TRANSLATING ELECTRIC KHFAC AND DC NERVE BLOCK

FROM RESEARCH TO APPLICATION

by

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Dedication

To my parents, Vera and Ulrich Franke, and my sister Katharina for their never-ending support. To Abi Murali, making this world a sunnier place. And to all who take on new paths in life to develop cures for diseases others deemed incurable.

KHFA\textsuperscript{C}™DC – Let there be Block
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Abstract

by

MANFRED FRANKE

Electric nerve block has many potential applications in the medical field for treating sensory (e.g. pain, constant tingling, paresthesia), motor (e.g. spasticity, dystonia or reflex over-activation of muscles) and autonomic disorders (e.g. reduction of blood pressure or heart rate) by reducing activity in neural pathways. Current medical treatments often involve the repeated injection of chemical agents (such as Phenol or Botox) or the surgical transection of nerves, muscles or tendons to provide symptomatic care. Electric nerve block, using the right waveform, can be initiated instantly for a controlled reduction of neural activity, which completely returns as soon as the block is turned off. There have been several acute studies exploring different forms of electric nerve block but only very few chronic studies, leaving the field with insufficient information regarding the safety and efficacy of this promising new technology.

Three studies are described here; one in-vitro and two preclinical. They provide descriptions of optimal waveforms and technological solutions to facilitate the translation of electric nerve block technology from research to clinical use. In the first study, we quantified the efficacy and reliability of KiloHertz-Frequency Alternating Current (KHFAC) block, enabling bladder voiding post chronic-stage spinal cord injury. In the other two studies, the feasibility and safety of using combined KHFAC and Charge-
Balanced Direct Current (CBDC) waveforms for an onset-response free nerve block are investigated in-vitro and in-vivo.

We report how electric-only, daily bladder maintenance was successfully achieved in awake subjects at the push of a button and how the induction of an onset-response-free nerve block without nerve damage was achieved. Essential alterations to the block and/or stimulation waveform generation circuit necessary to avoid unintended DC contamination are also described in this thesis. The data from the three studies suggest that many previously published nerve block experiments report insufficient information regarding the waveform generation circuitry to interpret the absence of harmful components in the block signal. The methods presented here and the technological solutions for measuring and preventing such harmful components are intended to provide nerve block researchers with clean waveforms, enabling them to achieve reproducible results in chronic experiments.
I. Chapter I Introduction

This thesis deals with two important aspects in the translational process of Electric Nerve Block from the research bench to patient bedside: Safety and Efficacy. In order to become a new tool in the physician’s future tool box, electric nerve block must be proven to “first, do no harm”, or “Primum non nocere!” in the modern version of the Hippocratic Oath. As “harm” can come in many forms, ranging from patient impressions of mild discomfort to injuring or killing of cells and parts of organ systems, side effects must be evaluated for their relative danger and discomfort for the patient. Secondly, and equally important, the tool must be effective, easy to control and its effects reproducible under varying patient conditions in order to ensure an applicability for a broad patient base. Both these aspects, safety and efficacy, of nerve block, using two different electric waveforms are investigated in this thesis providing information for the next steps in preclinical and clinical studies of this new technology.

Electric nerve block was first reported in 1885 and later summarized in detail in 1903 by Nicolai Evgenevich Wedensky (Vvedenskii) [1, 2]. It is a phenomenon that has not yet been understood in its entity, even though there are already first applications in the medical field intending to help patients with diseases that “can’t be treated with a pill” [3-6]. Some of these neuro-prostheses rely on effects that could be combinations of nerve activation and block, as well as different forms of nerve block in parallel. The physiological mechanisms causing different forms of nerve block are still in the process of being fully discovered and understood. In order to aid with the understanding what exactly is likely happening with specific nerve-block waveforms, the thesis at hand will address different forms of nerve block separately, discuss their differences and explain
which situations are likely to cause nerve block by direct or by indirect (unintentional) pathways.

The document at hand furthermore provides an example for a chronic implementation of kilohertz-frequency alternating current (KHFAC) nerve block and present results from unintentional interactions caused by additionally applied blocking waveform components. To better understand the specific waveforms and their nerve block effects, specific filters using capacitors and large value inductors were studied and are presented, providing “clean” nerve block waveforms. Using such clean KHFAC waveforms, a bilateral electric block of the pudendal nerve was combined with bilateral sacral nerve bladder stimulation to successfully provide daily bladder voiding in a preclinical chronic spinal cord injury model. The direct and indirect implications resulting from this study are then discussed in detail.

In a third study, a charge-balanced direct current (CBDC) nerve block, first described by Vrabec et al. [7] is used in combination with a clean-waveform KHFAC nerve block. This combined CBDC+KHFAC nerve block is able to provide a temporary interruption of peripheral nerve signal transmission using electric means. It can be achieved without an onset-response (typical for the KHFAC block) and does not change nerve conductivity post block, combating the two most significant side effects of KHFAC and DC block. Such a combined nerve block could provide an improvement in patient benefit in the future by aiding in the development of treatments for diseases that are caused by over-activity of the nervous system, with either central or peripheral origin.
I.1 Medical background: Need for an on-demand, electric nerve block

The human body utilizes two fundamentally different pathways to communicate information between its inner organs and organ systems: the humoral pathway utilizes the release and uptake of hormones to and from the blood lymph stream [8] to exchange hormones, cells and enzymes to communicate longer lasting states of information (e.g. level of comfort or stress), while the neural pathway transmits information as digital, frequency-coded packets of data at very high speeds [8-11]. The latter is the main path used for acquiring, transmitting, processing, and reacting to outside information that is presented to the body. In simplified terms, sensory information such as light, sound, taste, pressure, and temperature is translated into a series of pulses, called action potentials, and transmitted along axons of peripheral and cranial nerves in order to reach neurons in the central nervous system (CNS), where the data are analyzed and evaluated in order for the organism to respond in appropriate fashion. Once the response is calculated, an order to act is sent, again using axons of cranial and peripheral nerves, to various inner organs and organ systems such as muscles to achieve movement or glands to secrete specific fluids. By interfacing directly with the neural pathways of the body, information can be read from and written into or deleted from the central (CNS), the peripheral (PNS) or the autonomic nervous system (ANS) [8, 9]. In other words, communication with virtually any organ of the human body is possible by interfacing with axons of the nervous systems.

Electric means are the most common form of communication with neuronal pathways today. The technology of functional electrical stimulation (FES) relies on subjecting neurons to an externally applied electric field, which causes the opening of voltage-gated ion channels in the cell membrane and initiates an action potential (AP), a
self-propagating transient voltage change traveling along the neuron’s membrane. The externally applied electric field essentially mimics the changing field that ion-channels in the cell’s membrane experience when a naturally occurring AP depolarizes a neuron. This phenomenon was first described by Luigi Galvani in 1771 and is being used in many FES applications such as artificial activation of efferent motor nerves to provide functional restoration after paralysis [12], deep brain stimulation (DBS) for Parkinson’s disease, the cochlear implant to restore hearing [13-15], or more recently providing sensory input to amputees [16, 17]. Unfortunately, the act of stimulating the nervous system fundamentally provides only positive input, meaning an increase of neuronal activity when stimulation is applied (although DBS is in a grey zone as is described below). A way to subtract information from the nervous system, essentially a nerve block, has important implications for future medical technologies.

A variety of neurological diseases as well as direct injuries to the PNS or the CNS can result in reduced, unbalanced or over-activity in organs or organ systems connected with the severed neurons. Examples include spinal cord injury, stroke, cerebral palsy, multiple sclerosis and traumatic brain injury. The resulting symptoms include a loss of function or loss of control over the function, development of muscle spasms or rhythmic over-activity such as clonus, paralysis (loss of muscle function), paresthesia (feeling of burning, tingling) or uncontrolled reflexive over-activity (e.g. detrusor-sphincter-dyssynergia [18]). Technological advances in the field of neural engineering during the past few decades led to the development of implantable devices to mitigate said symptoms by means of neural stimulation. Examples include external and implantable stimulators for select nerves and muscle [19, 20], or entire muscle groups [12, 21], as well as devices providing deep brain stimulation (BDS) for mitigation of symptoms in
diseases such as Parkinson’s, dystonia, obsessive compulsive disorder (OCD) and depression, migraines [22-27] and spinal cord stimulation (SCS) for neuropathic pain [28, 29]. All these examples utilize neural stimulation that can be grouped into two main frequency bands: A lower frequency band from 1 to about 50 Hz and a higher band from roughly 100 to about 200 Hz. The lower band results in effects that are purely excitatory in nature, while the higher band can likely potentially result in a mixture of neural stimulation and neural block. The biological and physiological effects that provide the patient benefit in DBS and SCS applications are still not fully understood, as stimulation location can have just as much of an impact on the outcome of the treatment as waveform amplitude, frequency and the electrode shape.

Interfacing with the nervous system with electric stimulation can enhance, modulate, or inhibit the way certain organs and organ systems transmit information and thus one can influence their function. Aside from using electric means, neural modulation can be achieved with drugs via topical application or subcutaneous injection of local anesthetics such as lidocaine [30, 31], intra-muscular injections of botulinum toxin causing a chemical synaptic junction block [32-35], or intrathecal baclofen [33, 36, 37] to achieve chemically-mediated effects on the nervous system, be it the reduction of pain or spasticity, complete local or even general anesthesia. Aside from chemical means, temporary nerve block can be achieved by localized lowering or increasing of temperatures [38-40] or with a reduction of the oxygen supply to the nerve [41]. Surgical interventions as part of medical treatments, such as resecting a nerve, can partially be restored by the body in the peripheral nervous system (PNS) if given enough time, whereas lesions in the CNS are commonly understood as permanent [8]. In summary, the favored technology used to provide a temporary nerve block is a chemical
intervention, whereas surgical interventions are often the gold standard for permanent solutions. More recently, light waves are being investigated to provide inhibitory input to PNS and CNS neurons [42-45], with all of the in-vivo studies still in the preclinical phase.

In addition to providing their intended effect, all of the before-mentioned interventions are accompanied by local or systemic side effects. Combinations of electric nerve stimulation and nerve block have the potential to provide similar intended effects as the current clinical solutions but with fewer side effects by adding and subtracting neural activity specifically only where needed. Hence, in order to better understand the strengths and weaknesses of different forms of neural modulation, various modalities using chemical and physical means are compared against each other from different perspectives.
I.2 Comparing electric with other forms of nerve block

As with other interventions, electric nerve block can be applied locally and act systemically (e.g. Deep Brain Stimulation, DBS) and result in side effects of which some are local and some can be systemic. Of interest is thus the reversibility of the applied nerve block (primarily reversible or irreversible) as well as the specific site of intervention to assess how localized the effects of a certain intervention will be (figure I-1). Fully reversible electric nerve block, has the advantage of localized selectivity (i.e. affecting only the nerve inside a cuff electrode and thus only a specific muscle or organ function) and the advantage of temporal specificity as it could be applied only when necessary in time, limiting systemic side effects significantly when compared to e.g. chemical means that require a reasonable long time to begin and end showing effects, such as Botulinum Toxin (Botox) or Intrathecal Baclofen.

