smalley RE (2002) Band gap fluorescence from individual single-walled carbon nanotubes. Science 297(5581): 593–596
33. Kruss S, Hilmer AJ, Zhang J, Reuel NF, Mu B, Strano MS (2013) Carbon nanotubes as optical biomedical sensors. Adv Drug Deliv Rev 65(15):1933–1950
34. Reuel NF, Grassbaugh B, Kruss S, Mundy JZ, Opel C, Ogunniyi AO, Egodage K, Wahl R, Helk B, Zhang J, Kalcioglu ZI, Tvrdy K, Bellisario DO, Mu B, Blake SS, Van Vliet KJ, Love JC, Wittrup KD, Strano MS (2013) Emergent properties of nanosensor arrays: applications for monitoring IgG affinity distributions, weakly affined hypermannosylation, and colony selection for biomanufacturing. ACS Nano 7(9):7472–7482
35. Zhang J, Kruss S, Hilmer AJ, Shimizu S, Schmois Z, De La Cruz F, Barone PW, Reuel NF, Heller DA, Strano MS (2014) A rapid, direct, quantitative, and label-free detector of cardiac biomarker troponin T using near-infrared fluorescent single-walled carbon nanotube sensors. Adv Health Mater 3(3):412–423
36. Jin H, Heller DA, Kalbacova M, Kim JH, Zhang J, Boghossian AA, Maheshri N, Strano MS (2010) Detection of single-molecule H_2O^2 signaling from epidermal growth factor receptor using fluorescent single-walled carbon nanotubes. Nature Nanotechnol 5(4):302–309
37. Kruss S, Landry MP, Vander Ende E, Lima BM, Reuel NF, Zhang J, Nelson J, Mu B, Hilmer A, Strano M (2014) Neurotransmitter detection using corona phase molecular recognition on fluorescent single-walled carbon nanotube sensors. J Am Chem Soc 136(2): 713–724
38. Bisker G, Ahn J, Kruss S, Ulissi ZW, Salem DP, Strano MS (2015) A mathematical formulation and solution of the CoPhMoRe inverse problem for helically wrapping polymer corona phases on cylindrical substrates. J Phys Chem C 119(24):13876–13886
39. Freeman R, Bahshi L, Finder T, Gill R, Willner I (2009) Competitive analysis of saccharides or dopamine by boronic acid-functionalized CdSe-ZnS quantum dots. Chem Commun (Camb) 7:764–766
40. Mu Q, Xu H, Li Y, Ma S, Zhong X (2014) Adenosine capped QDs based fluorescent sensor for detection of dopamine with high selectivity and sensitivity. Analyst (Cambridge, U K) 139(1):93–98
41. Chen JL, Yan XP, Meng K, Wang SF (2011) Graphene oxide-based photoinduced charge transfer label-free near-infrared fluorescent biosensor for dopamine. Anal Chem 83(22):8787–8793
42. Yu C, Luo M, Zeng F, Zheng F, Wu S (2011) Mesoporous silica particles for selective detection of dopamine with beta-cyclodextrin as the selective barricade. Chem Commun (Camb) 47(32):9086–9088
43. Zhang X, Chen X, Kai S, Wang HY, Yang J, Wu FG, Chen Z (2015) Highly sensitive and selective detection of dopamine using one-pot synthesized highly photoluminescent silicon nanoparticles. Anal Chem 87(6):3360–3365
44. Zhou X, Ma P, Wang A, Yu C, Qian T, Wu S, Shen J (2014) Dopamine fluorescent sensors based on polypyrrole/graphene quantum dots core/shell hybrids. Biosens Bioelectronics 64C: 404–410
45. Jeon SJ, Kwak SY, Yim D, Ju JM, Kim JH (2014) Chemically-modulated photoluminescence of graphene oxide for selective detection of neurotransmitter by "turn-on" response. J Am Chem Soc 136(31):10842–10845
46. Schultz W (2007) Multiple dopamine functions at different time courses. Annu Rev Neurosci 30:259–288
47. Davie CA (2008) A review of Parkinson's disease. Br Med Bull 86: 109–127
48. Lynge ME, van der Westen R, Postma A, Stadler B (2011) Polydopamine—a nature-inspired polymer coating for biomedical science. Nanoscale 3(12):4916–4928
49. Liebscher J, Mrowczynski R, Scheidt HA, Filip C, Hadade ND, Turcu R, Bende A, Beck S (2013) Structure of polydopamine: a never-ending story? Langmuir 29(33):10539–10548
50. Cash KJ, Clark HA (2013) Phosphorescent nanosensors for in vivo tracking of histamine levels. Anal Chem 85(13):6312–6318
