Biocompatibility of neural electrodes

or:
Curbing the immune response against brain electrode implants

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Summary

A healthy functioning brain is crucial for proper sensory- and motor processing. Neurological disorders change normal neuronal activity, which can impair cognition, sensory and motor functions. Electrodes implanted in the brain improve these functions by interacting with electrically excitable tissue. The central nervous system (CNS) has limited regenerative potential, but seems tolerant with regard to biomaterial implants. However, the foreign body response (FBR) leads to structural and cellular changes around the implant, that affect both nervous tissue and electrode functionality.

Implantation of electrodes in the CNS activates the immune system through surgical damage and foreign material introduction. The cellular and molecular components can stimulate protective and toxic pathways, limiting the application of this therapy. Approaches for future development include inflammation prevention, mimicking of brain tissue characteristics and promoting recovery. This essay reviews strategies for new neural electrode applications and the interaction of electrodes with neurons and the resident immune cells of the nervous tissue.

Abbreviations

- BBB, blood-brain barrier
- CNS, central nervous system
- ECoG, electrocorticogram
- EEG, electroencephalogram
- FBR, foreign body response
- GFAP, glial fibrillar acidic protein
- MEMS, micro-electromechanical system
- NTFs, neurotrophic factors
- ROS, reactive oxygen species
- TGFβ, transforming growth factor beta
- TNFα, tumor necrosis factor alpha
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Introduction

A healthy central nervous system (CNS) allows us to interact with our environment via controlled behaviour. It functions as a biological interface, constantly converting sensory information into appropriate motor output. In spite of the plasticity and robustness of neuronal tissue, impairments can occur in signalling that leads to a multitude of neurological and psychiatric disorders. Neural implants can in theory take over any connection or function that is lost or damaged with artificial units. This includes sensory input, processing of information and control of motor functions. Implants have to imitate the main players of the CNS, neurons. This means directing incoming and outgoing signals, which is possible via neural electrodes. Neural electrodes are needles, that can conduct electricity and are implanted in the brain. Since the 1970s researchers have become increasingly better in monitoring and modifying brain activity by implanting electrodes in the CNS (2–4).

The use of these electrodes is both powerful, they can induce a strong response in neurons, and region-specific. However, the applications do require surgery and introduction of foreign material, at vulnerable sites. The resulting foreign body response (FBR) affects both the nervous tissue and the implant, especially when used for a longer period of time. Therefore, new therapeutic applications require biocompatibility of neural electrodes. This means interaction between the implant and neuronal tissue without a loss of function of either one over time.

Topics and questions

Firstly, how can the immune response limit the function of neural electrodes? To answer this question it is necessary to know what the neural implant applications depend upon to function. Interaction between electrodes and the CNS tissue around it is one of the biggest challenges, because we want a stable functioning implant. Secondly, how does neuronal tissue react to chronically implanted material? The FBR is a continually changing process during the lifetime of the implant. Therapies discussed in this essay are limited to electrode applications that interact with neuronal activity.

The main and final question is: how can the biocompatibility of neural electrodes be improved to allow for new therapeutic applications? This involves the integration of a variety of research fields from biochemistry and neurobiology to medical engineering.

Advantages of electrode implantation

The brain is shielded from the periphery by the blood brain barrier (BBB). The BBB restricts access of many molecules to the CNS. High doses or difficult targeting modifications are strategies to overcome this barrier. Since the body periphery uses receptors and signal molecules similar to those in the CNS, but with different functions, these strategies are already prone to complications (5). Without specific central targeting, the use of drugs that influence these systems is restricted. Neural electrodes act locally and direct on neuronal activity. More advanced applications can in fact measure activity and act thereupon precisely as much as needed. These advantages have led to more widespread use of neural implants (Table 1).
Table 1. Pharmaceutical intervention versus neural implant treatment.

<table>
<thead>
<tr>
<th>Topic</th>
<th>Neural implants</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjustments</td>
<td>Careful placement during implantation, followed by signal strength optimization.</td>
<td>Adjust dosage till optimal therapeutic window is found.</td>
</tr>
<tr>
<td>Cost</td>
<td>A costly procedure with low maintenance.</td>
<td>Expenditure increases once lower doses of medication fail.</td>
</tr>
<tr>
<td>Reversibility</td>
<td>Electrical effects are reversible, but initial tissue damage remains.</td>
<td>In general reversible, but patients can develop tolerance.</td>
</tr>
<tr>
<td>Cure</td>
<td>Complete remission of impairments is possible.* No restoration of damages.</td>
<td>Can slow down progression of the disease. Generally targets symptoms.</td>
</tr>
<tr>
<td>Effectiveness</td>
<td>Most patients show improvement; often dosage of medication can be decreased after implantation.</td>
<td>Less severe symptoms can be managed properly. The benefits do not always outweigh the side-effects.</td>
</tr>
<tr>
<td>Damage</td>
<td>Local tissue damage. Depends on size and type of implant.</td>
<td>At higher dosages widespread and systemic effects can be deteriorating.</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Local tissue damage and inflammation.</td>
<td>Physiological alterations can disturb homeostasis.</td>
</tr>
<tr>
<td>Localization</td>
<td>Local. Stimuli can affect connected brain regions through projections.</td>
<td>Depends on the delivery method and target, but often drug availability throughout CNS.</td>
</tr>
<tr>
<td>Control</td>
<td>Real-time control.</td>
<td>Careful maintenance of drug concentrations.</td>
</tr>
</tbody>
</table>

*Lost neural connections can be replaced, but do not stop or reverse disease progression. Even so, stimulation of cellular activity can support regenerative drug and stem cell therapies.

Interaction of electrodes and CNS tissue determines success

The neurodegenerative disorders and traumatic injuries targeted by implantation therapy involve neuronal loss, tissue degeneration or dysfunctional neurons. Brain tissue is compact and its functions are localized to precise regions. A first guideline follows: be very precise. In case the tissue surrounding the neural electrode becomes unresponsive, the implant fails. Stimulating electrodes might compensate for this by increasing their impulse strength. Other applications, that rely on specificity and durability, require careful insertion (6).