![Figure I-1: Forms of neural block: Localization of benefits and side effects](image)

Comparison of different means to provide nerve block, looking at the targeted benefit location vs. potential area affected by side effects in the body. Blue indicates electric means of intervention, green are chemical, red are surgical (including temperature increase during cautery) and grey is temperature reduction. The advantage of a localized electric nerve block acting locally and resulting in local side effects (e.g. blocking more or less fibers than intended temporarily) would put such an electric nerve block in an area between a topical and an injectable anesthetic. The DC* block mentioned here is not of the reversible kind, hence damaging tissue during application.
A second assessment of currently available techniques to reduce neural activity is the invasiveness of the procedure relative to how long the treatment is effective in addressing the symptoms or problems (figure I-2). Chemical means of interfacing with the nervous system provide a somewhat linear relationship between the required invasiveness to administer the treatment vs. the resulting duration of a nerve block. The dominating factor besides location is the type of interaction, blocking e.g. ion channels for quick acting and quick reversing topical or injectable blocks or interfacing with the neurotransmitter cascade allowing for longer lasting effects. The reversibility of chemical blocks is their major advantage over surgical approaches, as transection of neural tissue in the CNS generally does not reverse, while there is a partial reversal of function following a lesion in the PNS. Electric nerve block can provide both results, a temporary and short-term intervention (once the system has been implanted and allowed to heal) or a the repeated application of a damaging nerve block (e.g. DC) with a permanently installed electrode around a peripheral nerve to provide a localized, quasi-permanent nerve block for the reduction of spasticity.

![Figure I-2: Forms of neural block: Invasiveness vs. Block Duration](image)

Comparison of the invasiveness of an approach against the duration the nerve block is in effect. Blue indicates electric means of intervention, green are chemical, red are surgical (including temperature increase during cautery) and grey is temperature reduction. Once implanted, electric nerve block can provide short- and long term reduction of neural activity and thus treatment that can be very patient specific without the need for repeated injections or surgeries. The DC* block mentioned here is not of the reversible kind, hence damaging tissue during application. Vagal stimulation depending on the chosen placement and frequency range can activate or block bodily functions and thus have far-reaching means of interactions.
Third and last, the current status of human implementation of various nerve block techniques is charted (figure I-3). Deep Brain Stimulation (DBS) stands out as the clinically most established form of neural stimulation and block, followed by Spinal Cord Stimulation (SCS) and vagal stimulation. Peripherally and centrally applied forms of kilohertz-frequency stimulation are currently in the transition period with multicenter clinical trials being performed for “High-Frequency HF10” spinal cord stimulation by Nevro Medical (for pain control) and First-in-Man PNS trials by Enteromedics (for obesity) and Neuros Medical (for phantom limb pain). The graph illustrates how various nerve blocks are in different stages of the investigation and transition process towards becoming a treatment option in the future.

**Figure I-3: Forms of neural block: Stage of development vs. Application**

Electric forms of nerve block, achieved by different application locations and waveform parameters, are currently in the research and (further) development phase, even when already applied in the clinic. While deep brain and spinal cord stimulation are already providing treatment for varying neurological conditions, there is still a significant amount of research targeted at understanding the specific mechanisms and effects, similar to vagal stimulation and block. Kilohertz-frequency nerve stimulation and block applied in/near the spinal cord are currently being investigated in first-in-man and multi-center clinical trials, while peripherally applied reversible and irreversible forms of DC nerve block are still early in the investigational pre-clinical phase. All other means of nerve block shown here provide some form of clinically applied treatment.
I.3 The advantages of electric nerve block

An electric approach has several advantages over other means of nerve block:

1) **Clearly defined initiation and termination of the block effect.**

   In contrast to other forms of block, both KHFAC [46-48] and DC [49] conduction as well as “Wedensky” depletion [1, 2] nerve block have been shown to provide the potential to provide an immediate (<1s) interruption of nerve conduction as well as immediate recovery (KHFAC [46], multi-phased DC block [7, 50]). Immediate termination also means immediate reversibility without the need for an antidote as is necessary for some forms of various chemical forms of nerve block.

2) **Adjustable nerve block from graded to complete block possible on demand.**

   Partial as well as complete KHFAC [46, 51] and DC [49, 50] nerve block have been shown. There is a direct correlation between current amplitude and degree of the partial nerve block, as well as the ability to either reduce or increase an already existing block on demand.

3) **Selectivity for fiber size – larger fibers are blocked with smaller current amplitudes.**

   Selectivity for both, KHFAC [52-54] and DC [55-57] nerve block has been shown. This should similarly hold true for “Wedensky” depletion block (100..900 Hz) in peripheral nerves as only the synaptic terminals of the continuously activated nerve fibers are being depleted and thus a large fiber, being activated electrically first, could be depleted while smaller fibers should remain conduction, as long as their terminals are not being depleted as the smaller fibers are not activated continuously at 100..900 Hz.
4) **No need for the regular refill of chemical agents to achieve the nerve block.**

Energy can be provided by a battery that can be recharged transcutaneously, potentially requiring a replacement every few (5 to 7) years similar to cardiac pacemakers or DBS stimulators. These intervals are significantly larger than the time between refills for e.g. an intrathecal Baclofen pump, which are in the range of two to three months [33, 37].

5) **No signs of adaptation of the organism to electric nerve block known.**

In contrast to various chemical means of nerve block (esp. L-dopa in Parkinson’s or opioids for pain), electric nerve block has not yet been reported to cause the nervous system to get accustomed to the block and the need to increase the stimulation amplitude (“dose”) to achieve the same block effect after years of usage.

6) **No side effects from prolonged drug application over time.**

Many pharmaceutical solutions used to treat spasticity, muscular over-activity or chronic pain result in systemic side effects. Examples include respiratory and profound CNS depression after the administration of Botulinum toxin (Botox)

7) **Potential to provide a longer-lasting nerve block, if needed.**

Destructive DC nerve block could be used to provide a nerve block in the PNS that lasts well beyond the application of the electric waveform. Could be used to provide relief from phantom limb pain in amputees and would require less energy than a solution that utilizes temporary nerve block via KHFAC or neuro-transmitter depletion block. It is unknown if a neuroma, a
potential side-effect of PNS surgery, would form after the application of destructive DC block or if the neuroma growth could be influenced with a repeated application of DC nerve block.
I.4 Electric nerve block as a solution for current medical needs

From a physician’s point of view, finding an optimal treatment for a specific medical condition often involves weighing the advantages and potential disadvantages of different therapies. A specific therapy is likely to be chosen if it

- has a high probability for patient benefit while adding only small potential for unintended side effects (risk-to-benefit ratio),
- can provide a specific treatment for a specific localized condition (e.g. aspirin for headache),
- can provide a specific systemic treatment in case a systemic intervention offers less side effects than many localized interventions (e.g. general anesthesia for surgery),
- offers adjustable treatment (long dose-response curve: targeted benefit vs. side effect),
- offers easy reversibility if therapy success is not achieved in a reasonable time frame or side effects cause the patient to request a full reversal (e.g. DBS instead of surgical resection for Parkinson’s),
- or if it offers new hope in case all other (conventional) therapy approaches have failed (e.g. a new experimental therapies for pain treatment).

If all of these requirements can be incorporated into therapies involving neuro-modulation as a combination of neuro-stimulation and neuro-block, then they are likely to increase in importance in the coming decades. Electric modulation of neural activity can be dosed and provided with a high degree of specificity allowing local (e.g. on a PNS
nerve branch) as well as general effects (if targeting central nuclei for mood such as the amygdala or the sub-thalamic nucleus for movement disorders). Electric neuro-modulation is highly reversible and within certain limits can provide very specific results with different waveforms causing activation instead of block and vice versa.

Furthermore, by providing the neuro-modulation community with the tool of a reliable, safe and easily adjustable nerve block, new therapies would become possible or more likely to be implemented: nerve block could be used to locally filter and thus reduce or even completely inhibit the conduction of unwanted neural information, allowing the compensation of unintended side effects of neural stimulation. From a system-theoretical standpoint of view, neural block would provide a specific negative input to a neural control system, where neural stimulation has caused an unintended over-activation of certain neural populations.

In the document at hand describes one application of neuromodulation that would have a large impact on the treatment of CNS/PNS disorders, and addresses the challenges encountered and solutions found that allowed the successful implementation. Electric nerve block was used to prevent the conduction of neural information on a peripheral nerve of the lower urinary tract in order to increase the efficacy of the overall device for bladder voiding and prevent unintended side effects such as high bladder pressures. Specifically, KHFAC nerve block was used to prevent unintended external urethral sphincter (EUS) muscle contractions during electrically mediated bladder voiding. Following spinal cord injury, individuals often lose the ability to control the coordinated contraction of bladder and relaxation of the EUS to allow for bladder voiding. The voiding process is further impeded by reflexive EUS activation initiated by urethral stretch receptors which are activated when urine enters the
urethra, providing that information about urethral stretch to the spinal cord, which in turn initiates a guarding reflex, activating the EUS. A complete electric nerve block of the PN would prevent EUS spasms during bladder voiding, while retaining continence for the remainder of the time.

The herein described application of complete nerve block using KHFAC waveforms was chosen because of specific characteristics of this type of nerve block. Different medical applications would potentially require a different forms of electric nerve block as each one has specific characteristics. The following pages will describe these characteristics into details to aid in the understanding that mixing of electric waveforms will likely result in combined effects, requiring the investigator to be aware of intended and unintended effects.
I.5 Types of electric nerve block

Electric nerve block can provide the opposite effect of conventional neural stimulation: instead of adding information to the nervous system, electric nerve block reduces neural activity, either right at the (electrode) location of block application or at the terminal (interface) of the neuron’s axon to other cells, the synaptic junction. Seen from a standpoint of signal and systems theory, electric nerve block thus represents the negative input to a control system, while electric nerve stimulation equates to the positive input. With that in mind, new kinds of medical devices can be envisioned that allow the controlled modulation of nuclei in the central nervous system or the controlled activation of specific muscles in a patient without unintentionally activating other muscle groups or processing units in the nervous system.

There are several kinds of nerve block that can be generated through the application of neural “block” waveforms and an unintended simultaneous combination of different forms of block is possible. In order to properly interpret experimental results in a nerve block study, one is advised to understand all potential components of the applied waveform and how they can result in an attenuation of neural communication.

I.3.1 Types of nerve block resulting from different waveform parameters

First and foremost, the type of electric nerve block achieved depends on the applied frequency: if an unidirectional current is applied continuously, or the classic understanding of direct current (DC), then the block is achieved at the electrode location as nerve conduction block, first described by Pfluger 1859 [58], differentiated into depolarization [59, 60] and hyperpolarization [61] block by Hubbard and Willis, and more recently described in detail by Bhadra and Kilgore [49]. Solomonow [62, 63] on the
other hand investigated interrupted, or pulsed, DC at various frequencies from 100 Hz to 20 kHz and concluded that an incomplete (90+%+) block is easiest achieved at 500 to 600 Hz. He attributed the effect to a synaptic junction depletion block, although an additional, localized nerve conduction block due to DC delivery at the electrode location is likely (figure I-4).

If the current polarity is continuously changed (e.g. a sinusoidal or rectangular waveform), and the applied frequencies are in a range of about 100 Hz to about 1 kHz, then a true neuro-transmitter depletion block can be achieved: Assuming that the waveform amplitude is above the nerve’s saturation threshold, then each electric input pulse is followed by the generation of an action potential in the axon at the location of the electrode, which technically does not block the nerve conduction there. Yet the arrival of these higher-than-normal frequency AP trains at the synaptic junction causes a release and eventually a depletion of neurotransmitters such as Acetylcholine (ACh). The depletion can be achieved within seconds, as first described by Wedensky in 1885 [1] and later explained in detail in 1903 [2]. As synaptic junctions function on the one hand as a biological equivalent of a diode, only passing information in one direction, and on the other hand as the biological equivalent of an integrator, summing incoming information on the post-synaptic end from several pre-synaptic inputs, depleting of neurotransmitters from the pre-synaptic end or flooding the post-synaptic receptors can reduce the firing rate at the post-synaptic end significantly, resulting in what is commonly referred as synaptic depletion block [64]. Ivy and Barry [65] tested frequencies up to 6 kHz applied to the CNS in acute preclinical studies and were able to show a “stunning” (analgesia) effect on awake dogs: “The most efficient current is one of 72 volts-interrupted 6000 times a minute and passing 8/10 of the total time, the
application lasting fifteen seconds. The average time that elapsed before a pain reaction was elicited after application of this current was 4 minutes and 14 seconds. The shortest time was 2 minutes and the longest 12 minutes. The minimum effective time for application of the current is 10 seconds, the optimum being 15 seconds.” And “Spinal analgesia can be produced in dogs by placing one electrode on the back of the neck and the other over the lower lumbar region and passing a current of 4 to 12 volts, interrupted 6000 times per minute, one-tenth on and nine-tenths off. This analgesia is accompanied with more or less muscular rigidity in most dogs. When applied daily for one week it caused no disturbances of the function of the spinal cord” [65].

