Selective Targeting of Neurons with Inorganic Nanoparticles: Revealing the Crucial Role of Nanoparticle Surface Charge

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Supporting Information

ABSTRACT: Nanoparticles (NPs) are increasingly used in biomedical applications, but the factors that influence their interactions with living cells need to be elucidated. Here, we reveal the role of NP surface charge in determining their neuronal interactions and electrical responses. We discovered that negatively charged NPs administered at low concentration (10 nM) interact with the neuronal membrane and at the synaptic cleft, whereas positively and neutrally charged NPs never localize on neurons. This effect is shape and material independent. The presence of negatively charged NPs on neuronal cell membranes influences the excitability of neurons by causing an increase in the amplitude and frequency of spontaneous postsynaptic currents at the single cell level and an increase of both the spiking activity and synchronous firing at neural network level. The negatively charged NPs exclusively bind to excitable neuronal cells, and never to nonexcitable glial cells. This specific interaction was also confirmed by manipulating the electrophysiological activity of neuronal cells. Indeed, the interaction of negatively charged NPs with neurons is either promoted or hindered by pharmacological suppression or enhancement of the neuronal activity with tetrodotoxin or bicuculline, respectively. We further support our main experimental conclusions by using numerical simulations. This study demonstrates that negatively charged NPs modulate the excitability of neurons, revealing the potential use of NPs for controlling neuron activity.

KEYWORDS: inorganic nanoparticles, surface potential, membrane depolarization, neural networks, neural excitability

Nanoparticles (NPs) are original tools to treat brain diseases. Given their high surface to volume ratio, NPs enable efficient loading of therapeutic molecules, while their material-specific intrinsic properties offer alternative therapeutic opportunities. For instance, for patients affected by glioblastoma multiforme, cisplatin-tethered gold NPs act as drug carriers and as radiosensitizers, emitting ionizing photoelectrons and Auger electrons in radiotherapy. Similarly, the heat generated by iron oxide NPs under an oscillating magnetic field has been exploited (i) to perform hyperthermia treatment on human glioblastoma, (ii) to temporally damage the brain barrier thus facilitating NP crossing, and (iii) to promote NP uptake by opening heat-sensitive ion channels. However, the use of NPs as drug carriers or therapeutic materials requires a deeper understanding of the principles governing their interaction and functional effects in neuronal circuits. Given the increasing number of NPs available and the increasing interest in studies pertaining to NP–cell interactions, there has been a substantial effort to correlate the NP material features (e.g., composition, size, shape, coatings, etc.) to their neuronal toxicity and activity. Since aberrant neuronal electrical activity is associated with most neurological diseases and can be an early marker of neurotoxicity, it is crucial to understand how NPs can modulate brain electrical function.

Controlled NP-induced bioelectric activity could be exploited to design nanotools that can regulate the imbalanced excitation/inhibition phenomena observed in many brain diseases. For instance, both single- and multiwall carbon
nanotubes (CNTs) boost electrical activity of neurons cultured on CNT-coated substrates. Since functional changes were also observed with “non-targeted” CNTs, the authors proposed that the intrinsic electrical conductivity of CNTs might determine neuronal electrophysiological effects. More recent studies performed with NPs of various compositions appear to contradict the initial hypothesis that only the electrical properties of the materials determine functional effects on neuronal activity. For example, neurons cultured on multi-electrode arrays (MEAs) exposed to carbon black (CB), hematite (Fe₂O₃), and titanium dioxide (TiO₂) NPs display an acute, concentration-dependent alteration of spontaneous electrical activity.¹⁵ An increased electrical excitability also occurs in mouse hippocampal slices exposed to star-shaped Au NPs,¹⁸ an observation confirmed at the single neuron level by patch clamp recordings.²⁴ Moreover, as shown in another study, quantum dots (QDs) with a negative coating were preferentially internalized by neurons with respect to astrocytes, oligodendrocytes, or microglia, suggesting that the surface charge is critical for neuron-specific uptake.²⁵ In this study, we have elucidated the mechanisms through which NPs modulate bioelectric activity from single neuron to neuronal networks.

We conducted our study on primary cultures of neurons since this experimental model facilitates access both to single-cell and large neuronal networks,¹⁵,²⁶,²⁷ and it allows complementary measurements at the subcellular, cellular, and network scale. We have tested the effects of NPs that differ in shape (spherical and cylindrical), dimensions (inorganic core material ranging from 5 to 75 nm), composition (cadmium- or iron-based), functionalization, and charge. By combining confocal microscopy and transmission electronic microscopy with single neuron and network-wide electrophysiology techniques on primary hippocampal neuronal cultures, we found that soon after administration of the NPs, the neuron–NP interactions are exclusively determined by the surface charge of the NPs. We observed that negatively charged particles were selectively localized on the neuronal membrane and induced electrophysiological alterations at both single neuron and network levels. Further, results obtained through the pharmacological manipulation of the electrical neuronal

Figure 1. The effect of NP surface charge on the interaction with neurons: Confocal microscope images (left panel; excitation wavelengths of 488, 560, and 647 nm) of primary hippocampal neural cells incubated with 1 nM of negatively charged fluorescent QRs (sample no.7 in Table S1.2) after 10 min of incubation at RT. Neurites and dendrites are covered by negatively charged QRs (A, green signal). Yellow represents the combination of NPs (green signal) and a neuronal marker (VGAT, red signal) and highlights the healthy condition of the entire neural network and the colocalization between QRs and synapses (MCC = 0.45 ± 0.06). (B) The same neuronal culture incubated with QRs identical in shape and size (sample no. 9 in Table S1.2), but with a positive zeta potential; note the absence of QR fluorescence. These results are independent of QR size. Functionalization effect on the neuron–QRs interaction (right panel; excitation wavelengths of 405 and 488 nm); the chemistry used to tune NP surface charge was varied. In functionalization type I (C), to the same polymer-coated (PC) QRs, amino-PEG derivatives bearing carboxyl (COOH) or methoxy (OCH₃) or amino (NH₂) as the other ending moieties were bound to the QR surface. In functionalization type II (D), to PC QRs, a fixed amount of amino-PEG-OCH₃ (via EDC chemistry) was attached, and different amounts of tertiary amine (N,N-dimethylethylendiamine) were covalently linked to the polymer shell. Negatively charged QRs always interacted with neurons independently of the functionalization protocol (E and G, sample nos. 3 and 4, respectively, in Table S1.2), while positive QRs did not interact (F and H, sample nos. 10 and 9, respectively, in Table S1.2). The blue signal in panels e–h indicates the cell nuclear staining with DAPI. Scale bars: 100 μm.
activity suggest that the neuron–NP interaction is mediated by neuronal activity.