The potential strengths and weaknesses of implantation therapy follow from Table 1. It is notable that most applications lack a feedback mechanism (Table 2). While sometimes adjustments controlled by patient or physician can fill this role, I think many applications would improve drastically with feedback from measuring electrodes (7,8). However, these kind of electrodes are the most sensitive to signal loss over time (6,9), one of the reasons they are not yet commonplace. Another major strongpoint, chronic effectiveness, is almost completely dependent on the immune response. Implants can only be as functional as the CNS allows them to be. Otherwise, application of these new technologies does more harm than good.
Table 2. Applications for biomaterial implantation in the CNS.

<table>
<thead>
<tr>
<th>Application</th>
<th>Function of implant:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep brain stimulation (DBS)(10)</td>
<td>Suppression, stimulation or simulation of electrical activity</td>
</tr>
<tr>
<td>Recording(11)</td>
<td>Measuring the activity of brain regions to identify targets for</td>
</tr>
<tr>
<td></td>
<td>surgery, indicate events or other diagnostics.</td>
</tr>
<tr>
<td>Sensory prosthetics</td>
<td>Stimulator of sensory cortex from a device with sensory</td>
</tr>
<tr>
<td></td>
<td>capabilities.</td>
</tr>
<tr>
<td>Pain treatment(12)</td>
<td>Direct suppression of pain circuitry.</td>
</tr>
<tr>
<td>Bladder control</td>
<td>Support autonomous functions.</td>
</tr>
<tr>
<td>Motor prosthetics</td>
<td>Record neural activity from the motor cortex to control a</td>
</tr>
<tr>
<td></td>
<td>prosthetic limb or device.</td>
</tr>
<tr>
<td>Motor support</td>
<td>Record neural activity and stimulate neural pathways or muscles.</td>
</tr>
<tr>
<td>Brain-computer interface(13)</td>
<td>Translate recorded brain activity to a computer and perhaps loop</td>
</tr>
<tr>
<td></td>
<td>back to a brain region to restore a connection.</td>
</tr>
<tr>
<td>Shunt for hydrocephalus</td>
<td>Drain excessive cerebrospinal fluid.</td>
</tr>
<tr>
<td>Drug carriers</td>
<td>Slow, local or on demand drug delivery.</td>
</tr>
<tr>
<td>Scaffolds(14)</td>
<td>Support for the regeneration of tissue.</td>
</tr>
<tr>
<td>Encapsulation of neural stem cells</td>
<td>Replace lost tissue. Support for cell transplantation.</td>
</tr>
</tbody>
</table>

Applications of neural electrodes

Neural electrodes target the electrical potential of one or more groups of cells. Electrical stimulation can depolarize neighbouring neurons to induce an action potential and thus increase neuronal activity. Interestingly, electrical stimulation can also have the opposite effect. Hyperpolarization inhibits (hyper)activity of neurons at the implant site. This manipulation of local membrane potentials can be adjusted in real-time by patient or doctor to provide an optimal configuration (15,16). Electrical stimulation is a unique approach, but insufficient knowledge of neuroanatomy and long term effects on a cellular level makes it still a bit unpredictable.

Patients with severe Parkinson’s disease have improved immensely with deep brain stimulation (DBS) therapy. In DBS, one or two macroelectrodes, which have a large area of effect, are implanted per hemisphere. Due to their size, they can largely overcome the effects of a chronic FBR (17). DBS is also applied against severe depression, chronic pain, obsessive compulsive disorders and other debilitating illnesses (see Appendix I).

Parkinson’s disease (PD)

In PD, neurons in the substantia nigra are affected, a brain structure in the midbrain. Since PD does not have a specific known origin, it is not yet curable (18,19). Local cell death in the substantia nigra reduces dopaminergic neurotransmission to the basal ganglia (20). Pharmacological intervention aims to increase the dopamine availability. Treatment is a continuous search for optimal symptom suppression. Medication ultimately leads to motor symptoms in itself. High concentrations of Levodopa disturb dopaminergic transmissions elsewhere (21).
<table>
<thead>
<tr>
<th>Neurological disorder</th>
<th>Role of implant:</th>
<th>Types of implants*:</th>
<th>Location*:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinson’s disease (22,23)</td>
<td>Stimulate dopaminergic projections, suppress tremors, relieve rigidity</td>
<td>Deep brain electrode</td>
<td>Thalamus, Subthalamus, Globus pallidus, Motor Cortex</td>
</tr>
<tr>
<td>Dystonia Tremors</td>
<td>Incapacitate regions that promote tremor</td>
<td>Deep brain electrode</td>
<td>Thalamus, Subthalamus, Globus pallidus</td>
</tr>
<tr>
<td>Tourette Syndrome</td>
<td>Reducing tic severity</td>
<td>Deep brain electrode</td>
<td>Globus pallidus, Thalamus</td>
</tr>
<tr>
<td>Obsessive-compulsive disorder (24)</td>
<td>Stimulate serotonergic projections, suppress anxiety, suppress compulsions</td>
<td>Deep brain electrode</td>
<td>Nucleus accumbens</td>
</tr>
<tr>
<td>Huntington (1)</td>
<td>Delivery of therapeutic factors to</td>
<td>Encapsulated cell delivery system</td>
<td>Striatum</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>Diagnostic: Identify epileptic focus Treatment: Predict seizure, Disrupt seizure</td>
<td>Electrode array, in or on top of region</td>
<td>Cortex, Epileptic focus</td>
</tr>
<tr>
<td>Depression (25,26)</td>
<td>Modulation of corticostriatopallido-thalamocortical circuits</td>
<td>Deep brain electrode</td>
<td>Nucleus accumbens, Subgenual area, Striatum</td>
</tr>
<tr>
<td>Traumatic brain injury (27)</td>
<td>Support recovery, Reestablish connections</td>
<td>Drug delivery system Multi-input multi-output prosthesis</td>
<td>Site of injury, Around injury</td>
</tr>
<tr>
<td>Nerve injury</td>
<td>Regeneration</td>
<td>Graft with growth factors, Transplant or artificial link</td>
<td>Injury site and connecting tissue</td>
</tr>
<tr>
<td>Sensory loss: Deafness (28)</td>
<td>New sensory system Sensors that stimulate functional brain regions</td>
<td>Cochlear implant Visual prosthesis Tactile feedback</td>
<td>Sensory systems Auditory system Visual system Somatosensory system</td>
</tr>
<tr>
<td>Blindness (29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of tactile sensation (30)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster headache Migraine (31)</td>
<td>Suppress the trigeminal-autonomic reflex</td>
<td>Radiofrequency electrode</td>
<td>Sphenopalatine ganglion</td>
</tr>
<tr>
<td>Chronic pain (32)</td>
<td>Interfere with pain signal propagation Stimulate release of opioids</td>
<td>Spinal cord stimulator Deep brain electrode</td>
<td>Spinal cord, Periaqueductal grey</td>
</tr>
<tr>
<td>Coma</td>
<td>Improve clinical status</td>
<td>Deep brain electrode</td>
<td>Thalamus</td>
</tr>
<tr>
<td>Paralysis</td>
<td>Provide means of interaction Brain-computer interface</td>
<td></td>
<td>Cortex</td>
</tr>
<tr>
<td>Motor system disorders</td>
<td>Gain control over limbs or prosthetic devices</td>
<td>Neuroprosthetics</td>
<td>Motor system Cortex</td>
</tr>
</tbody>
</table>