If biphasic currents at frequencies above ~1 kHz are applied to a nerve and the amplitude of the waveform is sufficiently high, above what Kilgore and Bhadra [47, 66] defined as the “Block threshold”, then a nerve conduction block at the location of the electrode can be achieved, first described by Cattell and Gerard [67], who reported inhibiting “Wedensky” effects at frequencies of 400-600 Hz and compared these to “blocking” effects experienced at frequencies of 2.5 and 5 kHz. The first publication showing a transition from “Wedensky” synapse depletion block to nerve conduction block by measuring compound action potentials (CAP) in frog sciatic nerves were Schaefer and Göpfert [68]. They measured CAP at “stimulation” frequencies from 50 to 512 Hz and saw that 1024 Hz resulted in a block effect in the frog preparation, indicated by diminishing CAP amplitudes. In recent years, Dowden et al. investigated the intra-fascicular implementation of both, neuromuscular junction depletion (at 500 to 8000 Hz) and conduction block (only at 16 kHz) to provide muscle-selective block using the Utah Slanted Array. While they were successful at achieving fascicle-specific nerve block, it is unclear why frequencies commonly associated with nerve conduction block
(>5kHz) did cause continuous activation or what was reported by Dowden et al. as neuromuscular junction block until 8kHz. A potential explanation is that unintentional DC contamination caused mixed effects between nerve activation and nerve conduction block, similar to what Ackermann et al [69] saw when kilohertz signal crosstalk caused a nerve activation at the DC-blocking electrode (figure 1-4).

The effect of nerve conduction block was described further by Tanner [70], Bowman and McNeal [71], and extensively summarized by Gildemeister [72]. Although quite subjective in experimental design, Gildemeister’s findings are interesting from a translational standpoint for KHFAC block: he studied frequencies from 9 to 500 kHz at current amplitudes up to 440 mA, provided by tube amplifiers with a power range of up to 400 Watts. The electrodes were handles that he touched with wetted hands. He described his personal experiences after initiating the waveforms at e.g. 150 kHz at low and at high current amplitude values: low current amplitudes did not cause any reaction upon initiation. Starting the waveform application beyond a certain threshold (e.g. 65 kHz at 122mA) caused a short shock feeling, followed by a tingly feeling that diminishes within 2 to 3 seconds (i.e. onset response?). He further reported a linear relationship for the “shock and relaxation” threshold, with examples from 57 mA for 12.6 kHz (very long tingle) and 360 mA for 136 kHz (very short tingle). He further reported that the location of the electrodes influenced where the tingle would be felt; holding the electrodes in the hands resulted in short tingle sensations at the wrist joint or the lower arm with signal initiation; putting the electrodes directly in contact with the lower arm caused the tingle at the electrode location. He further experimented with overlaying the kilohertz-frequencies with direct current and observed a prolonged tingle, making him the first to report intentional combinations of different waveforms.
More recently, Ackermann et al were able to demonstrate a direct current nerve block with a separated interface electrode that did not lead to nerve damage [73]. The underlying concept for the approach was based on the assumption that the damage resulting from DC is due to chemical byproducts produced during the application of the nerve block whenever the voltage across the electrode-electrolyte interface is leaving the water window. Thus, by physically distancing the nerve from the electrode-electrolyte interface such that the nerve only interacts with the electrolyte, the potential difference applied to the nerve was enough to cause a reproducible block for minutes while no change in nerve conductivity was observed. This opened the door for further investigation by the Kilgore-Bhadra Lab, first presented by Vrabec et al, showing how high charge injection capacity electrodes with charge-balanced (multi-phase) direct-current waveforms can produce a DC block that does not lead to signs of changes in nerve conductivity post block [7].

The current understanding of electric nerve block differentiates between effects that are achieved temporarily and those resulting in a permanent effect. Permanent electric nerve block can be achieved with waveforms of very high frequency and current density, such as cautery and coagulation during surgery, or with currents of one direction, commonly referred to as direct currents (DC). The former destroys cells by heating them due to the high amount of injected energy, the latter due to changes of pH-levels and the creation of peroxides as well as free radicals at the interface. Especially these changes in pH have been shown to provide acute and chronic nerve block [74], in part resulting from toxic salts caused by long-term DC-delivery, such as platinum-oxides which are being used as cytotoxic agents in carcinoma therapy.
Temporary electric nerve block on the other hand has been achieved with several different waveforms that can best be differentiated by the frequency of the applied signal. On the lower end, frequencies in the range of 1/10 Hz can provide a temporary nerve conduction block that has been shown to be fully reversible [7, 50, 75]. This block can be achieved as either depolarization or as hyperpolarization block [48, 49]. Next higher in frequency are waveforms that use the phenomenon of the anodic block on one side of an electrode to provide unidirectional stimulation pulses that can be used to “collide” with physiological action potentials and prevent them from reaching an end organ [76], though there are no reports of chronic studies reproducing promising acute results using block at frequencies of 1 to 30 Hz. Frequencies above 30 Hz caused the anodic block to become unstable [76]. Next, higher frequencies resulting in an effect that is sometimes confused with nerve block, are in the range of 30 to 70 Hz, resulting in the depletion of ATP in a muscle when applied over extended periods of time (tens of seconds to minutes). Frequencies from about 100 up to about 1 kHz, when applied in the peripheral nervous system, can result in the depletion of ACh and other neuro-transmitters at the synaptic cleft of neuro-muscular end-plate [1, 2, 46]. This means that the interruption of neural communication is not achieved at the location of the electrode (as “conduction block”) but instead at the synaptic terminal (as “depletion block”). The reason is that the bandwidth of axonal firing is generally higher than the maximum frequency for continuous conductivity of a synapse. This difference in transmission bandwidth is caused because chemical agents are used to transmit the information across the synaptic cleft which (1) provides a diode effect, as information travels only in one direction across the synapse, and (2) is used to provide a summation of information, as generally seen in the transition from PNS to CNS neurons, where several afferent input AP are chemically integrated, before the CNS neuron itself
releases an AP. Applied in the CNS, frequencies of about 135 Hz (range 100 to 150 Hz) are used to provide activation and suppression of multiple neural circuits simultaneously, summarized in the rate- and the gate theory of deep brain stimulation (DBS).

![Diagram of Charge delivery](image)

**Figure I-4: Different types achieved by varying frequency and charge delivery**

Depending on the chosen frequency and the type of charge delivery, different effects on the spectrum from neural stimulation to temporary to permanent nerve block are possible. Grey coloring represents stimulation, red the various forms of DC block, green the neuro-muscular or neuro-synaptic junction block, yellow mixed forms of block and blue pure KHFAC nerve block (if the amplitudes are above the HF block threshold). The “New SCS” is spinal cord stimulation utilizing waveforms in the Kilohertz-range where the mode of action is still debated. Charge-balanced direct current (CBDC*) block, described by Vrabec et al. in [7], uses the high charge-injection-capacities of high-surface roughness electrodes in order to provide a short-term direct current nerve block without electrode-electrolyte boundary potentials reaching the water window in order to avoid changes in nerve conductivity post block delivery.

Another parameter determining if a nerve block is achieved is the specific waveform amplitude of the applied signal. Kilgore and Bhadra defined the term “Block Threshold” as the linear relationship between applied KHFAC frequency and the necessary voltage amplitude to achieve a complete nerve conduction block [46, 47, 66]. They further showed how an incomplete block can be achieved with waveforms in the
kilohertz range whose amplitude first was set above the block amplitude and then carefully reduced to levels below the block threshold. Wodlinger et al. on the other hand used kilohertz waveforms at amplitudes that never reached the block threshold, resulting in sustained unsynchronized neural activation for their study of sensory electrode arrays [77, 78]. It is unclear how the sustained neural firing relates to the KHFAC onset-response, but Kilgore and Bhadra reported that a tight electrode fit around the nerve achieves lower block thresholds than a loose fit does.
### Table I-1: Comparison of neural block types by frequency.

<table>
<thead>
<tr>
<th>Block type</th>
<th>Frequency range</th>
<th>Effect at low amplitude</th>
<th>Effect at high amplitude</th>
<th>Implementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;pure&quot; DC-block</td>
<td>&lt; 0.1 Hz Not-alternating (monophasic on)</td>
<td>Partial block if ramped, partial activation &amp; block if stepped on, using ramps can prevent onset</td>
<td>Full block if ramped, partial activation and block if stepped, using ramps can prevent onset</td>
<td>Rats: [49] Note: <em>Safety is a huge concern.</em></td>
</tr>
<tr>
<td>&quot;multi-phased&quot; DC-block</td>
<td>0.1 Hz (with one cathodic and anodic phase)</td>
<td>Activation [7], partial block [75]</td>
<td>Neural block, assumed to be inactivation of sodium channels [49, 50]</td>
<td>Rats [7, 50] None.</td>
</tr>
<tr>
<td>Collision block</td>
<td>10 to 30 Hz</td>
<td>Stimulation of big fibers [76]</td>
<td>Simultaneous stimulation of small fibers and block of big fibers</td>
<td>Rabbits [20, 76] None.</td>
</tr>
<tr>
<td>Deka-Hz</td>
<td>100 HZ to ~ 1kHz</td>
<td>Activation [1, 2]</td>
<td>Neuro-muscular or neuro-synaptic junction block</td>
<td>Frog, human [1, 2] DBS and SCS [1, 2]</td>
</tr>
<tr>
<td>Radio kHFAC</td>
<td>100 to 500 kHz</td>
<td>cautery</td>
<td>coagulation</td>
<td>-</td>
</tr>
</tbody>
</table>
I.3.II Requirements for an optimal nerve block

Optimal qualities of an electric nerve block might differ for specific applications, but one can define a list of fundamental qualities a nerve block should provide in order to achieve the highest usability from a physician’s and a patient’s perspective. The following list is ordered by importance, first items being the most significant.

1) Safety:
   a. Absence of unintended permanent reduction in neural activity or conduction, unless specifically intended.
   b. No unwanted muscle activation upon initiation or post termination of the block.

2) Efficacy:
   a. Reproducible initiation and termination of the block, on demand and instantly.
   b. Stability of block parameters (same waveform input, same block result; chronic reproducibility).
   c. No side effects at initiation and termination of the block (e.g. nerve activation at block onset or partial block remaining post block; of interest for both, efficacy and safety).
   d. Full or graded reduction of neural communication (depending on application).
   e. Minimal requirements to initiate, maintain, and end the block (energy as the equivalent of a dosage).
   f. Ease of implantation/installation of the device, or transcutaneous approach if possible.
   g. Specificity to location (fascicles) and nerve fiber types (or sizes).
   h. Directionality, affecting only afferent or only efferent nerves fibers.