RESULTS AND DISCUSSION

NP Interaction with Neurons Depends on NP Surface Charge and Is Shape-, Size-, and Material Independent.

In a first series of experiments, rod-shaped semiconductor NPs made of a core-shell CdSe/CdS were added to the cell culture media of in vitro grown primary hippocampal neuronal cultures. These NPs, also known as quantum rods (QRs), with different length and diameter sizes (see Tables S1.1 and S1.2 and Figure S1.1 of the Supporting Information, SI) were initially chosen because of their bright fluorescent signal, enabling a straightforward investigation of interactions by confocal microscopy. The hydrophobic QRs, synthesized in nonpolar solvent with organic ligands, were transferred in water using an established amphiphilic polymer coating protocol; the binding of amino-modified polyethylene glycol (PEG) molecules provides increased stability to these NPs in physiological media.28,29 In a typical experiment, upon QR addition, neurons became fluorescent within 10 min (QRs at 1 nM, see Figure 1A and the time-lapse movie Movie M2.1). This suggests a fast localization of these NPs to the neuronal cell membrane, through the somatic and the dendritic neuronal subregions. A similar fluorescent signal was observed with different QR samples of the same composition and of different longitudinal lengths or diameters (see for instance Figure S2.1). However, in some experiments, when testing QR batches with similar sizes and surface coatings, QRs did not produce the same fluorescent signal (Figure 1B). These divergent results prompted us to carry out a systematic study on a wide array of QRs samples, with the aim of disentangling their physicochemical properties and their ability to interact with neurons.

We found that the NP surface charge, which is measured as zeta potential value, was crucial in determining their interaction with neurons. QR samples with zeta potential more negative than $-20$ mV adhered on the neuronal membrane and gave rise to the fluorescent signal, whereas QRs with a zeta potential value higher than $-20$ mV did not show this effect and resulted in no detectable neuronal fluorescence signal. To confirm this dependency on zeta potential, we designed an experiment that allowed tuning of the final zeta potential (Figure 1C, functionalization type I). An aliquot of the same pristine hydrophobic QRs was first transferred in water by the polymer wrapping procedure and later, the QR charge was tuned from positive to negative by attaching different amino-PEG derivatives (amino-PEG-metoxyl, amino-PEG-carboxyl, or diamino-PEG molecules) to the polymer-coated QRs. Contrary to the negatively charged QRs ($-35$ mV), which maintained their interaction with neurons, neither the positively charged (zeta potential of $+25$ mV) nor the neutral QRs interacted with neurons, as confirmed by confocal microscopy (compare Figure 1E and 1F). Almost neutral or slightly positive QRs also failed to interact with neurons; QR fluorescence was either visible on the substrate as tiny aggregates or not detectable (Figure S2.2, even in absence of neurons, positive QDs interact with the substrate resulting in tiny fluorescent spots; data not shown).

Interestingly, while the charge was tuned by changing the type of functionalization, we found that the final interaction of QRs with neurons primarily depends on the QRs’ zeta potential. We used, for instance, an alternative strategy to adjust the charge of the QRs from $-50$ mV to $+35$ mV by fixing the amount of amino-PEG-OCH$_3$ molecules and varying the amount of amino-alkyl-tertiary amine molecules bound to the QRs (Figure 1D, functionalization type II). Even in this case, the negative QRs still interacted with neurons, while the positively charged QRs did not (compare Figure 1G,H).

In addition to rod-shaped NPs, we also investigated spherical NPs (QDs) of similar composition (CdSe/ZnS) and with similar fluorescent properties to QRs, thus enabling confocal imaging. When comparing the interaction with neurons of negative, neutral, and positive QDs, the results are consistent with those obtained with QR (see Figure S2.3 and an example of a time-lapse recorded with neutral QDs, Movie M2.2). This supports the hypothesis that only negatively charged NPs interact with neurons, independent of their size and shape. These findings are in agreement with a previous study that reported the selective uptake of negatively charged QDs in brain slices after an overnight incubation.30

To directly image the QRs on neurons at higher magnification, we performed transmission electron microscopy (TEM) and scanning transmission (STEM). Negatively charged QRs localized close to the plasma membrane of cell bodies (Figure 2A). They were also frequently located at synapses, inside the synaptic clefts (Figure 2B,C), and also along neurites (Figure S3.1A,B). In contrast, positive QRs were never found on neurites, neuronal somata, or synapses (Figure S3.2A–C). Negative QRs were only occasionally observed in endocytic pits or enclosed in endocytic vesicles in the cell cytoplasm (Figure 2A). In fact at a short incubation time (10 min), endocytosis is not expected to yet occur. EM tomography enabled us to reconstruct the 3D distribution of QRs on neurons. QRs were mostly detected either contacting or in the close vicinity of the neuronal membrane. EM tomography unambiguously confirmed the presence of QRs at synapses, inside the synaptic cleft (Figure 2C and Movie M3.1).

All of these experiments were performed with cadmium-based NP material. Therefore, we could not exclude that the material composition might also affect the NP–neuron interaction. To eliminate this possibility and demonstrate the same phenomenon with a second material, we tested magnetic NPs made of iron oxide NPs, $\gamma$-Fe$_2$O$_3$, with either a spherical (12 nm in diameter) or rod (65 nm $\times$ 6 nm) shape. Since the magnetic NPs do not fluoresce, the imaging of NP interaction with neuronal cultures was performed exclusively by TEM and STEM. Similar to the QRs, both spherical and rod-shaped NPs with negative zeta potential interacted with neurons, independent of their length and shape (Figures 2D,F and S3.1C–D). Electron tomography further confirmed that negative Fe$_2$O$_3$ rods were randomly oriented on the neuron surface, lying parallel, obliquely, or perpendicular with respect to the cell membrane (Figure 2D and Movie M3.2). They were arranged in a more parallel fashion mainly in the synaptic cleft, likely due to steric hindrance effects (Figures 2B,D and S3.1C,D). Also in this case, positively charged iron oxide NPs were never found on neurites or neuron cell bodies (Figure S3.2D–F).