* Locations and types are neither complete nor precise. They are simplified to give a better understanding of current common strategies. See Appendix I or references for more information.
A neural implant can relieve motor symptoms of severely affected patients. What started as a last resort to improve quality of life, has developed into a reliable and safe alternative therapy (23). Areas that have altered activity due to PD were targeted previously for lesions with varied results. These targets are now candidates of DBS, with the advantage of reversibility and dynamic interaction.

Figure 1. General schematic of common DBS targets. (A) Sagittal view of DBS targets. (B) Coronal view of DBS. Schematic is not anatomically accurate. Image copied from Williams and Okun, 2013 (10).

Recording electrodes

Neural recording electrodes allow real-time or chronic supervision of brain activity by measuring signal currents (see Appendix IV). The advantage over common neuroimaging techniques is that it has higher specificity and allows for chronic use. They are applied in neuroprosthetics; systems that allow control through brain activity, with both monitoring and stimulation feedback capabilities. They require a high temporal and spatial resolution (33,34). Applications are in controlling prosthetic limbs, muscles or communication devices. An implant has little interference from muscle activity or overlapping signals from neighbouring neurons, because electrodes can be placed right next to the target. The immune response can interfere with exactly these qualities if cells isolate the electrodes, breakdown biomaterial or agitate local tissue.

Multiple electrode arrays

Neural recording implants often target the cortex and there is a trend towards multiple electrode arrays (35). Ideally, electrode arrays cover topographic organized regions of sensory systems or motor systems. These provide straightforward interpretation and are also relevant regions. Unfortunately, many neurons do contribute to multiple functions or are mixed and interconnected with other networks (36). Thus, for useful interaction with the brain, neural electrode applications need a large contact area to either comprehend signals or stimulate the right neurons. Figure 2 shows various approaches to measure local electrical potentials of the cortex.
Core components of the CNS and immune response

The brain is made up of many regions with specialized functions. Nuclei are clusters of neurons that act as a node in neuronal networks. Damage to even one such part of the brain can have major debilitating effects. Like in PD, where damage to a small part of the substantia nigra underlies a whole range of symptoms (20,38,39). So, its core components need to be preserved.

The CNS revolves around nervous tissue. There is grey matter, consisting mainly of the neuronal cell bodies and surrounding glial cells. Then there is white matter, which comprises of neuronal axons and other glial cells (5). Brain matter is shielded from the periphery by the BBB. Besides this structural protection, resident immune cells take care of local disturbances.

Neuronal tissue has limited plasticity

The CNS relies on its strong barrier and a modest inflammatory response, that prevents disturbances of the neuronal milieu as much as possible. There is evidence for neurogenesis from ependymal cells of the spinal cord or the subventricular zone, in the hippocampus and dentate gyrus (40–44). Unfortunately, in vivo it is not productive enough to regenerate all damaged tissue or to counteract cell death caused by neurodegenerative diseases. Once matured, this neuroregenerative potential is limited, but neuronal networks still show a high degree of plasticity. Nearby neurons can take over functions of damaged regions or make new connections to highly active neurons (45–47).

Neuroregeneration can support biocompatibility

Implantation surgery can lead to permanent damage. Some animal models do exist where lost connections are restored successfully, but only under special conditions, like in immature mammals (48). The challenge is that neurons should only connect to their targets, while avoiding malignant growth. Fortunately, many therapies are being developed to promote regeneration, especially in relation to spinal cord injuries (49,50). This research
might also be useful to support recovery from implantation surgery. Neurotrophic factors (NTFs) have already been shown to improve cochlear implant effectiveness (51). NTFs are signal molecules that can stimulate recovery of existing neurons. New neurons and glial cells would require pluripotent stem cells to regenerate from (52,53). Those need to be implanted together with the biomaterial or attracted to the injury site. Whether they can be guided to any region and become beneficial for recovery from implant surgery has yet to be determined (53,54). An optimal approach has to take the region, damage and pathology into account.

The CNS is a shielded environment

The brain is a big, soft and delicate organ. For electrode implantation, both the response to injury and foreign bodies is of extra importance. The immune response of the CNS is different and separate from those of in the periphery Figure 3. In the course of history many surgical procedures in the brain have been successful due to a high tolerance for foreign substances (23,55). Therefore some consider the CNS to be an immune privileged site, or at least partially. Rupert Billingham and Peter Medawar researched the phenomenon extensively with skin grafting (56). They thought some tissues are inaccessible by the immune system as a result of barriers that enclose privileged organs. It has now become clear that the CNS accommodates its own specialized immune cells and can communicate with cells of the periphery (57–60).

Under normal circumstances mainly glial cells have access to an inflammatory site in the CNS and leukocytes, white blood cells, are actively repelled (45,61–63). The CNS excludes dendritic cells that specialize in antigen presentation (Figure 3) and astrocytes actively dampen inflammation and intruded (59,64,65). Also no formation of foreign body giant cells occurs. Those normally appear when macrophages fuse in reaction to a large foreign object (57,66). CNS resident immune cells clearly behave differently and operate against autoimmune damage. This seems advantageous for neural implantation.