The three studies described here where designed to address the first five requirements to facilitate translation to human studies.
1.6 Specific Aims

To aid in the transition process by acquiring fundamental information about the safety and efficacy of electric nerve block, the thesis at hand addresses the six specific aims in the following three chapters:

Chapter II

Electric Bladder Voiding using KHFAC Pudendal Nerve Block in an awake chronic SCI model

Aim 1: Demonstrate that KHFAC PN block can provide reproducible reduction in sacrally evoked EUS spasms with stable block current thresholds.

Aim 2: Provide daily bladder voiding using only a combined KHFAC PN block and sacral bladder drive for three or more consecutive days.

Chapter III

Direct Current Contamination of Kilohertz Frequency Alternating Current Waveforms

Aim 1: Measure amplitudes of unintended DC offset caused by KHFAC application with different filters between signal generator and electrodes.

Aim 2: Demonstrate a filter that reduces the unintended DC below levels considered tolerable.
Chapter IV

Combined KHFAC+DC nerve block without onset or reduced nerve conductivity post block

Aim 1: Demonstrate electric, combined no-onset block with CBDC+KHFAC using Hi-Q electrodes.

Aim 2: Demonstrate Hi-Q DC+HFAC nerve block without a decrease in nerve conductivity post block.
II. Chapter II

Electric Bladder Voiding using KHFAC Pudendal Nerve Block

in an awake chronic SCI model

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II.1 Abstract

**Aims:** Spasms of the external urethral sphincter (EUS), after neurological dysfunction or injury such as spinal cord injury (SCI), can prevent bladder voiding and result in urological complications. Kilohertz Frequency Alternating Current (KHFAC) conduction block of the pudendal nerves (PN) can reduce EUS pressures and allow voiding acutely. This study evaluated the ability of KHFAC to prevent EUS contractions using chronically implanted PN electrodes and provide daily bladder voiding after chronic SCI.

**Methods:** Cuff electrodes were implanted on the sacral roots to activate the bladder and electrically block pudendal nerve conduction bilaterally in five cats. After chronic stage SCI, stimulus parameters for PN block and bladder voiding were evaluated bi-weekly. KHFAC PN block was used as the only means for weekday bladder voiding in unanaesthetized animals.

**Results:** KHFAC PN block reduced sacral-evoked EUS pressures from 71.4±63.3 to 22.7±18.3 cmH₂O. Bilateral block increased bladder voiding from 6.8±3.5% to 89.2±10.2% (continuous sacral stimulation) and from 33.7±18.0% to 90.8±8.1% (intermittent). Bladder voiding was provided only via KHFAC PN block during weekdays at least twice/day for 2-10 weeks in three animals. KHFAC voiding was equivalent to manual bladder expression. PN block thresholds remained stable, allowing consistent parameters to be used chronically. PN activation thresholds and evoked pressures did not change.
Conclusions: KHFAC PN block in conjunction with electric bladder drive can replace manual expression as the primary method of clinical bladder voiding after chronic SCI. The stability of the block parameters also suggests that an implantable neural prosthesis using KHFAC PN block to restore bladder voiding is feasible.

Keywords

Nerve block; spinal cord injury; SCI; micturition; neurogenic bladder; chronic study; high frequency alternating current; kilohertz frequency alternating current; KHFAC; HFAC
II.2 Introduction

Spasms of the External Urethral Sphincter (EUS) and Detrusor-Sphincter-Dyssynergia (DSD) can impede bladder voiding and result in significant medical complications for individuals after spinal cord injury (SCI) [93, 94]. Retention of urine in the bladder and the high residual bladder pressures can lead to autonomic dysreflexia (AD) and renal damage [95-99]. Since the Pudendal nerve (PN) provides the principal innervation of the EUS muscle [100, 101], bilateral neurotomy can reduce residual EUS pressure [102], which will allow an increase in bladder voiding. However, the chances of urinary incontinence will also increase [102-105]. Similarly, a sphincterotomy can allow low residual bladder voiding, but with increased chances of incontinence [106, 107]. The current neuro-stimulation standard of care, the Brindley system requires bilateral dorsal rhizotomy, resulting in the loss of reflexive erection and defecation [83, 97, 108-112].

Kilohertz Frequency Alternating Currents (KHFAC) can provide an immediate, on-demand and fully reversible conduction block in acute animal preparations [4, 47, 52, 54, 66, 71, 81, 82, 84, 86, 88, 91, 113] and humans [3]. Using KHFAC waveforms, a complete PN block can be achieved, reducing EUS pressures [82, 86]. If combined with sacral bladder drive, then complete bladder voiding can be achieved as seen in an acute SCI model of anesthetized cats [84, 85, 114]. Unfortunately, an anesthetized model can only provide a partial representation of an awake model with intact reflex pathways, which can be significantly inhibited in anesthetized models [115, 116]. Furthermore, to the best of our knowledge there have been no reports published about the stability of KHFAC block thresholds, the current needed to achieve a nerve block for a given nerve and kHz frequency, for an extended period in literature [46].
Demonstrating effective daily voiding in an awake, chronic SCI model with fully intact neural circuitry and musculature for normal continence would be a significant step in the development of a neuroprosthesis for bladder management post chronic SCI. Such a proof-of-concept would have to show efficacy in both, the anesthetized and in the awake model: first EUS spasms would need to be prevented during voiding, and secondly, bladder voiding would have to be demonstrated after chronic SCI. A final phase showing daily bladder maintenance with low residual volumes each time while using the neuroprosthesis alone would provide strong evidence for a clinically translatable device. This constitutes a necessary step in the development of a neural prosthesis using KHFAC PN block and is required to justify subsequent human studies.

The objectives of this study were to (1) determine reliable parameters for achieving reproducible KHFAC PN block with chronically implanted electrodes to reduce sacrally evoked EUS pressures; (2) demonstrate long-term, electric-only, KHFAC-aided bladder voiding in awake animals with chronic SCI; and (3) verify the absence of adverse effects of repeated application of KHFAC on the PN.
II.3 Methods

II.3.1 Study design

All surgical and testing procedures were reviewed and approved by the institutional animal care and use committee (IACUC) of the Cleveland Veterans Affairs Medical Center and Case Western Reserve University. The study was conducted in two stages on ten purpose-bred, sexually intact male cats (weight 4.2±0.6 kg, age 7.7±1.1 months). The first, exploratory, stage involved five animals to define and optimize the experimental procedure, the electronic setup, and waveforms used to achieve neural stimulation and block. Minimizing signal crosstalk between the two stimulation and two block channels and ensuring DC-free KHFAC waveforms used for nerve block represented a secondary, very important outcome that influenced the design of the electronic setup. The second stage involved a second group of five animals, utilizing the electronic setup and experimental design devised in the first stage.

II.3.2 Surgical procedures

All surgeries were performed under sterile conditions and general isoflurane anesthesia (1 to 3%, Baxter Healthcare Corp, Deerfield, IL) following ketamine (IM, Ketamine HCl 100mg/ml) induction. General pain management was provided during procedures with subcutaneous buprenorphine (0.01 mg/kg; Henry Schein, Melville, NY), injected at induction and prior to wound closure at the end of electrode implant and SCI surgery. During the terminal procedure, buprenorphine was further provided every 12 hours. Systemic pain management following implant and SCI surgery was provided with the application of a Fentanyl Transdermal patch (25mcg/hr; Cordium International, Grand Rapids, MI), changed twice after three days each. All wounds were closed in layers with 0.25% Marcaine (Hospira Inc., Lake Forest, IL) as local pain management
injected sub-dermally into the wound sites during closure. Following the first procedure, animals were fitted with jackets (Lomir Biomedical Inc., Malone, NY), to protect electrode leads and exit sites.

Vitals were monitored during surgical procedures and experiments using the SurgiVet V9200 (Smiths Medical PM, Inc., Waukesha, WI). Specifically, we observed EKG and heart rate, pulse SpO₂, expired pCO₂, blood pressure and body temperature, while maintaining the latter between 36 and 38°C using a heating pad. Knowledge of the specific depth of anesthesia was achieved by assessing blood pressure, heart rate, pCO₂ and the regular examination of eye blink and pain-withdrawal reflex. Respiration was controlled under isoflurane, Propofol and α-Chloralose anesthesia using a pressure-regulated respirator (ADS 1000, Engler Engineering Corporation, Hialeah, FL). Loss of fluids over time was compensated in both surgeries and the terminal procedure by administering 0.9% NaCl (iv; 5–20 ml kg/hr) with sodium bicarbonate (8.4 mg/ml) and dextrose (5%) additives.

**II.3.3 Electrode implant**

To provide sacrally evoked bladder drive (figure II-1), a laminectomy at the L7-S2 level was performed and two tripolar Case Spiral cuff-electrodes (Ardiem Medical, Indiana, PA) of 1.25mm inner diameter were placed on the left and right extradural S1/S2 root, electrode location was verified post-mortem. The electrodes were implanted on the root that yielded the highest, electrically evoked bladder pressure with 20cc slowly (2cc/min) infused bladder volume. The common pudendal nerve was accessed through a postero-lateral gluteal approach. On each side, a tripolar electrode (Ardiem Medical, Indiana, PA) of 1.00mm inner diameter was implanted on the common
PN proximal to the bifurcation of the deep perineal nerve [84, 100]. Electrode leads were looped and secured, then tunneled dorsally to the inter-scapular region, where they were exited and secured again. Impedance at 1 kHz (Z580, PROTEK Test and Measurement, Norwood, NJ) and nerve recruitment curves were measured for all the implanted electrodes under anesthesia.

II.3.4 SCI procedure and recovery

After consistent parameters for stable bladder drive and KHFAC PN block had been identified (3 to 6 weeks post implant), all five animals in the second group of the study underwent a complete surgical spinal transection at the T10-T12 vertebral level. A laminectomy was performed in a dorsal approach to expose the dura, which was opened with a small longitudinal cut. Following intradural application of 0.25% Marcaine, the cord was cut completely and separated by locally placing surgical-foam between the cut ends.

Following the procedure, the re-appearance of the bulbocavernosus, anal sphincter and lower urinary tract (LUT) reflexes were monitored to determine the transition from acute shock phase to chronic SCI. Characteristics of chronic SCI were observed on average about four weeks post SCI. Bladder care was provided by manual expression during the acute SCI phase and electrically with a combination of bilateral sacral bladder drive and KHFAC PN block in the chronic phase. In both cases, voiding was done 2-3 times per day in the awake animals. Animals did not require assistive bowel care due to reflex defecation, often directly following the assisted bladder emptying.
Four tripolar Case spiral electrodes were implanted bilaterally, two sacral electrodes to provide bladder drive and two PN electrodes for KHFAC PN block. The sacral electrodes were positioned on the S1 or S2 roots targeted for maximal evoked bladder pressure in addition to providing partial activation of the EUS (LHS). Starting with an empty bladder, sacral drive evoked EUS pressures “S” which were measured using a urethral pressure transducer (upper RHS). When KHFAC PN conduction block was activated, sacrally evoked PN-mitigated EUS pressures were reduced to residual urethral pressures (“pHFAC”) that were below the average urethral opening pressures measured during bladder voiding. After KHFAC PN block was deactivated, sacrally evoked EUS pressures returned immediately (“Sreturn”). Electric bladder voiding (lower RHS) was initiated with bilateral KHFAC PN block to prevent a KHFAC EUS Onset contraction from inhibiting bladder voiding, followed by sacral bladder drive. The resulting pressure difference provided continuous bladder voiding with high flow rates.