Interaction of Negatively Charged NPs with Neurons Is Driven by Neuronal Spiking Activity. Next, we studied the localization of NPs in cultures expressing both neuronal and glial cell types, the latter being nonexcitable cells that modulate neuronal activity.31 Ten minutes after the application of the negative fluorescent QRs ($L = 30$ nm, $d = 5$ nm, $Zp = -25$ mV), the fluorescence of negative QRs was selectively detected on neuronal cell bodies (Figure 3). This occurred even though the glial
cells were closely associated with neurons (identified in red by using glial fibrillary acidic protein, GFAP).

Figure 2. EM imaging of mouse primary hippocampal neurons treated with negatively charged CdS-CdSe QRs (A−C), Fe$_3$O$_4$ nanorods (D), and Fe$_2$O$_3$ dots (E,F). (A) TEM image of a cluster of −21 mV, 75 nm long QRs (sample no. 6 in Table S1.2), close to a neuron cell body plasma membrane (arrowheads). (B) TEM image of −35 mV, 32 nm long QRs (sample no. 2 in Table S1.2), inside the synaptic cleft and among neurites (arrowheads); (C) 3D model of −35 mV, 32 nm long QRs inside a synaptic cleft, representing the reconstruction of a 250 nm-thick tomogram acquired in HAADF STEM. The 3D model is set on a single tomographic section. (Right, C1) Single tomographic slices corresponding to a median section in the 3D reconstruction. Arrowheads point to QRs. (D) 3D model of nanorods with zeta potential of −21 mV, 65 nm long Fe$_3$O$_4$ (sample no. 11 in Table S1.2), close to a group of neurites, representing the reconstruction of a 100 nm-thick tomogram acquired in HAADF STEM (see Movie M3.2). The 3D model is set on a single tomographic section; n1−n5 are the five neurites present in the reconstruction. (Left, D1) Single tomographic slices corresponding to sections D1 and D2 in the 3D reconstruction. Arrowheads point to nanorods. (E) Projection image through dots close to neurite membranes (arrowheads). (F) STEM projection image showing the presence of Fe$_2$O$_3$ dots (sample no. 14 in Table S1.2), close to neurite membranes and inside the synaptic cleft (arrowheads). Note a cluster of dots entering a phagocytic pit (double arrowheads). Asterisks indicate presynaptic vesicles. Scale bars are 0.2 μm, except in A and B where they are, respectively, 0.5 and 0.1 μm. Abbreviations: cyt, cytoplasm; n, neurites; prs, presynaptic terminal; ps, postsynaptic terminal. Color code for 3D reconstructions: neurite, green; synaptic vesicles, orange; electron-dense bodies, red; cytoplasmic vesicles, blue; nanorods, yellow.

Figure 3. Negative NPs interact exclusively with excitable neurons. Neurons labeled with NeuN (blue) and glial cells labeled with GFAP antibody (red) were treated with 1 nM negatively charged QRs (green, −35 mV, sample no. 2 in Table S1.2). Note the QRs attach exclusively to the membrane of neural cells. Scale bar: 50 μm; excitation wavelengths: 488, 560, and 647 nm.

An explanation for such selectivity of negative NPs for neuronal cell types could be related to the electrical activity of neuronal cells, which might specifically attract NPs with a “charge-driven effect”. To test this hypothesis, we pharmacologically manipulated electrophysiological activity in neuronal networks, by increasing or reducing spiking activity using Bicuculline (BICU) or Tetrodotoxin (TTX), respectively, and characterized the NP−neural interaction with confocal microscopy. These experiments confirmed that the level of neuronal spiking activity modulates the attraction of negatively charged QRs to the neural cell membranes (Figure S2.5). An increase in network activity with BICU resulted in enhanced neuronal fluorescence, while suppression of the neuron network activity by TTX impaired the association of negative QRs with neurons. In contrast, positive QRs were never localized on neurons in the presence of either BICU or TTX. Therefore, negative NPs interact with neuronal membranes, and this interaction is mediated by neuronal spiking activity.

Negatively Charged NPs Affect Neuronal Bioelectric Activity in Networks. We next investigated whether these NPs might also interfere with electrical activity at the scale of single neurons or neuronal networks. First, we characterized the effects of NPs on network-wide electrical activity by exploiting high-density multi-electrode arrays (HD-MEAs) and by computing mean activity parameters. These devices (Figure 4A) provide 4096 simultaneously extracellular recording electrodes (i.e., 42 μm pitch, array arranged in a 64 × 64 layout) and allow computing with high statistical significance mean activity parameters to characterize network activity.27 After culturing dissociated hippocampal cultures on HD-MEA chips for 21 days in vitro (DIV), the typical experimental protocol consisted of recording the spontaneous activity for 10 min, followed by administration of 1 nM NPs, and then recording the activity after treatment for additional 10 min. Representative temporal traces of the detected spiking activity on single electrodes as a function of time, before and after addition of NPs, are shown in Figure 4B. Network activity changed upon administration of negative NPs (Figure 4B), while there was no clear variation for positive NPs. The effects...
on the network activity of the different NPs were quantified by computing four mean activity parameters: (i) the number of active channels (channels with >0.1 spike/s), (ii) the mean firing rate (MFR, the firing rate averaged on all the active channels), (iii) the mean burst rate (MBR, the average number of bursts per minute), and (iv) the interspike burst interval (ISBI, the average firing rate of spikes during the burst events). While the MFR and ISBI characterize the spiking synchronicity of the network, the MFR and the number of active channels define the global spiking activity of the network. Since in these measures, functional effects were also exclusively dependent on the surface charge of the NPs, the statistical analysis was performed by grouping all NPs belonging to the same zeta potential ($Z_p$, X-axis) interval ($i.e., Z_p < -40 \, mV, -40 \, mV < Z_p < -20 \, mV, -20 \, mV < Z_p < 0 \, mV, Z_p > 0 \, mV$) independent of their shape, material, or type of functionalization (Figure 4c). Each data point represents the normalized increase of the plotted parameters after NP administration with respect to basal conditions averaged from at least three independent measurements for each NP type. For all the parameters, there is a significant increase with respect to the basal network activity when $Z_p < -20 \, mV$, thus demonstrating a clear relationship between charge of the NPs and modulation of the network activity. This indicates that negatively charged NPs not only increase the global spiking activity of the network but also the spiking synchronicity of the network, up to a plateau of about $-20 \, mV$. These effects disappear when $Z_p$ is close to 0 mV and when positive NPs are administered.