Cerebrospinal fluid (CSF) and the blood-brain barrier (BBB)

The CNS is embedded in CSF. The CSF is an important drainage system for metabolic waste and protects the tissue against physical damage as well. The fluid provides buoyancy to the tissue. This means that the brain floats and the soft structure does not press down on itself. Moreover, it dampens sudden movements of the skull. A barrier of endothelial cells and choroid epithelial cells separates the CSF and the capillaries. This has major consequences for implantation surgery. The surgery itself punctures the system and neural electrodes have to be able to move along with the nervous tissue to prevent physical damage.

The arterial system supplies the CNS with glucose, oxygen and other nutrients. Blood capillaries that extend into the nervous tissue are enveloped by the BBB. The BBB consists of tightly connected endothelial cells, pericytes and end-feet of astrocytes (Figure 4). These support a controllable defensive barrier against homeostatic fluctuations of the periphery. In neurological autoimmune diseases a disrupted BBB is likely part of the pathology (67). Electrodes often permanently penetrate the BBB, which likely results in immunological issues. Once leukocytes infiltrate the CNS, they can intensify neurotoxic inflammation and managing the process becomes more complex (68).
Figure 3. Differences between periphery (A) and CNS (B) immune components. From Ransohoff and Brown, 2012. (66)

Figure 4. BBB transport. Endothelial cells regulate passage of molecules. O₂ and CO₂ enter the CNS via diffusion. Other molecules rely on specialized transport mechanisms. Tight junctions (TJ) and adherens junctions (AJ) lock the cells together. Left ↑: Efflux transporters, ATP-binding cassette transporters (ABC) prevent accumulation and entry of toxic compounds. Middle ↓: Membrane transport mechanisms mediate transport of nutrients and ions. Right ↓: Endocytosis. EC, endothelial cells; P, pericytes; A, astrocyte end-feet; M, microglia; Glut1, Glucose transporter; AQP4, Aquaporin water channel. Figure is adapted from Lampron et al, 2013. (58)
Almost all cells residing in and around the CNS play their part, both by responding to signals, and by secreting signal molecules (59,69). Local differences are common, because cells are not uniformly distributed throughout the CNS and can adopt various phenotypic states on top of that (58,70). Most notable are astrocytes and microglia.

**Figure 5. The cellular response to degenerating neurons.** These cell interactions may influence the outcome of therapeutic interventions. Microglia and astrocytes transform upon activation.
Adapted from Cicchetti and Barker, 2014.(1)

**Astrocytes**
Astrocytes, 30-65% of all glial cells, make close contact with both endothelial cells and neurons. They regulate many supporting functions and can even influence the activity of neurons, what makes them of special interest for implantation therapy (4,71). Astrocytes can transform into a "reactive" phenotype once the immune response is activated. This causes enhanced migration, proliferation and hypertrophy. Additionally, it triggers intracellular changes, like an increase in mitochondria and Golgi complexes. The reactive astrocyte also produces NTFs and inflammatory factors. This process of transforming cells is called reactive gliosis (69,72).

**Microglia**
Microglia make up 12% of the total number of glial cells. They normally have a long lifespan, but are also uniquely capable of self-renewal from brain-resident progenitors (52,73,74). Microglia act as macrophages and are the driving force of the CNS immune response (75,76). Unlike what the description implies, resting microglia are actively
surveying the surrounding tissue. When injuries or homeostatic disturbances are sensed, microglia can also transform to an activate state and proliferate rapidly. This includes morphological changes and the increased secretion of signal molecules. Secreted cytokines promote inflammation. Chemokines are signal molecules that attract immune cells, other microglia and astrocytes. NTFs can stimulate immune cells to activate and proliferate. Cytotoxic factors induce apoptosis of compromised cells or break down foreign cells and material (77–80). Much about their influence and interaction with neurons under normal circumstances has yet to be elucidated.

**The complement system**

Besides the cellular initiation of the immune response, a molecular system exists. The complement system consists of proteins circulating the extracellular fluid. The blood-brain barrier blocks passage of these proteins from the blood, but they are locally synthesized by microglia, astrocytes and neurons (60). One could say it is a primitive component of the innate immune system, because it can recognize intruders, induce an inflammatory cascade and is capable of damaging bacteria (81). Activation leads to cleavage of precursor proteins and cascade proteins, which can set inflammatory pathways in motion. Therefore screening for complement activation is an important step in evaluating biomaterials.

**Figure 6. Neurodegenerative pathways after electrode implantation.** Neurotoxicity can be caused by activity of microglia (top) or cells from the blood (bottom). Damaged vasculature and BBB disruption cause initial damages and promote secondary neurodegeneration. From Potter et al., 2012.(82)
A fine balance between neuroprotective and neurotoxic effects

Neuroinflammation can prevent regeneration, damage neurons, glia and electrodes. Microglia play their part herein, as they attack intruders by releasing reactive oxygen species (ROS), free radicals such as nitric oxide (NO) and superoxide. Although a powerful weapon, excessive secretion of such molecules can lead to neurotoxicity, damage of healthy neuronal tissue (Figure 6) (71,79,83). Previously mentioned complement system is pro-inflammatory, but has recently also been implicated with neurogenesis and protection (84). There have not yet been found ways to modulate these contrasting responses effectively for the benefit of electrode biocompatibility. A response is needed to repair damages and incorporate the implant into the tissue, but inflammation has to cede in time to return healthy neuronal activity around electrode sites.

Biomaterials

Neural electrodes are made in different shapes, sizes, numbers, materials (Table 4) and can be placed at different locations. All these characteristics have a different impact on the FBR and thus biocompatibility.

Soft and loose implants prevent physical damage
Brain tissue is soft and more pliable than most available biomaterials. Classic metal implants are rigid. While this is advantageous for the surgical procedure and conductance, it can lead to movement-induced damage long after implantation. Prolonged contact with neurons requires that the whole implant is flexible enough to follow small movements of the tissue without tension (85,86). Newly developed polymer electrodes are stiff at first, to allow controlled implantation, and become softer once placed. This not only reduces the inflammatory response in the long term, but also seems beneficial for a healthy BBB. A stiff polymer coating could be made to dissolve after implantation surgery and leave soft material behind (37,87). But these materials are still being developed and it is a real challenge to combine these characteristics with other requirements for neural electrodes.