Figure II-1: Experimental and measurement setup.
II.3.5 Terminal procedure, dissection and histological assessment

A supra-pubic 6F dual-lumen catheter (DLC-6D, Life Tech, Stafford, TX) was placed and secured in the bladder apex using a ventral midline approach, allowing the simultaneous measurement of bladder pressures during bladder filling and voiding as well as the measurement of residual volumes after voiding to account for additional urine production. The terminal procedure was conducted according to the protocol below. Following euthanasia (50mg/kg Pentobarbital IV, Virobac AH, Fort Worth, TX), both the PN were harvested with the electrodes intact and approximately 1 cm of neural tissue proximal and distal to the electrode and preserved in 10% Formalin (10% w/v Phosphate Buffer, Thermo Fischer Scientific, Waltham, MA). The completeness and level (T10-T12) of SCI and the location of the sacral electrodes (S1 or S2) were verified and the bladder and urethra with EUS were examined for macroscopic signs of trauma or hypertrophy. Formalin solution was changed after 24 and 72 hrs post initial immersion. Macroscopic histology was performed approximately three months post immersion to analyze for tissue discoloration near the electrodes and on the back of the electrodes were the stainless steel wire was bonded to the platinum contact using a 10x microscope. Micro-histology was performed on slides sectioned every 30 µm, 0.5 cm proximal and distal to, as well as at the electrode location. Microscopic assessment was provided by a surgeon familiar with the experiments and trained in peripheral nerve histology.
II.3.6 Data acquisition

Data were acquired in three phases. This approach allowed a progressive development of the final electronic setup and voiding protocol to achieve daily bladder maintenance and the acquisition of data that allowed comparisons between the phases.

In the first phase, data were acquired every two weeks under isoflurane (0.5-1.5%) or Propofol (0.6mg/kg IV; PropoFlo 28, Abbott Animal Health, Libertyville, IL) anesthesia, prior to the induction of SCI. The main objectives for this phase were to acquire (1) unilateral and bilateral KHFAC block thresholds for waveform frequencies of 6, 8, 12.5 and 25 kHz, (2) parameters for optimal bladder drive with and without KHFAC block, finding (3) optimal parameters for bladder voiding under anesthesia with chronically implanted electrodes using bilateral electric sacral drive and bilateral electric KHFAC PN block. Additionally, (4) activation and saturation thresholds were acquired for each electrode as well as (5) electrode impedance measures. Furthermore, the longitudinal location of the EUS inside the urethra, as well as maximal intra-urethral EUS and bladder pressures were recorded as described in [84]. These data were acquired using a 3F catheter mounted microtransducer (CT3F, MMI-Gaeltech, Hackensack, NJ), and verified with a fluid-column based, external pressure-transducer-mounted 3.5 F perfusion catheter, attached to an infusion pump (0.05 ml/min, Genie YA-12, Kent Scientific, Torrington, CT) for controlled bladder filling. Typical bladder filling volumes used in biweekly tests were 30 ml, infused at 1.5 to 2ml/min, preventing distension-evoked bladder contractions during filling. Data 1-5 were acquired fall all ten animals. SCI was induced only in those animals in whom (1) sacrally evoked bladder pressures of at least 80 cmH2O (value derived from unpublished data) and (2) bilateral KHFAC block was achieved reproducibly allowing bladder voiding under anesthesia. One of these two
criteria were unmet in the first five animals, which experienced unintended DC damage to the PN during KHFAC delivery. The five animals of the second stage all met both criteria and underwent SCI, though two of the five secondary stage animals were later prevented from electric awake bladder maintenance: one experienced PN damage via transcutaneous electrode lead trauma and in the other one, the SCI procedure also became the terminal procedure as sacrally evoked bladder pressures only reached sufficient levels for reliable bladder voiding during that procedure.

The **second phase** began following SCI. Biweekly data acquisition was continued following the same protocol as during the first stage. The main objective was to find parameters for both, sacral drive and pudendal KHFAC block that would allow reproducible bladder voiding volumes and percentages, now with reflexes from chronic SCI stage present. Voiding results were acquired for (1) continuous pulse trains of 30 s duration and (2) trains of intermittent stimulation (15 times 2s-on/4s-off). Continuous stimulation resulted in sustained activation of the bladder and the co-activation of the EUS. Intermittent stimulation resembles the stimulation pattern used in the Brindley approach [108].

The three animals receiving daily electric bladder maintenance were initially maintained by manual expression for the duration of the acute spinal shock. Reappearance of LUT reflexes indicated the gradual change from the acute SCI shock to the chronic stage SCI, with EUS spasms increasingly inhibiting daily manual bladder expression of the awake animals. At that point, a final set of parameters for electric bladder maintenance was chosen under anesthesia to then be used in the awake animals. With the full return of EUS reflexes between 28 and 35 days post SCI, awake, electric-only, KHFAC-aided bladder maintenance was initiated. A pre-determined
The bladder maintenance protocol defined the randomization between (I) continuous or (II) intermittent sacral bladder drive and (III) KHFAC nerve block or (IV) no nerve block. Additionally, animals were (V) manually expressed providing a control measure defined by the clinical standard [99, 114]. For each case (I-V), the recorded voiding volumes were measured while residual volumes were estimated following manual palpation of the bladder post voiding. Animals were voided on average 2 to 3 times daily. Animals were voided more often if bladder volumes (=voided+residual) fluctuated on certain occasions reaching 100 ml and more. In compliance with research facility’s ability to host animals post SCI, electric voiding was performed during weekdays and select weekends, while manual expression was used for the remainder of the days.

Concluding the second phase, a terminal experiment lasting approximately 48 to 65 hours was conducted to collect data under different anesthesia conditions. Main goal was to record voiding volumes and percentages under isoflurane anesthesia, intravenous Propofol and α-Chloralose (iv. 65 mg/kg; Sigma, St. Louis, MO), randomizing the type of electronic voiding performed (I-IV) using the same optimal voiding parameters formerly applied during awake maintenance. Voiding with these parameters were compared under each anesthesia condition, while alternating the average bladder filling volumes between a small (30ml) and a large (72ml) values. The small value represented the one used in the first stage under anesthesia and the large value was the average volume observed during awake bladder maintenance. More voiding trials (again with 30 and 72 ml filling) were acquired after (VI) the bilateral application of a chemical PN block (Lidocaine 2% solution, Hi-Tech Pharmacal Co. Inc., Amityville, NY), followed by (VII) bilateral transection of the PN, providing comparable data between voiding achieved with electric KHFAC, chemical and surgical PN block.
II.3.7 Signal generation

Two sacral root stimulation channels and two KH FAC block channels were provided using a DS8000 Digital Stimulator (World Precision Instruments WPI Inc., Sarasota, FL) with DLS100 stimulus isolators. To prevent unintended ground loops between channels, the internal capacitor C47 in the DLS100, coupling the floating to the DS8000 ground, was removed following consultation with WPI. BNC cables were used to connect the DLS100 to electrode lead connectors at animal, further minimizing capacitive and inductive crosstalk between the four channels and the surrounding environment. Floating oscilloscopes (Agilent TDS2004c on electrically floating, battery-powered power supply, Agilent Technologies, Santa Clara, CA) with 100 MΩ probes were used to verify signal integrity before and in certain cases during waveform application. A combination of two 1 µF capacitors and two 8.2 Henry DC-shunting inductors was used in each KH FAC channel to prevent unintended DC contamination of KH FAC block waveforms [117].

II.3.8 Sacral bladder drive and block waveforms

All electrical stimulation signals were biphasic, symmetric, charge-balanced, square-pulse, current-controlled waveforms. Three types of stimulation waveforms were applied: (1) A “twitch” stimulation was provided using pulses of 100 µs pulse-width of increasing amplitude (0.1 to 10mA, 0.1mA steps) at 1 Hz to acquire nerve activation and saturation thresholds. (2) Tetanic bladder and EUS contractions were achieved with 100 µs pulse trains at 20 Hz (above saturation threshold); two variations of tetanic bladder drive were used: Continuous pulse trains of 30 s duration and intermittent stimulation trains of 15 times 2s-on/4s-off. Continuous stimulation provided sustained activation of the bladder and co-activation of the EUS. Intermittent stimulation
resembles the stimulation pattern used in the Brindley approach [108]. (3) The waveform used for achieving KHFAC PN conduction block was a current controlled, charge balanced, continuous square-wave of 6, 8, 12.5 or 25 kHz.

II.3.9 Data acquisition

Pressure data were acquired using a 3F catheter mounted microtransducer or fluid-column based, external pressure-transducer-mounted 3.5 F perfusion catheter for intra-urethral pressures and bladder pressures during biweekly experiments. Additionally, bladder pressures during voiding were acquired in the terminal procedure using a fluid-column based, external pressure-transducer-mounted supra-pubic catheter. Volume data were acquired as liquid weight using the single point load cell (s215, 0.9 kg; Strain Measurement Devices, Meriden, CT or ESP 0.6 kg, Load Cell Central, Monroeton, PA). Applied current and voltage amplitudes, waveform frequencies and electrode impedances were manually recorded.
II.3.10 Definitions and terminology

(1) Sacrally and pudendally evoked EUS pressures were measured relative to baseline before applying any stimulation.

(2) Successful KHFAC PN block was defined as the minimum current amplitude required, for each tested KHFAC frequency, that achieved a reduction of sacrally evoked EUS pressure by at least 50% after ten seconds of KHFAC application and below the threshold for urethral opening pressure of 40 cmH2O. Block thresholds were determined with an empty bladder and for both, unilateral and bilateral EUS activation and PN block: using continuous sacral stimulation to evoke EUS pressures (figure II-1 right, pressure increase from “Base” to “S”). After three seconds of sacral stimulation alone, KHFAC PN block waveforms were applied for ten seconds. The initiation of the block waveform caused a pressure onset response, followed by a subsequent reduction in sacrally evoked EUS pressure (figure II-1 right, from “S” to “pHFAC”). For all four KHFAC frequencies tested, the onset response would wear off within less than ten seconds, indicated by a plateau in the remaining EUS pressure (“pHFAC”). Two main pressures were analyzed: the maximal reduction in sacrally evoked EUS pressure with the aid of KHFAC ($P_{EUS_{maxDiff}}$) and the minimal pressure achieved during KHFAC application ($P_{EUS_{min}}$). Both pressures could be the same but could also be different for repeated trials if the sacrally evoked pressures differed while the same EUS pressure was measured with KHFAC application, hence providing a measure of how independent the neuroprosthesis would be of sacrally evoked pressures. After the end of KHFAC PN block application, sacrally evoked EUS pressures returned immediately indicating the reversibility of KHFAC nerve conduction block. As a secondary measure of block
threshold, the current providing maximum reduction of sacrally evoked EUS pressure was also identified.

The “Block Ratio” was the ratio in percent of sacrally evoked EUS pressure, reduced by KHFAC PN block, thus providing a measure of completeness of nerve block, as calculated in [82, 84]. (3) Sacrally evoked bladder pressures were recorded across a range of current amplitudes (0.1 to 3 mA) and either 20 or 30 ml filling volume as unilateral and bilateral continuous drive. (4) The combination of optimal sacral bladder drive and KHFAC PN block current/frequency combination maximizing bladder voiding were identified. Optimal bladder drive was determined as the minimum current amplitude evoking iso-volumetric peak bladder pressure of 80 cmH₂O or more. The optimal KHFAC amplitude and frequency for bilateral block reduced sacrally evoked EUS pressures to a minimum pHFAC <40 cmH₂O and provided a short onset response duration of no more than 10s. Voiding trials were performed with and without KHFAC PN Block (control) and with intermittent (ISS) and continuous (CSS) sacral stimulation bladder drive, providing four electric means of bladder voiding.
II.3.11 Data reduction

Custom-written Matlab (Mathworks, Natick, MA) routines were used to analyze raw data and to extract the following data: nerve activation and saturation thresholds for each of the four electrodes, maximal sacrally and PN evoked pressures of EUS and bladder, and KHFAC block threshold currents for each tested frequency in unilateral and bilateral application. Voiding data acquired under anesthesia were used for comparison with data acquired during awake bladder maintenance. Flow was calculated as the first derivative of voiding volume over time (figure II-1 right). All data were monitored and compared across biweekly experiments in each animal to assess trends over time as well as across animals.