Second, we investigated the effects of NPs on single-neuron activity with the patch clamp technique in the voltage clamp mode. In this experimental configuration, it was possible to record postsynaptic currents (i.e., electric signals below the action potential threshold) from an individual neuron within the neuronal network. This electrophysiological approach is complementary to HD-MEA recordings and provides high-quality intracellular signals from single neurons. Interestingly, these patch clamp experiments revealed that negatively charged NPs induced a significant increase in the amplitude and frequency of spontaneous postsynaptic currents, thus resulting in an enhanced charge transfer to the postsynaptic element (Figure 4D–F). In contrast, neutral or positive NPs did not significantly alter synaptic parameters (Figure 4F). These results corroborate the HD-MEA data, indicating that negative NPs are able to trigger an overall increase in neuronal and synaptic activity.

**Negatively Charged NPs Depolarize Neuronal Membrane: A Numerical Simulation.** In order to estimate the effect of negative NPs on the membrane potential, numerical simulations were performed with the finite element method (Comsol Multiphysics). Figure 5A,B shows the results for CdSe/CdS QR and CdSe/ZnS QD, respectively. To build the model, zeta potential and the hydrodynamic radius ($R_{H}$) of the NPs were used as input parameters to determine the charge...
distribution around each NP. Furthermore, the static permittivity for both CdSe/CdS and CdSe/ZnS was considered.\textsuperscript{32,33} We first considered the simplest case of spherical QDs. Starting with the experimental measurements of the zeta potential (Zp) equal to a negative or a neutral value (0 V), respectively. In (A2), for QD with Zp = −22 mV, there is a depolarization effect corresponding to $\Delta V = 65$ mV. The same result occurs in case (B2) for QR with Zp = −25 mV. For both structures (A3, B3), upon Zp = 0 V, there is no variation of the membrane potential. The 2D electric potential distribution is also shown for both structures. It is interesting to note that the effect of the charges is noticeably stronger along the axis passing through the center of QR/QD. Finally, the colored mapping images (under A2, A3, B2, and B3) represent the charge distribution along the zeta axis on the membrane once in contact with negatively charged QD or QR.

![Figure 5. Numerical finite element method calculations resolving the effect of a CdSe/ZnS QD (A) and CdSe/CdS QR (B) on the membrane potential. For both types of particles, the hydrodynamic radius $R_{hy}$ is equal to 23 nm, as suggested by our experimental estimation. For both the QD (A2, A3) and QR (B2, B3), two situations are shown: zeta potential (Zp) equal to a negative or a neutral value (0 V), respectively. In (A2), for QD with Zp = −22 mV, there is a depolarization effect corresponding to $\Delta V = 65$ mV. The same result occurs in case (B2) for QR with Zp = −25 mV. For both structures (A3, B3), upon Zp = 0 V, there is no variation of the membrane potential. The 2D electric potential distribution is also shown for both structures. It is interesting to note that the effect of the charges is noticeably stronger along the axis passing through the center of QR/QD. Finally, the colored mapping images (under A2, A3, B2, and B3) represent the charge distribution along the zeta axis on the membrane once in contact with negatively charged QD or QR.](image-url)
to Z positions that are further from the membrane (corresponding to the white dotted lines in the 2D electric potential distributions below).

For both QR and QD, we observed a clear shift of the membrane potential from $-70$ mV to $-65$ mV. This suggests an increased neuronal action potential firing once the membrane is in contact with negatively charged NPs. Such a potential reduction is not uniform over the contact area between the QR/QD and the membrane, as shown by the 2D distributions of the electric potential. In particular, for the zeta potentials of $-22$ mV (QD) and $-25$ mV (QR), the 2D plots describe the depolarization effect with the brightest colors associated with the strongest occurring depolarization. Conversely, when the zeta potential is equal to zero, there is no reduction of the membrane potential. Finally, the two images showing the charge distributions around the membrane upon contact with negatively charged particles explain why, experimentally, our negative QD and QR could easily adhere to the cell membrane: A net attractive Coulomb interaction has pushed the negative NPs to move toward the membrane. A positively charged NP would not promote the attraction toward an outer positive membrane.

**DISCUSSION**

The present study identifies the key determinants of NP interactions with neuronal functional activity at both synaptic and network levels. The NP surface charge is crucial for the modulation of neuronal electrical activity, while NP size, shape, and material play a negligible role in the association and functional interaction with neurons. These results are in agreement with the literature on single material studies. For instance, similar NP–neuron interactions were observed for negative carbon black, hematite iron oxide, titanium dioxide, and gold NPs. However, the interactions in those studies, unlike those reported in this work, were investigated over prolonged time exposure (from few hours to days) and at different concentration ranges within them. In this work, we have conducted a systematic study on different NP materials, of different size, shape composition, and coating, and we have investigated the immediate effects of NPs just after their administration, within the first few minutes and at a rather low-concentration range. Our experimental data demonstrate four main results: (i) only negative NPs (zeta potential $< -20$ mV) adhere to neurons (bodies, neurites and synaptic clefts); (ii) negative NPs selectively interact with neuronal excitable membranes; (iii) negative NPs on neurons cause a depolarization of neurons and measurable changes in neuron and neuronal network electrical activity; (iv) the suppression of neuronal activity by TTX hinders the interaction of negative NPs with neurons.

Our theoretical model explains how a Coulombic potential on the outer neuron surface retains only negative NPs. Importantly, these simulations predict membrane depolarization that supports the experimental evidence of increased neuronal activity, as measured at the single neuron and at the whole network levels. However, this simulation model does not account for the selective interactions of NPs with neurons and not with nonexcitable glial cells. Based on our data, three key factors appear to determine the selective localization of negatively charged NPs on neurons and the electrophysiological activity change. First, neuronal spiking activity attracts NPs, as demonstrated by experiments with TTX and by the lack of localization on glial cells. Second, Coulombic attraction retains only negatively charged NPs at the cell membrane, as observed in our simulations. Third, the presence of negatively charged NPs on neurons induces a depolarization, as measured in our electrophysiological experiments and also predicted by our model, which in turn facilitates the crossing of the threshold for firing an action potential.