Texture can be adapted with coatings
Texture can either increase the immune response or allow growth of regenerative tissue close to the implant (86). Minimal interaction is required or it should be recognized as an endogenous structure. Immune cells can be activated by the material itself, but also texture or physical qualities, like stiffness, density, oxidation. Other research aims at developing permeable structures with nervous tissue properties. Polymers seem the most versatile at this time and can be combined with other materials (table 4).

A pro-active approach is to incorporate bioactive molecules and layers of protective material. For example, Laminin, a structural component of the CNS tissue, combined with polyethylenimine, which attaches to polymers. They do not seem to affect impedance, but durability of this surface is not yet known (14,88). Coatings provide all kinds of favourable surface characteristics, both for the electrode function and biocompatibility (89,90).
<table>
<thead>
<tr>
<th>Material</th>
<th>Electrode type</th>
<th>Applications and characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductive</td>
<td>e.g. with platinum, tungsten, gold, iridium, stainless steel, alloys (91, 11).</td>
<td>Stiff, easy production, three-dimensional, reaches deep brain regions. Needs insulation, e.g. teflon or polyimide.</td>
</tr>
<tr>
<td></td>
<td>Single electrode</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microwire array</td>
<td></td>
</tr>
<tr>
<td>Silicon</td>
<td>Micro-electromechanical system (MEMS)</td>
<td>Easiest production, most versatile design options in shape, size, texture, electrical properties. Multiple recording sites per probe. Microfluidic channel possible. Needs coating for flexibility, durability and against FBR.</td>
</tr>
<tr>
<td></td>
<td>High-density electrode arrays</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Michigan array (9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Utah array (94)</td>
<td></td>
</tr>
<tr>
<td>Ceramic</td>
<td>Ceramic array</td>
<td>Beneficial electrical properties. Electrochemical recordings, biosensor capability, enzyme coating breaks up biomolecules for measurement.</td>
</tr>
<tr>
<td></td>
<td>Biomolecule sensor</td>
<td></td>
</tr>
<tr>
<td>Polymer</td>
<td>e.g. with polyimide, silk, parylene, silicone, liquid crystal (96,90,97).</td>
<td>Adjustable flexibility, dielectric properties, allows multilayered design, microfluidic channel possible. Also used as coatings. Modifications with biomolecules. Biodegradable or in vivo polymerization.</td>
</tr>
<tr>
<td></td>
<td>Multi-electrode arrays</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flexible array</td>
<td></td>
</tr>
<tr>
<td>Glass</td>
<td>Micropipette electrodes (99)</td>
<td>Fragile, durable. Intracellular recording, introduction of cells or biomolecules.</td>
</tr>
<tr>
<td></td>
<td>Neurotrophic cone</td>
<td></td>
</tr>
<tr>
<td>Nanomaterials</td>
<td>silicon nanowires</td>
<td>Very small, close contact to neurons, also use in coatings. High signal-to-noise ratio. Still under development.</td>
</tr>
<tr>
<td></td>
<td>carbon nanotubes</td>
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<td>conducting polymer nanostructures (37)</td>
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Complications with electrode implantation

The immune response to damage normally resolves within a month. With electrodes still present after initial surgery, microglia and astrocytes sometimes stay active. This ongoing response is called frustrated phagocytosis (100). Astrocytes then isolate the material with their processes and do not return to their supportive functions. That is exactly the opposite of what a good functioning implant needs, the increased impedance caused by a scar is implicated in electrode implant failure (101,102). When the target region does not return to homeostasis, this severely affects the resolution of electrode measurements as well. Thus, in a way, the biomaterial has to delude immune cells and proteins.

Implantation of electrodes can also cause cognitive impairments, through surgical damage (21), an adverse reaction against biomaterials (101,103), side-effects of electrical activity or altered neuronal activity (104). Many of these consequences are influenced by the immune system. Neuroinflammation by itself has been shown to affect cognitive functions and damage the CNS as well (61,105). An infection is a worst case scenario. The immune system has to react aggressively, which damages recovering neurons and glia. If the health of the patient or device functionality is compromised, the implant has to be replaced or removed with another invasive surgery.

Altered immunology in patients complicates biocompatibility

Pathologies of the CNS are unpredictable. Even for the same neurological disorder interindividual differences are often high. Brain anatomy is one of the variables, the response to drug treatment another. The immune system and the brain are dramatically different in elderly people or the very young (106–109). The application of neural implants is aimed especially at such cases. This makes it that much harder to get applications on the market.

For instance, FDA regulations require extensive evaluations for chronic use, toxicity and tissue pathology. Although known biocompatibility of materials will provide insight, each medical device needs individual assessments (110). While at the same time, the behaviour of immune cells changes considerably in both the aging brain and under influence of NDDs (70,111). In regions with heightened immunological activity, there is an increased risk of neurotoxicity (112,113). The clinical outcome depends on the biocompatibility of the implant in context of the targeted neurological disorder (7). If we knew better how these conditions affect the immune system and how they influence local responses, better protocols could be set up to test for the safety of neural electrode therapies.

Course of the FBR after implantation surgery

The surgical event initiates a cellular response even before any material is inserted. Haemorrhaging introduces serum and leukocytes to the CNS tissue. Monocytes and perivascular cells enter the site of trauma. Damaged glial cells excrete chemokines and together with addressins from endothelial cells, these signals attract immune cells. These molecules are candidates to influence cellular migration around the electrode (114). Platelet-derived factors and transforming growth factors set the cascade of events of the acute immune response in motion (63). This also involves blood clotting and repair of
barriers. The sponginess of brain tissue allows for some displacement. In theory many regions of the cortex can be accessed via sulci without much damage to other tissue. This is one of the advantages of ECoG implants. They lie on top of the cortex without breaching the BBB (115,116). Intruding monocytes from the blood transform into macrophages and mix with microglia, performing similar functions (101). But the behaviour of these peripheral immune cells is not tailored to nervous tissue, which can result in increased neurotoxicity. Perforation of the BBB drastically changes the inflammatory response (67,82,117).