II.3.12 Statistical analysis

Matlab routines and JMP (SAS, Cary, NC) were used. One way analyses of variance (ANOVA) were performed on (1) minimal EUS pressure during combined sacral drive and KHFAC PN block ($P_{HFAC\_min}$, $P_{EUS\_maxDiff}$), (2) the KHFAC block threshold currents, and (3) the voiding volumes and percentages achieved during awake bladder maintenance. Factors examined for their influence on (1) and (2) were the choice of the specific pudendal nerve for unilateral (n=8) and bilateral (n=4) PN block, the applied KHFAC frequency, and the number of days post implant; the ANOVA for (3) included the specific cat id, initial bladder volume, the day post implant and the voiding method (intermittent or continuous sacral drive, KHFAC PN block or control, manual expression) as independent variables. The ANOVA was followed up with the Tukey-Kramer multiple comparison test to assess the influence of KHFAC frequency on HF threshold currents (2) with (alpha=0.05), and the influence of voiding method (3) on voiding volumes and percentages (a=0.01). All values reported here are mean ±1 standard deviation.
II.4 Results

II.4.1 Phase one

**KHFAC Block achieved with chronically implanted electrodes.**

In the first group of five subjects, KHFAC block was achieved in all animals, at all frequencies (6, 8, 12.5 and 25 Hz), from first to terminal test (4 to 9 weeks). Unfortunately, PN conduction was reduced in all animals by more than 50% after first or second biweekly experiment due to direct current (DC) contamination of the KHFAC signal (range approximately 30 to 50µA), despite the use of inline-capacitors intended to prevent DC from reaching the electrodes. Loss of PN conduction in all animals of this first stage after the first two to four experiments resulted in a loss of EUS contractions during sacral stimulation and continence prior to bladder voiding. Hence, the voiding volumes achieved in this group of animals without nerve block (control condition) were essentially the same as those using nerve block. Based on this result, DC-shunting inductors were installed across both KHFAC channels in addition to DC-blocking inline-capacitors, to prevent unintentional DC contamination of KHFAC signals and to help reduce signal crosstalk between channels.
II.4.2  Phase two  
KHFAC Block aids in bladder voiding in anesthetized and awake animals  

II.4.2.1  Reduction of EUS pressures with KHFAC Block  

KHFAC PN block was achieved in all animals of the second group, in each biweekly test under anesthesia until the terminal experiment, (69 to 125 days after the initial test, figure II-2). Sacrally evoked EUS pressures returned after KHFAC block ended (figure II-1), indicating that KHFAC waveforms were no longer contaminated by DC as we no longer observed a loss in nerve conduction post block. During KHFAC block application, bilateral-sacral stimulation evoked EUS pressures were reduced from 66.8±68.1 to 18.4±12.8 cmH2O (n=342) resulting in 83.4±12.2% (ratio of n=342, mean+/-SE) reduction across all KHFAC block trials with all frequencies (figure II-2 left). The residual $P_{EUS_{\text{min}}}$ (18.4±12.8cmH2O) was slightly higher than the basal pressure (12.8±5.2cmH2O; p<0.001), but significantly smaller than the urethral opening (59.6±23.6cmH2O, p<0.001) and closing (28.5±9.2cmH2O, p<0.001) pressures during bladder voiding. The Block Ratio was 0.93±0.15 (n=926, mean+/-SE) and the main effect of nerve (left/right and animal number), KHFAC frequency, and days post implant were not significant. The reduction of sacrally evoked $P_{EUS}$ remained consistent throughout the study for each of the animals (figure II-2 right). Neither the days post implant (unilateral p=0.8375, bilateral p=0.2400), nor the choice of KHFAC frequency significantly influenced $P_{EUS_{\text{min}}}$ (unilateral p=0.0886, bilateral p=0.0469; significance level was set to 0.001 due to large sample size (n= 926)). The choice of PN was significant (unilateral p=0.0002, bilateral 0.0015). Statistically same results were found for $P_{EUS_{\text{maxDiff}}}$.
Bilateral (unilateral) application of KHFAC PN block reduced the sacrally evoked EUS pressure from 96.2±93.5 cmH2O (53.6±47.4 cmH2O) to 24.1±15.9 cmH2O (16.0±10.2 cmH2O), p<0.001 (*). The basal pressure (**) indicates residual pressure without any electrical drive. For bilateral KHFAC PN block the (dotted) fit line resembling the reduced EUS pressure over time is y = 0.126 * x + 19.0; for unilateral block the (continuous) fit line is y = 0.0849 * x + 14.2. All pressures measured with an empty bladder. Data from four cats (8 unilateral nerves, 4 bilateral nerve combinations). The cases the reduced EUS pressure would have been above typical urethral opening pressures (50 cmH2O), were maximum pressures measured with currents formerly determined to completely block PN conduction. Subsequent bladder voiding trials acquired the same day confirmed complete, unimpeded bladder voiding.

Figure II-2: KHFAC PN Block consistently reduced sacrally evoked EUS pressures over time.
II.4.2.2 KHFAC block thresholds

The KHFAC PN Block threshold currents remained stable for the duration of the study for all the animals in the second group (figure II-3). While the KHFAC PN Block threshold currents to achieve $P_{EUS_{\text{min}}}$ showed a frequency dependence (2.9±1.4 mA for 6kHz, 3.1±1.6 mA for 8kHz, 3.2±0.9 mA for 12kHz, and 4.6±1.2 mA for 25kHz), but only the block threshold for 25kHz proved to be statistically different from all the other frequencies (Tukey HSD, alpha=0.05). The two factors with significant effect on the HF block threshold were the KHFAC frequency (p<0.001) and the blocked pudendal nerve (p<0.001), while the days post implant (unilateral p=0.4164, bilateral p=0.4020) had no impact. Results for achieving the maximal reduction in sacrally evoked pEUS ($P_{EUS_{\text{maxDiff}}}$) were not statistically different from those for achieving the minimal pEUS during KHFAC block ($P_{EUS_{\text{min}}}$). The current thresholds for bilateral KHFAC PN block were comparable to the block thresholds for unilateral PN block.

![Graph showing the current thresholds to achieve KHFAC PN block remained stable throughout the study.](image)

**Figure II-3:** The current thresholds to achieve KHFAC PN block remained stable throughout the study. Tested were four frequencies 6, 8, 12 and 25kHz. Depicted are the thresholds to achieve minimum PEUS during application of unilateral (grey, closed) and bilateral (black, open) KHFAC PN block. Data from four cats, 342 threshold trials out of 947 KHFAC PN Block trials. Duration from first test to terminal test 125 days (140 days post implant). The lines for best fit show minimal change in threshold over time x in days: 6kHz fit line = 0.0011*x + 4.2mA (4.3 ± 2.5 mA, n = 48); 8kHz fit line = 0.0089*x + 4.5mA (4.7 ± 2.3 mA, n = 77); 12kHz fit line = 0.0083*x + 4.3mA (4.5 ± 1.6 mA, n = 172); 25kHz fit line = 0.010*x + 5.7mA (6.1 ± 1.7 mA, n = 45); (mean±1sd).
II.4.2.3 Bladder pressures

During the biweekly experiments under anesthesia, average evoked bladder pressures $P_{\text{VES}}$ under iso-volumetric conditions were not increased by applying KHFAC waveforms to the PN (table II-1). With pooled data from both sacral drive types (ISS and CSS data), the difference between the bladder pressures recorded with (83.9±33.1 cmH$_2$O) and without (95.2±29.6 cmH$_2$O; p=0.2301) the KHFAC block were not significant. Similarly, there was no significant difference between pressures achieved with continuous (CSS alone 93.4±29.6 cmH$_2$O; CSS and KHFAC 86.0±33.3 cmH$_2$O; p=0.4613) and intermittent (ISS alone 108.2±36.0 cmH$_2$O; ISS and KHFAC 74.5±33.4 cmH$_2$O; p=0.3880) sacral drive as well as between CSS only vs. ISS only (p=0.6642) or CSS+KHFAC vs. ISS+KHFAC (p=4786). Bilateral sacral drive provided sufficient evoked bladder pressures for voiding in three of the five animals and allowed to proceed towards SCI and awake bladder maintenance. No bladder pressures were recorded during daily, awake bladder maintenance voiding trials as urethral catheters would have obstructed the voiding process (table II-1).

During the terminal experiment, average bladder pressures measured with the supra-pubic catheter during voiding (non iso-volumetric condition) were comparable to the measurements collected in biweekly experiments for CSS alone (92.6±45.4 cmH$_2$O). Bladder pressures recorded with the application of KHFAC block, in contrast, were significantly smaller (CSS+KHFAC = 52.7±20.3 cmH$_2$O; p<0.0001). For intermittent bladder drive, evoked average bladder pressures during voiding differed only slightly from the treatment condition involving KHFAC block (31.4±8.5 vs. 34.7±11.1 cmH$_2$O; p=0.1014). For pooled ISS and CSS data, the KHFAC aided approach achieved voiding
with significantly lower evoked bladder pressures (44.9±19.1 vs. 63.5±45.3 cmH$_2$O; p=0.0002).

Following bilateral application of Lidocaine to the PN, average bladder pressures measured during voiding were comparable to bilateral KHFAC PN Block: with CSS alone, pressures of 56.3±0.7 cmH$_2$O (p=0.1593 vs. CSS+KHFAC) were evoked while intermittent bladder drive resulted in 35.4±10.6 cmH$_2$O (p=0.8889 vs. ISS+KHFAC). When pooled data from the two sacral drive types was analyzed, average evoked bladder pressures measured after applying the lidocaine block (43.3±13.5 cmH$_2$O) were found to be not statistically different from the bladder pressure during KHFAC block CSS+ISS+KHFAC (44.9±19.1 cmH$_2$O; p=0.7469) prior to Lidocaine application, but were different compared to the control (no KHFAC) prior to Lidocaine application (63.5±45.3 cmH$_2$O; p=0.0051). The bladder pressures during voiding with bilaterally transected PNs were comparable to bilateral KHFAC PN block as well as the lidocaine block of the PN: the 55.6±20.2 cmH$_2$O measured with CSS alone were not statistically different from those measured with the aid of KHFAC (p=0.9586), voiding with ISS alone gave similar results at 36.2±11.3 cmH$_2$O (p=0.9580 vs. ISS+KHFAC). For the pooled CSS and ISS data, the 43.7±17.9 cmH$_2$O were not very different from values measured with CSS or ISS and KHFAC (p=0.2309), but they were significantly smaller than values with CSS or ISS only (p<0.0001).
Table II-1: Evoked bladder pressures during different voiding conditions.