To explain the attraction of NPs, which appears to be induced by neuronal spiking activity, we speculate that the strong depolarization during the firing of an action potential (up to $+40$ mV) could transiently remove the counterions surrounding the negatively charged NPs, thus allowing a tight and stable electrostatic interaction between the negatively charged NP surface and the positively charged outer side of the neuronal membrane. This mechanism could explain the selective, activity-dependent interaction of NPs with neurons, as we observed with imaging, EM, and electrophysiology. Besides the Coulombic interactions at the cellular membrane, the presence of NPs at the synaptic cleft could also be due to the matching of NP size with the cleft space. NPs at synapses are indeed expected to be strategically located to operate a further “fine modulation” of neuronal excitability.

The selective NP–neuron interaction shown here paves the way to innovative applications of NPs in neuroscience. The key role of NP surface charge for neuronal interactions is a fundamental finding for the future design of NPs for neuron targeting. The specific interaction with neurons, but not with glial cells, could represent an excellent opportunity to discriminate between these two cell types for selective drug delivery. In addition, the higher sensitivity to neuronal activity suggests that negatively charged NPs could be used as markers of active neurons for long-term imaging, easily measured by NP fluorescence. This approach could be exploited, for instance, to visualize aberrant increased neuronal activity in neurological disorders. In the same conceptual framework, negatively charged NPs could also reveal alterations of excitability in different subregions of individual neurons (e.g., soma vs distal dendrites), an important factor for the emergence of specific brain states, including network oscillations and selection of cell assemblies. Along the same lines, the increased neuronal activity could be exploited to increase the activity of inhibitory neurons with reduced excitability that are responsible, for example, for severe forms of epilepsy. More generally, given an efficient neuronal subtype-specific targeting, in principle, NPs could be exploited to finely modulate the balance between excitation and inhibition in the brain, a major determinant for most neurological diseases.

HD-MEA experiments have revealed that the increase in the overall spiking activity by NPs is accompanied by a change in the network synchronicity. This could be induced by the uniform increase of the neurons’ activity, reaching a high level of network stability. Alternatively, NPs could differentially affect neurons in the network by, for example, preferentially activating neurons with high connectivity (hub neurons) that may be more likely to interact with NPs due to longer dendritic/axonal length and more complex arborization. Such activity-dependent behavior of negative NPs might allow the design of nanomedicine tools for the control of neuronal activity or for the selective delivery of drugs to firing neurons.

**CONCLUSIONS**

NPs with negatively charged surfaces rapidly localize on neuronal membranes and induce electrophysiological alterations. In contrast, the same type of NPs with positive or
neural zeta-potential shows no or low nonspecific interaction with the cellular membrane and no effects on bioelectric activity. These effects are material independent because similar results occurred with magnetic NPs as well as Cd-based semiconductors. Importantly, the interaction of negative NPs is not only selective for excitable neuronal cells but also dependent on neuronal activity. These observations suggest that electric activity most likely plays a role in the specificity of the NP–neuron interaction. Finally, numerical simulation supports the proposed mechanism for the selectivity of negative NPs and the modulation of neural electric activity.

MATERIALS AND METHODS

Materials and Cell Method Protocols. Materials for the NP Synthesis. Poly(maleic anhydride-alt-1-octadecene) MW 20,000–25,000, poly(isobutylene-alt-maleic anhydride), poly(styrene-co-maleic anhydride) cumene terminated, dodecylamine, oleylamine, bis(6-aminoehexyl)amine, N-(3-dimethylamino)propyl)-N-’-ethylenediamine hydrochloride (EDC), methoxy(polyethylene glycol) amine MW 750 (NH2-PEG-OCH3), O-(2-aminoethyl)-O’-(2-carboxyethyl)-polyethylene glycol hydrochloride MW 3000 (NH2-PEG-COOH), poly(ethylene glycol) bis(amine) MW 2000 (NH2-PEG-NH2), N,N’-dimethylthelylenediamine (DMEDA), poly-n-lysine hydrobromide (PDL), tetrodotoxin (TTX), bicuculline (BICU), diethylzinc, 1.0 M in hexane, hexamethyldisilathiane, toluene, and methanol anhydrous were purchased from Sigma-Aldrich. Trioctyolphosphine oxide 99% (TOPO), cadmium oxide 99.999% (CdO), tri-n-octylphosphine 97% (TOP), sulfur 99%, selenium powder 99.99%, Fe(CO)5, and trioxyporphosphine were purchased from Strem Chemicals. n-Octadecylyphosphonic acid (ODPA) and hexylphosphonic acid (HA) were obtained from Sigma-Aldrich.

NP Synthesis. All QDs, QRs, and magnetic NPs used were prepared by the seeded growth approach as already reported.36,39 The surfactant-coated colloidal NPs were transferred from organic solvent into water by implementing a previously developed polymer-coating procedure.25,40 To tune the surface charge of the NPs, further surface surfactant-coated colloidal NPs were transferred from organic solvent to an aqueous solution. For the surface modification of NPs, various functional groups were attached to the polymer shell by using different coupling agents, such as 3-amino propyl triethoxy silane (APTES), to prepare bi-functional amino-functionalized NPs. The following linkages were used for the functionalization of NP surfaces: primary amino groups of the functional group were reacted with carboxylic acid groups of the polymer in a solution containing either 1 M sodium hydroxide or 1 M sodium bicarbonate.

Electron Microscopy (EM) Analysis. TEM Methods. TEM and high-angular annular dark-field (HAADF) STEM were performed on mouse primary hippocampal neurons doped for 10 min with Fe3O4 nanorods and dots differing exclusively in their overall surface charge in a range from ~40 mV to ~250 mV. Similar experiments have been conducted on a plethora of nanorods differing in composition, overall surface charge and dimensions (see Tables S1.1 and S1.2). The neurons were plated on glass coverslips and fixed with 0.66 M cacodylate buffered 1.25% glutaraldehyde solution for 1 h at room temperature. After extensive rinsing in the same buffer, the samples were postfixed in a solution of 1.5% potassium ferrocyanide and 1% osmium tetroxide in 0.1 M cacodylate buffer, stained overnight in the dark in a 0.5% aqueous solution of uranyl acetate, dehydrated in a graded ethanol series, and embedded in EPON resin. Sections of 70 nm were cut with a diamond knife (Diatome) on a Leica EM UC6.
ultramicrotome and stained with lead citrate and uranyl acetate. EM tomography was performed in bright-field TEM and HAADF STEM on thick sections (ranging in thickness from 100 to 250 nm). Computation weighted back-projection (WBP) of tomograms was done with the IMOD software package. Segmentation and three-dimensional visualization were performed using the Amira software package. (FEI Visualization Science Group, Bordeaux, France). EM projection images and HAADF STEM tilted series were collected using a FEI Tecnai G2 F20 equipped with a Schottky field-emission gun and recorded with a 2k x 2k Gatan BM UltraScan charge-coupled device (CCD) camera.