51. Liang R, Broussard GJ, Tian L (2015) Imaging chemical neurotransmission with genetically encoded fluorescent sensors. ACS Chem Neurosci 6(1):84–93
52. Okumoto S, Looger LL, Micheva KD, Reimer RJ, Smith SJ, Frommer WB (2005) Detection of glutamate release from neurons by genetically encoded surface-displayed FRET nanosensors. Proc Natl Acad Sci U S A 102(24):8740–8745
53. Hires SA, Zhu Y, Tsien RY (2008) Optical measurement of synaptic glutamate spillover and reuptake by linker optimized glutamate-sensitive fluorescent reporters. Proc Natl Acad Sci U S A 105(11):4411–4416
54. Marvin JS, Borghuis BG, Tian L, Cichon J, Harnett MT, Akerboom J, Gordus A, Renninger SL, Chen TW, Bargmann CI, Orger MB, Schreiter ER, Demb JB, Gan WB, Hires SA, Looger LL (2013) An optimized fluorescent probe for visualizing glutamate neurotransmission. Nat Methods 10(2):162–170
55. Brun MA, Tan KT, Nakata E, Hinner MJ, Johnsson K (2009) Semisynthetic fluorescent sensor proteins based on self-labeling protein tags. J Am Chem Soc 131(16):5873–5884
56. Masharina A, Reymond L, Maurel D, Umezawa K, Johnsson K (2012) A fluorescent sensor for GABA and synthetic GABA(B) receptor ligands. J Am Chem Soc 134(46):19026–19034
57. Brun MA, Griss R, Reymond L, Tan KT, Piguet J, Peters RJ, Vogel H, Johnsson K (2011) Semisynthesis of fluorescent metabolite sensors on cell surfaces. J Am Chem Soc 133(40):16235–16242
58. Brun MA, Tan KT, Griss R, Kielkowska A, Reymond L, Johnsson K (2012) A semisynthetic fluorescent sensor protein for glutamate. J Am Chem Soc 134(18):7676–7678
59. Schena A, Johnsson K (2014) Sensing acetylcholine and anticholinesterase compounds. Angew Chem Int Ed Engl 53(5):1302–1305
60. Takikawa K, Asanuma D, Namiki S, Sakamoto H, Ariyoshi T, Kimpara N, Hirose K (2014) High-throughput development of a hybrid-type fluorescent glutamate sensor for analysis of synaptic transmission. Angew Chem Int Ed Engl 53(49):13439–13443
61. Okubo Y, Sekiya H, Namiki S, Sakamoto H, Iinuma S, Yamasaki M, Watanabe M, Hirose K, Iino M (2010) Imaging extrasynaptic glutamate dynamics in the brain. Proc Natl Acad Sci U S A 107(14):6526–6531
62. Nguyen QT, Schroeder LF, Mank M, Muller A, Taylor P, Griesbeck O, Kleinfeld D (2010) An in vivo biosensor for neurotransmitter release and in situ receptor activity. Nat Neurosci 13(1):127–132

63. Pradhan T, Jung HS, Jang JH, Kim TW, Kang C, Kim JS (2014) Chemical sensing of neurotransmitters. Chem Soc Rev 43(13): 4684–4713
64. Omiatek DM, Cans AS, Heien ML, Ewing AG (2010) Analytical approaches to investigate transmitter content and release from single secretory vesicles. Anal Bioanal Chem 397(8):3269–3279
65. Kim D, Koseoglu S, Manning BM, Meyer AF, Haynes CL (2011) Electroanalytical eavesdropping on single cell communication. Anal Chem 83(19):7242–7249
66. Keighron JD, Ewing AG, Cans AS (2012) Analytical tools to monitor exocytosis: a focus on new fluorescent probes and methods. Analyst (Cambridge, U K) 137(8):1755–1763
67. Sames D, Dunn M, Karpowicz RJ, Sulzer D (2013) Visualizing Neurotransmitter Secretion at Individual Synapses. ACS Chem Neurosci 4(5):648–651
68. Gubernator NG, Zhang H, Staal RG, Mosharov EV, Pereira DB, Yue M, Balsanek V, Vadola PA, Mukherjee B, Edwards RH, Sulzer D, Sames D (2009) Fluorescent false neurotransmitters visualize dopamine release from individual presynaptic terminals. Science 324(5933):1441–1444
69. Secor KE, Glass TE (2004) Selective amine recognition: development of a chemosensor for dopamine and norepinephrine. Org Lett 6(21):3727–3730
70. Hettie KS, Liu X, Gillis KD, Glass TE (2013) Selective catecholamine recognition with NeuroSensor 521: a fluorescent sensor for the visualization of norepinephrine in fixed and live cells. ACS Chem Neurosci 4(6):918–923
71. Klockow JL, Hettie KS, Glass TE (2013) ExoSensor 517: a dual-analyte fluorescent chemosensor for visualizing neurotransmitter exocytosis. ACS Chem Neurosci 4(10):1334–1338