<table>
<thead>
<tr>
<th>Evoked bladder pressures in cmH₂O</th>
<th>CSS (control)</th>
<th>CSS KHFAC</th>
<th>ISS (control)</th>
<th>ISS KHFAC</th>
<th>CSS+ISS (control)</th>
<th>CSS+ISS KHFAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biweekly (regular) (iso-volumetric)</td>
<td>mean 93.4</td>
<td>86.0</td>
<td>108.2</td>
<td>74.8</td>
<td>95.2</td>
<td>83.9</td>
</tr>
<tr>
<td></td>
<td>stddev 29.6</td>
<td>33.3</td>
<td>36.0</td>
<td>33.4</td>
<td>29.6</td>
<td>33.1</td>
</tr>
<tr>
<td>Terminal (regular) (non iso-volumetric)</td>
<td>mean 92.6</td>
<td>52.7</td>
<td>31.4</td>
<td>34.7</td>
<td>63.5</td>
<td>44.9</td>
</tr>
<tr>
<td></td>
<td>stddev 45.4</td>
<td>20.3</td>
<td>8.5</td>
<td>11.1</td>
<td>45.3</td>
<td>19.1</td>
</tr>
<tr>
<td>Terminal Lidocaine (non iso-volumetric)</td>
<td>mean 56.3</td>
<td>-</td>
<td>35.4</td>
<td>-</td>
<td>43.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>stddev 0.7</td>
<td>10.6</td>
<td>13.5</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terminal - Cut PNs (non iso-volumetric)</td>
<td>mean 55.6</td>
<td>-</td>
<td>36.2</td>
<td>-</td>
<td>43.7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>stddev 20.2</td>
<td>11.3</td>
<td>17.9</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
II.4.2.4 Voided volumes

During the biweekly experiments under anesthesia, average voiding achieved with KHFAC PN block was significantly higher than volumes achieved with sacral drive alone (table II-2).

Table II-2: Voiding volumes achieved under different conditions.

<table>
<thead>
<tr>
<th>Voided volumes in ml</th>
<th>CSS (control)</th>
<th>KHFAC</th>
<th>ISS (control)</th>
<th>KHFAC</th>
<th>CSS+ISS (control)</th>
<th>KHFAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biweekly (regular)</td>
<td>8.1</td>
<td>24.8</td>
<td>17.0</td>
<td>33.5</td>
<td>10.3</td>
<td>27.9</td>
</tr>
<tr>
<td>(30 ml filling)</td>
<td>4.1</td>
<td>15.3</td>
<td>11.8</td>
<td>4.6</td>
<td>7.4</td>
<td>13.1</td>
</tr>
<tr>
<td>Terminal (regular)</td>
<td>27.2</td>
<td>33.7</td>
<td>33.8</td>
<td>45.9</td>
<td>30.2</td>
<td>39.4</td>
</tr>
<tr>
<td>(30 or 72 ml filling)</td>
<td>20.9</td>
<td>20.7</td>
<td>28.0</td>
<td>28.2</td>
<td>24.1</td>
<td>25.0</td>
</tr>
<tr>
<td>Terminal - Lidocaine</td>
<td>56.0</td>
<td>-</td>
<td>74.4</td>
<td>-</td>
<td>62.0</td>
<td>-</td>
</tr>
<tr>
<td>(30 or 72 ml filling)</td>
<td>52.5</td>
<td>59.1</td>
<td>-</td>
<td>-</td>
<td>44.5</td>
<td>-</td>
</tr>
<tr>
<td>Terminal - Cut PNs</td>
<td>47.1</td>
<td>-</td>
<td>65.6</td>
<td>-</td>
<td>58.7</td>
<td>-</td>
</tr>
<tr>
<td>(30 or 72 ml filling)</td>
<td>24.8</td>
<td>18.2</td>
<td>-</td>
<td>-</td>
<td>22.1</td>
<td>-</td>
</tr>
</tbody>
</table>

For both sacral drive types (pooling the data for ISS and CSS), voiding with the aid of KHFAC (27.9±13.1 ml) was significantly higher than voiding results without the aid of electric nerve block (10.3±7.4 ml; p<0.0001). Similarly, voiding volume achieved with
continuous sacral drive alone were significantly smaller (8.1±4.1 ml) than those resulting from adding KHFAC PN block (24.8±15.3 ml; p<0.005). The difference for intermittent sacral stimulation with and without block (p=0.1279), difference between the two controls (CSS and only; p=0.3202) and the difference between the two voiding conditions involving electric nerve block (i.e. CSS+KHFAC vs. ISS+KHFAC; p=0.1027), were not significant.

Bladder voiding during daily electric bladder maintenance: KHFAC-aided bladder voiding was significantly higher than those resulting from sacral stimulation alone (control) (figure II-4). Bilateral KHFAC PN block improved the voiding percentage for continuous sacral stimulation (CSS) from 6.8±3.5% (control) to 89.2±10.2% (p<0.0001) and for intermittent sacral stimulation (ISS) from 33.7±18.0% (control) to 90.8±8.1% (p<0.0001). Manual expression, the method used to maintain an animal on low residual bladder volumes post SCI, provided a voiding percentage of 75.1±17.1% (p<0.0001 vs. all four methods of bladder voiding tested here). There was no significant difference in the percentage of volume voided with CSS and ISS in the presence of KHFAC PN block (p=0.86) indicating that voiding becomes independent of the choice of bladder drive when KHFAC PN block is used. The initial bladder volume and the voided volume were different between animals (p<0.0001). The voiding method used also had a significant effect on the voided volume (p<0.0001) and percentage (p<0.0001). The days post implant had no significant effect on the voiding volume (p=0.5729) and percentage (p=0.8217).
Figure II-4: Bilateral KHFAC PN Block increased bladder voiding volumes during awake-animal voiding following chronic-SCI.

Animals received electric bladder voiding on weekdays and select weekends, manual expression during remaining weekends. Electric control voiding utilized solely bilaterally applied sacral drive, either as continuous or intermittent (intervals of 2s-on/4s-off) stimulation pattern with parameters chosen prior based on the ability to evoke at least 80cmH2O of bladder pressure in iso-volumetric runs under anesthesia. KHFAC aided voiding utilized the same sacral drive parameters and in addition, continuous, bilateral KHFAC PN Block with parameters determined prior under anesthesia providing full bilateral PN block. Voiding volumes were measured, residual bladder volumes estimated and on occasion, estimates confirmed following urethral catheterization and bladder drainage. While being *not* statistically different between each other (**), voiding volumes and percentages using KHFAC-PN block were significantly greater than all other means of bladder voiding (p<0.001).

Post hoc testing using the Tukey-Kramer test showed that KHFAC aided voiding percentages were statistically higher than all other methods of bladder emptying (p<0.0001). The voiding volumes and percentages achieved during daily bladder voiding with the aid of KHFAC were independent from the choice of bladder drive. The volumes and percentages achieved without the aid of KHFAC PN block were all significantly smaller (figure II-4) and statistically different from each other (CSS control vs. ISS control vs. manual expression; all p<0.0001). KHFAC aided voiding volumes achieved with either bladder drive (ISS or CSS) were statistically different from both types of sacral drive achieved without KHFAC PN block (control), as well as manual expression (p<0.001) (figure II-4).
The three animals receiving electric bladder voiding post chronic SCI received a combined electric and manual (on select weekend days) bladder maintenance of cumulative 10, 27 and 71 days for animals 1,2 and 3, respectively. Of these days, 7 (of 10), 22 (of 27) and 44.5 (of 71) were days when the animals received only electric bladder voiding (figure II-5). The longest consecutive electric-only maintenance durations were 3.5, 8.5 and 12 days for animals 1, 2 and 3, respectively. A total of 309 electric-only bladder voiding runs were performed during the study with the animals awake. The KHFAC block parameters used during awake bladder voiding were 8 kHz at 8 mA, 12 kHz at 5 mA, and 12 kHz at 6 mA in animals 1-3, respectively. The bladder drive used was 0.5 mA, 2mA and 3 mA in animals 1-3, respectively.

The voiding data measured during the terminal experiments (table II-2) were acquired after the animal had been on the table for about 24 hrs and did show a significant weakening of the EUS during control voiding trials not involving electric nerve block: for both the sacral drive conditions (pooled ISS and CSS data), voiding with the aid of KHFAC (39.4±25.0 ml) was not significantly higher than voiding without the aid of electric nerve block (30.2±24.1 ml; p=0.1303). Similarly, volumes achieved with continuous sacral drive alone were only slightly smaller (27.2±20.9 ml) than those resulting from adding KHFAC PN block (33.7±20.7 ml; p=0.3618). The difference between intermittent sacral stimulation with and without block was also not significant (ISS vs. ISS+KFAC; p=0.2506).
Voiding data acquired following bilateral application of Lidocaine to the PN: voiding volumes with CSS alone were larger but not significantly different (56.0±52.5 ml; p=0.5388), similar results were obtained for ISS (p=0.6171) as well as the pooled data (ISS+CSS = 62.0±44.5 ml; p=0.6403). The volumes achieved after bilaterally transected PNs were comparable to bilateral-KHFAC and lidocaine block of the PNs: the average voiding volume of 47.1±24.8 ml measured with CSS alone was not statistically different from those measured with the aid of KHFAC (p=0.2663), although voiding with ISS only was significantly larger at 65.6±18.2 cmH₂O (p<0.05 vs. ISS+KHFAC). Pooled CSS and ISS voiding data was significantly larger than the values measured with CSS or ISS and KHFAC (p<0.05), the same was true for CSS or ISS only (p<0.0001).

![Figure II-5: Stability and reliability of KHFAC aided voiding over time superior to sole-sacral-drive bladder maintenance.](image)

Voiding percentage with aid of KHFAC showed much less variance (NN±MM) than manual expression or voiding without KHFAC (NN±MM). Bladder drive was provided either as continuous or intermittent (“Brindley”) drive, graph shows data irrespective of choice of sacral stimulation. Animals received electric-only bladder care whenever it was possible to host them at the research facility, which included one weekend to provide up to 12 (8) days of KHFAC-aided bladder voiding with low residual bladder volumes (10cc) for animal one (two). Voiding percentage with aid of KHFAC stabilized above 90% at about 50 days post SCI.
Peak flow data did not depend on the application of KHFAC nerve block. Flow recorded during the terminal experiments with the aid of KHFAC was 0.41±0.47 ml/s and not significantly different from flow without KHFAC 0.32±0.41 ml/s (p=0.1736). Flow rate was not significantly different with bilateral Lidocaine block (0.33±0.27ml/s), and bilateral PN transection, (0.54±0.57ml/s). A differentiation between voiding conditions was provided by the time to reach maximum flow after sacral drive had been initiated (table II-3). Typically, flow began in CSS only trials after sacral stimulation had ended, hence providing an indirect measure for the outlet resistance. As a result, the average time to maximal flow was 25.3±9.0 s, significantly larger than the time for trials with the aid of KHFAC PN block (15.7±7.4 s; p<0.0001). The flow measured for interrupted sacral stimulation started generally during one of the post-stim-off-times (4sec), resulting in a slight increase in the times to reach maximal flow, that was not significant relative to the with block condition (i.e. ISS vs. ISS+KHFAC, p=0.2282). The difference for the pooled (i.e. CSS and ISS) with and without block data was significant (p<0.001). Following the application of Lidocaine, the measured flow was not different from CSS and KHFAC (p=0.4522) or ISS and KHFAC (p=0.8335). The merged results were similar with no statistical difference in comparison to KHFAC aided voiding (p=0.9293), but a significantly shorter time than CSS+ISS alone (control, p<0.01). Following bilateral PN transection, the shortest time to maximal flow with CSS was recorded at 9.5±2.6 s; even significantly shorter than CSS and KHFAC combined (p<0.0001). For ISS post transection, the difference was not significant relative to ISS and KHFAC combined (p=0.5218). The pooled CSS and ISS data showed statistical significance in comparison to earlier CSS or ISS only voiding trials (p<0.0001), but no statistical difference vs. earlier pooled CSS or ISS with added KHFAC block trials (p=0.1362). Urethral opening pressures measured during the terminal experiments were 64.4±34.2 cmH₂O with and 59.8±28.5 cmH₂O
without KHFAC (p=0.2914). After Lidocaine the measured opening pressures were 69.5±17.7 cmH₂O (p=0.4258 vs. KHFAC) and after transection 62.2±25.9 cmH₂O (p=0.8377 vs. KHFAC).