Electrophysiological and Neural Network Activity Recording. Electrophysiological Recordings. Inhibitory spontaneous post-synaptic currents (iPSCs) were recorded in the whole-cell configuration of the patch-clamp technique. External recording solution contained (in mM): 145 NaCl, 2 KCl, 2 CaCl2, 2 MgCl2, 10 glucose, and 10 HEPES, pH 7.4. Patch pipettes, pulled from borosilicate glass capillaries (Hilgenberg, Malsfeld, Germany), had a 4–5 MΩ resistance when filled with intracellular recording solution containing (in mM): 150 KCl, 1 CaCl2, 2 MgCl2, 1 EGTA, 10 HEPES, and 2 Na2ATP (300 mM and pH 7.2 with KOH). Currents were acquired using Clampex 10.0 software (Molecular Devices, Sunnyvale, CA). iPSCs were recorded at room temperature from a holding potential of −60 mV in the presence of CNQX (10 mM) to isolate GABAAergic events. Currents were sampled at 20 kHz and digitally filtered at 3 kHz using the 700B patch amplifier (Molecular Devices). iPSCs were detected by using the “scaled sliding template” detection algorithm implemented in pClamp10, first described by Clements and Bekkers and by setting the “detection criterion value” to 5. QRs and QDs were bath applied in the recording chamber following the recording of a baseline trace of at least 5 min. The effects of QRs and QDs were examined 10 min after application. The stability of the patch was checked by repetitively monitoring the input and series resistance during the experiments. Cells exhibiting 10–15% changes in the duration of the whole experiment were excluded from the analysis.

Neural Network Activity Recording with High-Density Multi-Electrode Array (HD-MEA). Extracellular electrophysiological in vitro experiments were carried out by means of a recently developed HD-MEA. These devices, thoroughly reviewed in ref 46, provide a matrix of 4096 electrodes (sensing area 21 x 21 μm², pitch 42 x 42 μm²) arranged in a 64 x 64 layout and covering an area of 2.7 by 2.7 mm². After seeding and growing dissociated neuronal cultures on top of the active area, extracellular recordings of the spontaneous network activity were performed simultaneously from all the 4096 electrodes at 7 kHz sampling rate. Before seeding, HD-MEA chips were sterilized 20 min in 70% ethanol, rinsed abundantly in double deionized sterile water (DDW), filled with neurobasal complete medium (NB complete medium) and kept overnight in incubator to increase hydrophilicity. Then the active clefts were filled with neurobasal complete medium (NB complete medium) and 200 nm ethanol, rinsed abundantly in double deionized sterile water (DDW), placed in the QD/QR shell at this stage. The second calculation solved the Poisson equation by placing a charge density (in order to provide a surface potential matching the measured zeta potential) within the QD/QR shell. No membrane potential was set at this stage. The total potential was then plot by adding potential values from the two calculations at each point of the domain.

For both electrostatic calculations, the designed elements were surrounded by a 200 nm x 200 nm x 200 nm box terminated with 10 nm of infinite elements layer to truncate the calculation and to mimic an effectively more extended simulation domain. The outer surfaces of the box were set as ground for both calculations.

ASSOCIATED CONTENT

Supporting Information
Supporting Information contains time lapse movies showing the interaction of negative and positive NPs with neural networks; The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.7b00397.

Tables with summary of parameters and TEM images of all NPs; supplementary figures related to confocal microscopy and TEM imaging of NPs interaction with neural networks; TEM tomograms and relative 3D models of negative NPs inside a synaptic cleft and among neurites (PDF)

Movie M2.1: 10 min time lapse movie taken just after the administration of negative QRs (1 nM) to primary hippocampal neuron network (ZIP)

Movie M2.2: 10 min time lapse movie taken just after the administration of neutral QDs (1 nM) to primary hippocampal neuron network (ZIP)

Movie M3.1: TEM WBP tomogram and relative 3D model of QRs (~35 mV, 32 nm long) inside a synaptic cleft as shown in Figure 2C (AVI)

Movie M3.2: HAADF STEM WBP tomogram and relative 3D model of Fe2O3 nanorods (~40 mV, 65 nm long) among neurites as shown in Figure 2D (AVI)

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Author Contributions

These authors have contributed equally to this research. S.D., T.P., A.B., and L.B. conceived the experiments. S.D. and A.P. cultured the cells and performed the confocal imaging. R.M. performed electron microscopy. S.D. and A.M. performed the MEAs recording and analyzed the data. E.M.P. and T.R. performed the patch-clamp experiments. A.Q., F.D.D., and A.S. synthesized the NPs. A.P. and A.Q. functionalized the NPs and performed DLS and zeta potential measurements. A.A. and R.P.Z. performed the modeling. T.P., S.D., A.B., L.B., R.M., R.P.Z, and R.C. wrote the manuscript, and all the authors have revised the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Carvalho-de-Souza, J. L.; Treger, J. S.; Ding, B.; Kent, S. B.; Pepperberg, D. R.; Bezanilla, F. Photosensitivity of neurons enabled by cell-targeted gold nanoparticles. Neuron 2015, 86 (1), 207–17.
(2) Kotov, N. A.; Winter, J. O.; Clements, I. P.; Jan, E.; Timko, B. P.; Campidelli, S.; Pathak, S.; Mazzatenta, A.; Lieber, C. M.; Prato, M.; Bellamkonda, R. V.; Silva, G. A.; Kam, N. W. S.; Patolsky, F.; Ballerini, L. Nanomaterials for Neural Interfaces. Adv. Mater. 2009, 21 (40), 3970–4004.

(3) Nel, A. E.; Madler, L.; Velegol, D.; Xia, T.; Hoek, E. M. V.; Somasundaran, P.; Klaessig, F.; Castranova, V.; Thompson, M. Understanding biophysicalchemical interactions at the nano-bio interface. Nat. Mater. 2009, 8 (7), 543–557.