72. Hettie KS, Klockow JL, Glass TE (2014) Three-input logic gates with potential applications for neuronal imaging. J Am Chem Soc 136(13):4877–4880
73. Bucher ES, Wightman RM (2015) Electrochemical analysis of neurotransmitters. Annu Rev Anal Chem 8(1):239–261
74. Trouillon R, Passarelli MK, Wang J, Kurczy ME, Ewing AG (2013) Chemical analysis of single cells. Anal Chem 85(2):522–542
75. Mirzaei M, Sawan M (2014) Microelectronics-based biosensors dedicated to the detection of neurotransmitters: a review. Sensors (Basel) 14(10):17981–18008
76. Jackowska K, Krysinski P (2013) New trends in the electrochemical sensing of dopamine. Anal Bioanal Chem 405(11):3753–3771
77. Weltin A, Kieninger J, Enderle B, Gellner AK, Fritsch B, Urban GA (2014) Polymer-based, flexible glutamate and lactate microsensors for in vivo applications. Biosens Bioelectron 61:192–199
78. Sanghavi BJ, Wolfbeis OS, Hirsch T, Swami NS (2015) Nanomaterial-based electrochemical sensing of neurological drugs and neurotransmitters. Mikrochim Acta 182:1–41
79. Hafez I, Kisler K, Berberian K, Dernick G, Valero V, Yong MG, Craighead HG, Lindau M (2005) Electrochemical imaging of fusion pore openings by electrochemical detector arrays. Proc Natl Acad Sci U S A 102(39):13879–13884
80. Wang J, Trouillon R, Lin Y, Svensson MI, Ewing AG (2013) Individually addressable thin-film ultramicroelectrode array for spatial measurements of single vesicle release. Anal Chem 85(11):5600–5608
81. Yakushenko A, Katelhon E, Wolfrum B (2013) Parallel on-chip analysis of single vesicle neurotransmitter release. Anal Chem 85(11):5483–5490
82. Wang J, Ewing AG (2014) Simultaneous study of subcellular exocytosis with individually addressable multiple microelectrodes. Analyst (Cambridge, U K) 139(13):3290–3295
83. Carabelli V, Gosso S, Marcantoni A, Xu Y, Colombo E, Gao Z, Vittone E, Kohn E, Pasquarelli A, Carbone E (2010) Nanocrystalline diamond microelectrode arrays fabricated on sapphire technology for high-time resolution of quantal catecholamine secretion from chromaffin cells. Biosens Bioelectron 26(1):92–98
84. Chen P, Xu B, Tokranova N, Feng X, Castracane J, Gillis KD (2003) Amperometric detection of quantal catecholamine secretion from individual cells on micromachined silicon chips. Anal Chem 75(3):518–524
85. Wang L, Xu H, Song Y, Luo J, Wei W, Xu S, Cai X (2015) Highly sensitive detection of quantal dopamine secretion from pheochromocytoma cells using neural microelectrode array electrodeposited with polypyrrole graphene. ACS Appl Mater Interfaces 7(14): 7619–7626
86. Yakushenko A, Schöps V, Mayer D, Offenhäusser A, Wolfrum B (2014) On-chip fast scan cyclic voltammetry for selective detection of redox active neurotransmitters. Phys Status Solidi 211(6):1364–1371
87. Zhang B, Heien ML, Santillo MF, Mellander L, Ewing AG (2011) Temporal resolution in electrochemical imaging on single PC12 cells using amperometry and voltammetry at microelectrode arrays. Anal Chem 83(2):571–577
88. Wu WZ, Huang WH, Wang W, Wang ZL, Cheng JK, Xu T, Zhang RY, Chen Y, Liu J (2005) Monitoring dopamine release from single living vesicles with nanoelectrodes. J Am Chem Soc 127(25): 8914–8915
89. Li YT, Zhang SH, Wang L, Xiao RR, Liu W, Zhang XW, Zhou Z, Amatore C, Huang WH (2014) Nanoelectrode for amperometric monitoring of individual vesicular exocytosis inside single synapses. Angew Chem Int Ed Engl 53(46):12456–12460
90. Li X, Majdi S, Dunevall J, Fathali H, Ewing AG (2015) Quantitative measurement of transmitters in individual vesicles in the cytoplasm of single cells with nanotip electrodes. Angew Chem Int Ed Engl 54:11978–82
91. Liu Y, Li M, Zhang F, Zhu A, Shi G (2015) Development of Au disk nanoelectrode down to 3 nm in radius for detection of dopamine release from a single cell. Anal Chem 87(11):5531–5538