Improvements in implantation surgery methods and precision, has been one of the conditions for the increased effectiveness of implantation therapy. When damage to non-target areas can be avoided and blood vessel ruptures and tissue lesions as well, both initial damage and chronic stability are improved (118,119). Even in ideal conditions though, the current implant designs will cause displacement of nervous tissue and damage some cells and connections. Modulation of the initial response is a sound strategy towards more successful implantation.

The acute immune response can determine the survival of neurons

Immune cells detect injury by recognizing molecules that are released by damaged tissue. Right after surgery, microglia stretch processes towards the lesion and retract others, suggesting they sense a gradient of biochemical molecules (120). The microglial functional response depends on what pathways become activated. These are induced by membrane recognition receptors. Some recognize pathogen-associated molecular patterns, others damage or molecules associated with disturbances. Although microglia seem to be the most reactive cells, also astrocytes, endothelial cells, oligodendrocytes, and neurons express such receptors (see Appendix II) (61).

At the implant site inflammatory mediators and signalling molecules spread. Immune cells promote a hostile environment by lowering pH, releasing ROS and cytolytic enzymes. When brain trauma disrupts the blood flow, it can make astrocytes lose their connections with the vasculature. Thereupon, astrocytes cannot excrete buffered ions and swell. This is another trigger for immune activation (59,72). Microglia are the first cells to respond and can therefore also be decisive in the direction an inflammation takes. Either neuroprotective or neurotoxic pathways will gain the upper hand (79,83,121). For survival of neurons and optimal coexistence with the implant, an approach requires understanding of the triggers that drive microglia, in order to prevent secondary injury. Furthermore, therapeutic interventions can aim to inhibit detrimental effects or support neuroprotective mechanisms.

From repairs towards recovery

Within three days microglia and astrocytes will swarm the inflammation site and evaluate the damages (Figure 7). Microglia, supported by cytokines and the complement system, will transform to highly active immune cells. Their aim is to eliminate or neutralize threats, remove debris and recover homeostasis. The therapeutic aim is to regain a functional, healthy environment suitable for the implanted electrode. These goals are not mutually exclusive, but involve some conflicting strategies. Most notably, a homeostatic milieu suits recovering neurons and glia, but at the same time might allow latent microorganisms to survive as well. With an infection as a result.
Besides these injury-related reactions, activity of the implant can also invoke an immune response in surrounding tissue. Higher electrical stimuli can reach a broader region of cells, while the risk of damage to closer tissue is increased. Luckily, the necessary parameters perform well within the safety limits for long-term use (122,123). If an implanted device has a power source or complex electronics, increases in temperature can affect the immune response and might exacerbate implant related stress. In retinal implants a significant morphological change of microglia is observed above the upper limit of normal body temperature (124). International standards for implantable devices allow for a 2°C increase in temperature, but small temperature changes might nevertheless disturb tissue unnaturally.

**Inflammatory effects over longer distances and interactions between them**

Even if the immune response is contained, distant tissue can still be affected via signal molecules and the complement system. A ruptured blood vessel can cause oxygen deprivation in another region and immune cells from the periphery promote inflammation (82,120,125), whereas disturbed BBB permeability compromises CNS homeostasis. The other way around, unrelated immunological events can predispose the behaviour of immune cells before they come into contact with implant material.

Neurons, which depend on active connections, might not be able to send or receive signals anymore (49,126). Bloodflow and glial support have to recover to meet the demands of the neuronal population again. Microglia and astrocytes need to provide extra lipids and molecules to neurons for repair. Understandably, these cells have shifted their attention to clearance and other immunological functions. One of them, phagocytosis of apoptotic cells, is actually considered anti-inflammatory, because it is accompanied by an increase of the anti-inflammatory cytokine transforming growth factor beta (TGFβ) and a decreased secretion of the pro-inflammatory tumor necrosis factor alpha (TNFα) (100). Support of these processes can lead to a faster resolve of the inflammation and less neurons lost.

What has been looked at is the effect of multiple implant sites. This is of importance in many of the new designs. With a foreign body response at multiple sites or both hemispheres the functional side-effects might be cumulative. Fortunately, no effect on the immune response is found so far. In rats, no interaction between hemispheres was found and multiple electrodes next to each other seemed to actually reduce scarring around the middle electrodes. Perhaps this is due to a migratory effect (127).
**Chronic immune response**

An immune response to stab injury will eventually resolve for the lack of a persistent stimulus. With chronically implanted electrodes, the persistent foreign body and often permanent penetration of the meninges can lead to a chronic and more complex response (82,128–130). Although a large heterogeneity exists throughout the CNS with regard to cellular presence, glial activation states and signal factors (131), no distinct differences in the foreign body response between different brain regions have been found (119).

**Chronic inflammation and frustrated phagocytosis**

It is important which regions are passed by to reach the target site. Those will relate to the functions affected by potential impairing effects of the immune response. Once implanted, the material can still aggravate the surrounding tissue. The immune system machinery has specialized mechanisms that mediate opsonisation (marking foreign objects), degradation and removal of foreign material. Any implant that has surface markers that activates this system will likely induce a continuous inflammation (86). A biomaterial with optimal characteristics allows the nervous tissue to recover and return to a functional non-inflammatory state. In order to promote the most favourable outcome, a better understanding of the chronic FBR is still needed. Many biomaterials are tested, but a lot of this immune cell behaviour is still unknown.

In general, it is best to prevent a chronic inflammation altogether. When active immune cells continue to secrete signal molecules, they prevent complete recovery of the tissue surrounding the material. Immune cells try to phagocytose all debris and foreign substances. An inability to clear the substances can lead to an enduring inflammation. When a foreign material cannot be broken down, microglia continue to secrete inflammatory signals, a state called frustrated phagocytosis (72,100,132). Such a continuous inflammation disturbs the homeostasis locally (4).

![Image](image_url)

**Figure 8. Time course of scar formation around electrodes.** Glial fibrillar acidic protein (GFAP) staining at different time points after implantation A, B, C. From Polikov et al, 2005.(4)

**An unresolved FBR can lead to a glial scar, which isolates the implant**

A permanent breach in the BBB and other protective layers can stimulate repair of this barrier along the surface of the implant. While a severe rejection does not develop, due
to the immune privileged nature of the endogenous cells, a nonfunctional layer of immune
cells and endothelial cells can envelop the implant. Cells from the blood can provide fibrotic
molecules that form barriers and are implicated to stimulate scar formation (133). FBR
scarring prevents the stable performance of chronic implants by blocking the interaction
with neurons. Maybe chronic neuroinflammation continues as long as a cause is present,
which would mean that better biocompatibility of the material and design could circumvent
it. But perhaps such a chronic response is self sustained (92,134) and it has to be actively
suppressed. As there are so many possible causes, I think it should be studied which
mechanisms are pivotal and can be targeted.