(4) Setua, S.; Ouberei, M.; Piccirillo, S. G.; Watts, C.; Celli, M.; Cisplatin-tethered gold nanoparticles for multimodal chemo-radiotherapy of glioblastoma. Nanoscale 2014, 6 (18), 10865–73.

(5) Jordan, A.; Scholz, R.; Maier-Hauff, K.; van Landeghem, F. K.; Waldoeiner, N.; Teichgraeber, U.; Pinkernelle, J.; Bruhn, H.; Neumann, F.; Thiesen, B.; von Deimling, A.; Felix, R. The effect of thalamotomy using magnetic nanoparticles on rat malignant glioma. J. Neuro-Oncol. 2006, 78 (1), 7–14.
(6) Tabatabaei, S. N.; Giroud, H.; Carret, A. S.; Martel, S. Remote control of the permeability of the blood-brain barrier by magnetic heating of nanoparticles: A proof of concept for brain drug delivery. J. Controlled Release 2015, 206, 49–57.

(7) Chen, R.; Romero, G.; Christiansen, M. G.; Mohr, A.; Anikeeva, P. Wireless magnetothermal deep brain stimulation. Science 2015, 347 (6229), 1477–80.
(8) Dobson, J. Remote control of cellular behaviour with magnetic nanoparticles. Nat. Nanotechnol. 2008, 3 (3), 139–43.

(9) Cai, E.; Ge, P.; Lee, S. H.; Jeyifous, O.; Wang, Y.; Liu, Y.; Wilson, K. M.; Lim, S. J.; Baird, M. A.; Stone, J. E.; Lee, K. Y.; Davidson, M. W.; Chung, H. J.; Schulten, K.; Smith, A. M.; Green, W. N.; Selvin, P. R. Stable small quantum dots for synaptic receptor tracking on live neurons. Angew. Chem., Int. Ed. 2014, 53 (46), 12484–12488.

(10) Clarke, S.; Pinaud, F.; Beutel, O.; You, C.; Piehler, J.; Dahan, M. Covalent multifunctionalization of peptide-coated quantum dots for single-molecule assays. Nano Lett. 2010, 10 (6), 2147–54.

(11) Pinaud, F.; Clarke, S.; Sittner, A.; Dahan, M. Probing cellular events, one quantum dot at a time. Nat. Methods 2010, 7 (4), 275–85.

(12) You, C.; Wilmes, S.; Beutel, O.; Lochte, S.; Podopelewowa, Y.; Roder, F.; Richter, C.; Seine, T.; Schlabale, D.; Uze, G.; Clarke, S.; Pinaud, F.; Dahan, M.; Piehler, J. Self-controlled multifunctionalization of quantum dots for multiplexed protein tracking in live cells. Angew. Chem., Int. Ed. 2010, 49 (24), 4108–12.

(13) Arvizo, R. R.; Miranda, O. R.; Thompson, M. A.; Pabelick, C. M.; Bhattacharya, R.; Robertson, J. D.; Rotello, V. M.; Prakash, Y. S.; Mukherjee, P. Effect of nanoparticle surface charge at the rhl membrane and beyond. Nano Lett. 2010, 10 (7), 2543–8.

(14) Barbu, E.; Molnar, E.; Tsiboukis, J.; Gorecki, D. C. The potential for nanoparticle-based drug delivery to the brain: overcoming the blood-brain barrier. Expert Opin. Drug Delivery 2009, 6 (6), 553–65.

(15) Gramowski, A.; Flossdorf, J.; Bhattacharya, K.; Jonas, L.; Lantow, M.; Rahman, Q.; Schimmel, D.; Weiss, D. G.; Dopp, E. Nanoparticles induce changes of the electrical activity of neuronal networks on microelectrode array neurochips. Environ. Health Perspect 2010, 118 (10), 1363–9.

(16) Vuillermet-Beandre, L. The targeted delivery of cancer drugs across the blood-brain barrier: chemical modifications of drug or drug-nanoparticles? Drug Discovery Today 2008, 13 (23–24), 1099–106.

(17) Malvindi, M. A.; Carbone, L.; Quarta, A.; Tino, A.; Manna, L.; Pellegrino, T.; Tortiglione, C. Rod-shaped nanocrystals elicit neuronal activity in vivo. Small 2008, 4 (10), 1747–55.

(18) Salinas, K.; Kereselidze, Z.; Deluna, P.; Peralta, X. G.; Santamaria, F. Transient extracellular application of gold nanostars increases hippocampal neuronal activity. J. Nanobiotechnol. 2014, 12, 31.

(19) Dube, C.; Richichi, C.; Bender, R. A.; Chung, G.; Litt, B.; Baram, T. Z. Temporal lobe epilepsy after experimental prolonged febrile seizures: prospective analysis. Brain 2006, 129, 911–922.

(20) Sachdev, P. S. Alternating and postictal psychoses: review and a unifying hypothesis. Schizophr Bull. 2007, 33 (4), 1029–37.

(21) Yuan, P.; Grutzendler, J. Attenuation of beta-Amyloid Deposition and Neurotoxicity by Chemogenetic Modulation of Neural Activity. J. Neurosci. 2016, 36 (2), 632–41.

(22) Mazzatenta, A.; Giugliano, M.; Campidelli, S.; Gambazzi, L.; Businario, L.; Markram, H.; Prato, M.; Ballerini, L. Interfacing neurons with carbon nanotubes: electrical signal transfer and synaptic stimulation in cultured brain circuits. J. Neurosci. 2007, 27 (26), 6931–6.

(23) Lovat, V.; Pantarotto, D.; Lagostena, L.; Cacciari, B.; Grandolfo, M.; Riggi, M.; Spalluto, G.; Prato, M.; Ballerini, L. Carbon nanotube substrates boost neuronal electrical signaling. Nano Lett. 2005, 5 (6), 1107–10.

(24) Jung, A.; Bang, M.; Kim, B. S.; Lee, S.; Kotov, N. A.; Kim, B.; Jeon, D. Intracellular gold nanoparticles increase neuronal excitability and aggravate seizure activity in the mouse brain. PLoS One 2014, 9 (3), e93160.