Conclusion and future directions

It is evident that the immune response plays a pivotal role in the success of neural
electrode applications. The initial response after surgery, the biocompatibility of the implant
and the durability of its functioning, all are affected by the tissue response. Neural
electrodes should mimic brain tissue characteristics, take in as little space as possible and be
tailored to the patient case. Knowledge of the neuroanatomy, structures, cells and FBR is
behind recent engineering capabilities. Then again, there is always need for better and more
versatile biomaterials. The electrode design has many hurdles to overcome. A large surface
area causes more damage, but impedance and reach suffer with smaller electrodes.
Anchoring to the skull has a negative impact on biocompatibility (135). Indeed, evaluations
of free-floating, wireless implants compared to a fixed design, indicate better recovery of the
meninges and neuroregeneration (136). Tiny wireless particles, wireless electrodes, wireless
multi-electrode arrays, flexible materials or electrodes with flexible wiring are just some of
the proposed designs (7,116,130,137–143). Thus far, activity of electrodes and small
temperature changes have not been found problematic (124), so this provides some
leniency. The unresolved part of these developments is effective data processing and energy
transfer. Defining parameters should be proximity to neurons and reach.

Instead of betting it all on biomaterials and design, I think even more progress can be gained
by understanding and modulating the FBR. Multi-electrode arrays made from polymers are a
capable platform to test therapeutic interventions. Integration of bioactive molecules is very
interesting to modulate the FBR. NTFs promote survival and regeneration of neurons and
their axons (144). Cytokines can favour neuroprotective processes to protect target tissue
and suppress inflammation (143,145). Because during initial surgical damage the cells cope
with an overload of radicals, antioxidants are under investigation to protect endogenous
cells (72). Curcumin has protected against negative inflammatory effects for instance (146).
A biodegradable polymer can act as a slow drug release site or change the surface along with
the progression of the immune response (53,147). Unfortunately, against a chronic FBR and
glial scar formation, there are not yet as many strategies. These cannot be prevented by
regulation of the early response alone.

Besides signal molecules, also introduction of whole cells together with the biomaterial
could stimulate regeneration of the damaged site. Cells can play a role as mediators by
secreting neuroprotective signals (20,49,148,149), or cells themselves could actively fulfil
immunological functions. Perhaps it is advantageous to add potent neurons and glial cells to
restore neuronal circuits. Future implants could function a double role: one function via
electrodes, namely stimulation or recording of neuronal activity, another function as drug or cell delivery system (14,87,147). All in all, the brain is an ideal organ to benefit from bioengineered applications. If only we can manage the FBR, we will be able to measure the activity of tens of thousands of neurons in real time. Because, that is what is needed for normal motor control of limbs or functional cognitive support.

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Appendix

Figure I: Representation of cellular activity around brain electrode, which is part of a neural implant directed at the hippocampus. Figures adapted from Berger et al, 2012 (Left; 1) and Kozai et al, 2015 (Right; 2).

I: Examples of neural electrode applications

**Obsessive compulsive disorder (OCD)**

OCD patients suffer from anxiety, intrusive thought obsessions and motor compulsions. One of the causes is altered neuronal activity in the striatum, which plays a role in motivation (24). Biological causes are associated with abnormal serotonergic transmission. Many patients benefit from medication that elevates serotonin levels, like serotonin reuptake inhibitors. Unfortunately, it takes a long time for this beneficial effect to become apparent and treatment requires higher dosage than other major depressive disorders. Often drug treatment only provides a partial reduction in symptoms and a significant minority is unresponsive to pharmaceuticals, in which it is similar to PD patients (151).

Thus far, OCD pathology is poorly understood. A range of brain circuitry seems affected. Patients with persistent compulsions are eligible for neurosurgery. Some patients benefit from anterior capsulotomy, surgery of the internal capsule, the connection between two regions in the striatum. This is irreversible and no success is guaranteed (152). Complications included paralysis and the resulting mortality rates after surgery were not acceptable. A more flexible approach is warranted. DBS stimulation of the nucleus accumbens has been successful. Although some studies cannot find altered activity in this region in OCD patients, DBS there has relieved symptoms of some patients (24,153,154).

**Epilepsy**

Epilepsy is a disorder with such severe abnormal neuronal activity that patients suffer from seizures. Many patients cannot control seizures with medication alone. To account for
interindividual differences, profound analysis is required to find the exact epileptic location and distinguish it from healthy tissue. These patients cannot be adequately diagnosed with existing neuroimaging techniques (155,156).

Surgery is an option for patients with recurring epileptic activity that originates from one location in the brain. A proper preceding diagnosis is thereby essential. Current neuroimaging does not provide precision in combination with long-term monitoring. Many surgically implanted recording devices have been developed that do have such diagnostic capabilities (157) Following diagnosis, a lesion permanently eliminates all brain functions of the target region and may also disturb functions in connecting tissue due to damage or an inflammatory response. A less drastic approach that does not involve removing part of the nervous tissue would be to follow up with a neural implant as well. Chronic stimulation of the anterior thalamus has shown to be partially beneficial in patients with persistent epilepsy (16). Better still, an implant that detects oncoming seizures and disrupts the subsequent epileptic activity accordingly.

**Specialized diagnostics**

A group of biosensors has seen applications, especially in research. Various methods have been devised to measure neurotransmitters or other neurochemicals via microdialysis probe implants. These implants excel in detailed measurements and can combine sensors for different targets both electrophysiological and biochemical (150,158). While not yet studied extensively, the high sensitivity of such techniques implies that they are even more prone to disruptions by the immune response.

**Sensory disabilities**

Sensory capabilities can be affected by many causes. Genetic or developmental disorders can impair the functioning of senses from early on and accidents or illnesses can damage sensory organs later in life. Either way, neural implants promise to provide an answer. Chronic pain or extreme somatosensory sensitivity can be suppressed by electrodes directed at pain related brain regions, for instance the periaqueductal grey area (12). Multi-electrode arrays have been proven superior in pain relief applications and opened up a range of new possibilities (12).