(25) Walters, R.; Kraig, R. P.; Medintz, I.; Delehanty, J. B.; Stewart, M. H.; Susumu, K.; Huston, A. L.; Dawson, P. E.; Dawson, G. Nanoparticle targeting to neurons in a rat hippocampal slice culture model. ASN Neuro 2012, 4, AN20120042.

(26) Amin, H.; Maccione, A.; Marinaro, F.; Zordan, S.; Nieus, T.; Berdon, L. Electrical Responses and Spontaneous Activity of Human iPSC-Derived Neuronal Networks Characterized for 3-month Culture with 4096-Electrode Arrays. Front. Neurosci. 2016, 10, 121.

(27) Berdon, L.; Infeld, K.; Maccione, A.; Tedesco, M.; Neukom, S.; Kowalski, S.; Hep, M.; Martoniss, S. Active pixel sensor array for high spatio-temporal resolution electrophysiological recordings from single cell to large scale neuronal networks. Lab Chip 2009, 9 (18), 2644–2651.

(28) Sperling, R. A.; Pellegrino, T.; Li, J. K.; Chang, W. H.; Parak, W. J. Electrophoretic separation of nanoparticles with a discrete number of functional groups. Adv. Funct. Mater. 2006, 16 (7), 943–948.
(29) Pellegrino, T.; Manna, L.; Kudera, S.; Liedl, T.; Koktysh, D.; Rogach, A. L.; Keller, S.; Radler, J.; Natlie, G.; Parak, W. J. Hydrophobic nanocrystals coated with an amphiphilic polymer shell: A general route to water soluble nanocrystals. Nano Lett. 2004, 4 (4), 703–707.

(30) Walters, R.; Medintz, I. L.; Delehanzy, J. B.; Stewart, M. H.; Susumu, K.; Huston, A. L.; Dawson, P. E.; Dawson, G. The Role of Negative Charge in the Delivery of Quantum Dots to Neurons. ASN Neuro 2015, 7, 175909141559238.

(31) Araque, A.; Carmignotto, G.; Haydon, P. G.; Oliet, S. H.; Robitaille, R.; Volterra, A. Gliotransmitters travel in time and space. Neuron 2014, 81 (4), 728–39.

(32) Alabastri, A.; Toma, A.; Liberale, C.; Chirumamilla, M.; Giugni, A.; De Angelis, F.; Das, G.; Di Fabrizio, E.; Zaccaria, R. P. Interplay between electric and magnetic effect in adiabatic polaritonic systems. Opt. Express 2013, 21 (6), 7538–7548.

(33) Giugni, A.; Das, G.; Alabastri, A.; Zaccaria, R. P.; Zanella, M.; Franchini, I.; Di Fabrizio, E.; Krahne, R. Optical phonon modes in ordered core-shell CdSe/CdS nanorod arrays. Phys. Rev. B: Condens. Matter Mater. Phys. 2012, 85, 115413.

(34) Klausberger, T.; Somogyi, P. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. Science 2008, 321 (5885), 53–7.

(35) Mantegazza, M.; Catterall, W. A. Voltage-Gated Na+ Channels: Structure, Function, and Pathophysiology. In Jasper’s Basic Mechanisms of the Epilepsies, 4th ed.; Noebels, J. L., Avoli, M., Rogawski, M. A., Olsen, R. W., Delgado-Escueta, A. V., Eds.; Oxford University Press: Oxford, 2012.

(36) Carbone, L.; Nobile, C.; De Giorgi, M.; Sala, F. D.; Morello, G.; Pompa, P.; Hytch, M.; Snoeck, E.; Fiore, A.; Franchini, I. R.; Nadasan, M.; Silvestre, A. F.; Chiodo, L.; Kudera, S.; Cingolani, R.; Krahne, R.; Manna, L. Synthesis and micrometer-scale assembly of colloidal CdSe/CdS nanorods prepared by a seeded growth approach. Nano Lett. 2007, 7 (10), 2942–50.

(37) Dabbousi, B. O.; RodriguezViejo, J.; Mikulec, F. V.; Heine, J. R.; Mattoussi, H.; Ober, R.; Jensen, K. F.; Bawendi, M. G. CdSe/ZnS core-shell quantum dots: Synthesis and characterization of a size series of highly luminescent nanocrystallites. J. Phys. Chem. B 1997, 101 (46), 9463–9475.

(38) Peng, S.; Wang, C.; Xie, J.; Sun, S. Synthesis and stabilization of monodisperse Fe nanoparticles. J. Am. Chem. Soc. 2006, 128 (33), 10676–7.

(39) Das, R.; Alonso, J.; Porshokouh, Z. N.; Kalappattil, V.; Torres, D.; Phan, M. H.; Garaio, E.; García, J. A.; Sanchez Llamazares, J. L.; Srikanth, H. Tunable High Aspect Ratio Iron Oxide Nanorods for Enhanced Hyperthermia. J. Phys. Chem. C 2016, 120 (18), 10086–10093.

(40) Di Corato, R.; Quarta, A.; Piacenza, P.; Ragusa, A.; Figuerola, A.; Buonsanti, R.; Cingolani, R.; Manna, L.; Pellegrino, T. Water solubilization of hydrophobic nanocrystals by means of poly(maleic anhydride-alt-1-octadecene). J. Mater. Chem. 2008, 18 (17), 1991–1996.

(41) Quarta, A.; Curcio, A.; Kakwes, H.; Pellegrino, T. Polymer coated inorganic nanoparticles: tailoring the nanocrystal surface for designing nanoprobes with biological implications. Nanoscale 2012, 4 (11), 3319–34.

(42) Marconi, E.; Nieus, T.; Maccione, A.; Valente, P.; Simi, A.; Messa, M.; Dante, S.; Baldelli, P.; Berdondini, L.; Benfenati, F. Emergent Functional Properties of Neuronal Networks with Controlled Topology. PLoS One 2012, 7, e34648.

(43) Kremer, J. R.; Mastronarde, D. N.; McIntosh, J. R. Computer visualization of three-dimensional image data using IMOD. J. Struct. Biol. 1996, 116 (1), 71–76.

(44) Mastronarde, D. N. Automated electron microscope tomography using robust prediction of specimen movements. J. Struct. Biol. 2005, 152 (1), 36–51.

(45) Clements, J. D.; Bekkers, J. M. Detection of spontaneous synaptic events with an optimally scaled template. Biophys. J. 1997, 73 (1), 220–9.