Cochlear implants have already been developed into successful applications against deafness. One reason is that the cochlear nerve is quite accessible. More complex applications, for instance against blindness, are on the way as well. Artificial sensors can replace hearing or vision when they connect to their corresponding processing areas in the cortex. Therefore the primary and secondary sensory cortices are interesting targets, but also the thalamus or nodes that relay the sensory input. However, the latter regions are hard to reach with surgery (55,159). And this is just one example of how characteristics of a disorder, in combination with restrictions of the brain anatomy and required specificity for an acceptable solution, could influence implant design. On the other hand, it also sparks the imagination of the many possibilities neural implants have to offer.

The increasing interest for these implants is a result of better surgical procedures and the increased need for personal solutions. Therefore, the advantages outweigh the risks. Many different designs are developed from single electrodes to multiple electrode arrays, varying in depth, size and material. Here, especially chronic applications can benefit greatly from a
biocompatible design. And any diagnostic application should definitely not disturb its targets.

Making artificial replacements for our sensory and cognitive abilities is a delicate act and depends on compatible and precise interaction with the brain. Illustrative are the many layers that have to be breached in order to reach the brain:

![Layers of the skull that protect the brain from the outside world.](http://homepage.smc.edu/wissmann_paul/physnet/anatomynet/anatomy/neurolink.html)

Other applications

There are many other applications. Some of them are not uncommon at all. Shunt systems for hydrocephalus do not interact with neuronal activity, but instead regulate intracranial pressure with a shunt that can drain cerebrospinal fluid. These systems also deal with the risk of infection and immune related complications, similar to those discussed here (160,161). Sometimes electrical stimulation per se is not ideal, but then drug delivery or electrochemical stimulation can be an alternative. Many diagnostic applications and neural implants for research purposes are much more effective with specialized input.

II: Glia, signals and membrane recognition receptors

While neurons perform the cognitive tasks, glia actually make up most of the nervous tissue. These non-neuronal cells have a broad range of supporting functions. Together with endothelial cells and pericytes, they support the BBB and regulate blood flow. Other functions of glia include maintaining homeostasis and structural integrity of the CNS. Oligodendrocytes specialize in myelination of axons and thereby greatly increase signal propagation. Astrocytes and microglia are especially equipped to mediate the immune response. Until the influence of regional differences is elucidated, I will have to assume that the immune response mainly depends on extrinsic conditions.

Mechanically induced depolarization causes the opening of voltage-dependent ion channels of neurons and a flood of neurotransmitters is released thereafter. During initial damage,
neurons and glia release glutamate as a consequence of the mechanical depolarization. Astrocytes can take up glutamate, $K^+$ and eliminate free radicals. If they fail to do so or release them later on, this causes glutamate excitotoxicity, a detrimental outcome of inflammation. Glutamate opens ion channels, which results in intracellular $Ca^{2+}$ overload. Once mitochondria dysfunction, reactive oxygen species (ROS) form and cause lipid peroxidation (72). Then the cell is doomed. These disturbances can rapidly lead to secondary pathologies (155).

Nearly all cells have membrane recognition receptors. For instance, some Toll-like receptors (TLRs) can recognize microbial associated patterns. Via NF-κB activation the transcription of IL-1 family cytokines is upregulated. Receptors of the nucleotide-binding domain leucine-rich repeat containing family (NLRs) have to activate caspase-1, which can activate these cytokines (66). The absence of inhibitory signalling can also activate microglia in case of unknown threats or stress (162,163). Interestingly TLRs and NLRs can also recognize damage patterns. NLRs monitor cytosolic ion fluxes, aggregated peptides and endogenous cellular products. The following response cascade is tailored to the disturbance, since there are many types of receptors (58). Signals from other cells and the complement system can all modulate the cellular response. This underlies the versatility of the system.

III: CNS biomolecules with a different role peripherally:
- Histamine promotes inflammation in the periphery, while it acts as a neurotransmitter in the CNS, involved with the regulation of sleep and wakefulness (164).
- The catecholamines norepinephrine and dopamine are known as neurotransmitters in the CNS, but act as hormones in the blood circulation (155).
- Acetylcholine plays a major role in the peripheral nervous system, mediating muscle control. Centrally however, it is associated with plasticity, arousal and attention (5).

IV: Signal recordings via electrode impedance

"A commonly used equivalent circuit model (Robinson Model) of metal microelectrode recording in the brain. signals at the tip of the microelectrode ($V_{sig}$) generate currents ($I$) that flow to ground through the microelectrode and effective amplifier circuit, creating the potential ($V_{in}$) at the input of the amplifier before being recorded ($V_{rec}$);

$R_e$ is the resistance of the electrolyte; $R_c$ is the leakage resistance which models the flow of the charge carriers crossing the electric double layer; $C_e$ is the capacitance of the microelectrode-electrolyte interface; $R_m$ is the resistance of the microelectrode; $C_s$ is all the shunt capacitance to ground; and $Z_{in}$ is the input impedance of the amplifier.

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Thus, the effective impedance of the microelectrode is comprised of the resistance of the electrolyte ($R_s$), the resistance and capacitance of the double layer interface of the electrolyte ($R_e$ and $C_e$) and the (negligible) resistance of the microelectrode ($R_m$). The impedance of the microelectrode is frequency dependent. At low frequencies, the impedance is dominated by the series combination of $R_s$ and $R_e$, whereas at high frequencies $C_e$ bypasses the effect of $R_e$ so that the impedance is now close to $R_s$. Thus, by measuring the impedance of an electrode at high and low frequencies, it is possible to determine the component values for the equivalent circuit.

Figure and text from Jorfi et al., 2015 (92).

“Lowering the impedance is fundamental to enhance the signal recording quality, reduce the background noise, and, consequently, increase the signal-to-noise ratio. At the same time a lower impedance with no dimensional increase enables the injection of relatively large capacitive currents whilst minimizing electrode degradation due to Faradaic effects.” For instance increase the surface area with coatings.

Text from Castagnola et al., 2014 (34).