Table II-3: Time to reach maximum flow during voiding with different conditions.

<table>
<thead>
<tr>
<th>Time to max. flow in seconds</th>
<th>CSS (control)</th>
<th>KHFAC</th>
<th>ISS (control)</th>
<th>KHFAC</th>
<th>CSS+ISS (control)</th>
<th>KHFAC</th>
<th>CSS+ISS (control)</th>
<th>KHFAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal (regular) (30 or 72 ml filling)</td>
<td>Mean</td>
<td>25.3</td>
<td>15.7</td>
<td>19.0</td>
<td>17.2</td>
<td>22.9</td>
<td>16.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stdev</td>
<td>9.0</td>
<td>7.4</td>
<td>6.4</td>
<td>4.6</td>
<td>8.6</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Terminal - Lidocaine (30 or 72 ml filling)</td>
<td>Mean</td>
<td>14.2</td>
<td>-</td>
<td>16.6</td>
<td>-</td>
<td>15.7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stdev</td>
<td>2.6</td>
<td>5.3</td>
<td>4.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Terminal - Cut PNs (30 or 72 ml filling)</td>
<td>Mean</td>
<td>9.5</td>
<td>-</td>
<td>16.1</td>
<td>-</td>
<td>13.6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stdev</td>
<td>2.6</td>
<td>-</td>
<td>6.4</td>
<td>-</td>
<td>5.8</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
II.4.3 Absence of adverse events

Pudendal nerve activation thresholds remained at 302±198µA (range 80 to 1080 µA, n=56) and did not increase with time. One PN in the first maintained animal showed an increase in activation threshold from 400 to 1080 µA after receiving daily KHFAC block for bladder maintenance. This animal was tested with an electronic setup that temporarily showed large levels of crosstalk (issue of unintentional grounding) between the stimulation channels, which was fixed before implanting and testing the other four animals. Sacral activation thresholds remained at 245±149 µA (range 40 to 640 µA, n=56). The nerve activation thresholds for the pudendal nerves were in the same range as the thresholds recorded from the sacral roots with the exception of one PN. For this specific PN, the activation threshold had increased from 400µA before to 1080µA after electric maintenance. The evoked EUS pressures from supramaximal PN or sacral stimulation on the other hand had not changed. The PN activation thresholds in the animal maintained for 71 days changed from 240µA (R) and 320µA (L) to 280µA (R and L). Long term nerve viability was further confirmed through histology and is comparable to data presented by Camilleri et al [3].

The parameters for the KHFAC pudendal nerve block were chosen such that the onset response did not generally last longer than ten seconds; in most cases the onset-response duration was approximately two seconds. With such a short KHFAC onset response in place, the bladder voiding could be started without unnecessary delays, as the onset itself did not contribute to residual EUS pressures. Furthermore, as the SCI was a complete spinal transection, the animals did not show signs of discomfort during the KHFAC onset or during sacral stimulation. Any reflexive muscle activity (e.g. leg clonus) observed shortly after initiating sacral stimulation or KHFAC waveforms was of
short duration (<10sec) and well tolerated by the animals. The choice of electric voiding type (CSS/ISS with or without KHFAC) did not affect the animals’ general behavior during voiding or thereafter.

The electrode impedances stabilized during the first three weeks post implant and remained at $8.2 \pm 1.6 \, \text{k}\Omega$ for all animals. In the second group of animals, no signs of electrode corrosion or abrasion were found in examinations following explantation. In contrast, electrodes explanted from the first group of five animals did show signs of corrosion that were likely related to the unintentional DC contamination.

II.4.4 Histology

Pudendal nerves explanted from the second group of animals did not show macroscopic or microscopic histological signs of nerve damage. In contrast, some of the pudendal nerves explanted from the first group did show macrophage ingrowth indicating chronic inflammation, which correlated with electrodes that did show corrosive damage. This coincided with tissue discoloration seen during the macroscopic analysis. The discoloration was located adjacent to the platinum contacts of the electrodes. In three cases, a discoloration of the back of the platinum contact was noted where the stainless steel lead wire had been bonded to the platinum contact. All the animals with tissue or electrode discoloration were in the first group of subjects, where inductors to shunt DC currents had not been used yet.
II.5 Discussion

This is the first study showing daily bladder voiding in a chronic SCI model, on multiple days, using only an implantable neuroprosthesis without the need to transect neural or muscular tissue, which is the main shortcoming of the current standard of care in the clinic [99, 107, 118-120]. Secondly, we were able to show that a current-controlled KHFAC nerve block can be achieved reliably with stable parameters, which is an important requirement for implantable neuroprostheses based on KHFAC nerve block technology [46]. Furthermore, the study showed that KHFAC nerve block can be implemented chronically multiple times a day without leading to changes in functional nerve viability, once “clean” KHFAC waveforms were used without unintended DC contamination [117].

II.5.1 Reduction of EUS pressure with KHFAC Block

KHFAC nerve block was achieved in male cats with chronically implanted, human-grade electrodes for up to 125 days (140 days post implant). In contrast to Camillieri et al [3], we are reporting the block of nerve conduction during application of KHFAC as a direct measure of efficacy by showing that sacrally evoked PN activity did not cause spastic muscle contractions when the KHFAC nerve block is applied. Camillieri et al. only reported indirect measures such as reduction in appetite or loss in weight over periods of time, which could just as well have resulted from permanent stimulation or permanent injury to the pancreatic branch of the vagal nerve [121]. We were able to show that KHFAC nerve block leads to an immediate reduction of spastic EUS muscle contractions, an effect that was independent of the choice of frequency (6, 8, 12.5 or 25 kHz) and that the effect was stable over time. KHFAC PN block was achieved with all frequencies and in all animals throughout the study. The residual $P_{EUS, min}$ was
independent of the choice of KHFAC frequency once that block was successful. Sacrally evoked intra-urethral EUS pressures were reduced reliably and significantly with both unilateral and bilateral KHFAC PN Block.

Current thresholds to achieve PN block were stable over the study duration. The optimal voiding parameters determined under anesthesia provided consistent reduction of sacrally evoked EUS pressures in anesthetized and awake animals for KHFAC-aided, electric-only bladder voiding without the need to adjust block parameters over time (“set and forget”). In addition to blocking sacrally evoked EUS activity, bilateral KHFAC PN block was shown to prevent spontaneous EUS muscle contractions (“spikes”), observed under α-Chloralose anesthesia, indicating full PN motor block. Even though the PN is the primary innervation to the EUS, there are non-PN pathways that can lead to sacrally evoked intra-urethral EUS pressures even with bilaterally transected PNs [14]; this phenomenon was observed in this study both during bilateral KHFAC PN block and with bilaterally transected PNs. However, these residual PEUS_min during KHFAC PN block did not seem to impair voiding as they were still significantly lower than the recorded urethral opening and closing pressures. This was confirmed by residual pressures measured during KHFAC application, which too were lower than the urethral opening and closing pressures. The relatively small, remaining increase in pEUS due to pelvic floor muscles did not impede bladder voiding and did not change with the bilateral application of Lidocaine to the PN as well as PN transection. The achieved reduction of sacrally evoked EUS pressures using KHFAC nerve block was a very stable and reproducible effect over time.
II.5.2 Bladder maintenance post SCI

We provided bladder care with an electric-only approach for extensive periods of time without changing the parameters of the neuro-prosthesis. This is the first study to demonstrate successful KHFAC nerve block for over four months post electrode implant. Our voiding results are comparable to those presented by Tai [114], Sawan et al. [122, 123] and Boger et al [84, 124]. However, the major difference between this study and the others is that we are the first to report a successful implementation of a functional neuroprosthesis prototype that allowed bladder maintenance in awake animals, without the need for neural transection (Brindley [99, 110, 125]). In contrast, the animal models in other chronically implanted studies were anesthetized during bladder voiding [114, 122, 123]. The Boger et al study was done in an acute model of SCI and also reported significant reduction of sacrally evoked EUS pressures by the end of the study. Bladder voiding achieved with the aid of KHFAC was independent of the choice of sacral stimulation and was significantly better than sacral stimulation of either type (continuous / intermittent) and manual expression. In fact, as detrusor-sphincter-dyssynergia led to prolonged voiding times for manual expression, electric voiding became the main choice from a user’s perspective providing the manual expression. This observation correlates with lower bladder pressures that were observed during KHFAC-block aided voiding, especially when compared to continuous sacral drive, and presumably continuous pressures during manual expression, which would activate the guardian reflex [126]. The other advantage of this approach is that unlike the Brindley approach [108, 110], there is no necessity for nerve or muscle transection. With the KHFAC block nerve conduction returned immediately when the application of the block waveform was stopped. We were able to maintain three animals for long periods of time (>3 consecutive days) by relying on the described neuro-prosthetic only.
We maintained 3 animals for up to 71 days post chronic-SCI (44 days electric only). During that time, electric bladder voiding using bilateral KHFAC PN block was used as the only means to provide micturition for a maximal continuous duration of 3.5 to 12 days using the *same parameters* to achieve sacral drive and PN block in awake animals. These animals had transitioned from the acute spinal shock into the chronic-SCI phase with fully intact reflexive pathways, showing symptoms of DSD during manual bladder expression. Electric bladder maintenance was statistically more effective than manual expression which is likely an effect of the DSD-evoked EUS contractions during manual expression. The choice between continuous vs. intermittent bladder drive did not change the efficacy of the voiding approach. The difference between intermittent (Brindley [110]) and continuous sacral stimulation alone (both used as control measures) showed that most of the voiding without electric PN block occurred right at the end of sacral stimulation, likely resulting from evoked bladder pressures being larger than urethral pressures at the EUS without sacral activation leading to EUS spasms. The residual bladder volume after voiding with the aid of KHFAC PN block was independent of the choice of sacral bladder drive (ISS/CSS). Our voiding results (voided volumes and percentages, residual volumes) are comparable to those reported in other studies [84, 114, 122-124].

Furthermore, lower bladder pressures were measured during voiding with aid of KHFAC PN block, indicating that the bilateral nerve block resulted in less outlet resistance, which was further confirmed with shorter times to reach the maximum flow during voiding. Subsequently, voiding results achieved under anesthesia and in awake animals were comparable to voiding results achieved in a former acute study [84], further providing an indirect measure of completeness of PN block. During awake
bladder voiding, the combination of either choice of sacral stimulation with KHFAC PN block led to continuous flow. While electric control voiding (CSS or ISS) alone did provide bladder voiding during the awake maintenance, residual volumes and percentages were higher and the majority of the flow happened more towards the end of the voiding trials, indicating that high bladder pressures were necessary to produce the control voiding results. In contrast, most of the voiding during the KHFAC aided voiding runs occurred earlier and generally as continuous flow.

We did notice a training effect in both the bladder and EUS over time: sacrally evoked bladder pressures kept increasing throughout the study, especially after SCI, with the highest bladder pressures measured towards the end of the four-month period post SCI and after several days or weeks of bladder maintenance. Similarly, sacrally evoked EUS pressures increased over the duration of the study. The dissection postmortem showed an enlarged EUS muscle in two out of the three animals that underwent awake bladder maintenance, especially the animal that received 44 days of electric voiding. We hypothesize that the “electric training” received during experiments under anesthesia as well as the strong contractions caused by the onset-response induced by the KHFAC waveform, several times a day, might have caused the strengthening of the EUS muscle. Manual expression became harder and took longer (often 45-60 minutes and required five or more voiding attempts) in these animals, likely a result due to DSD and the stronger EUS muscles, which was not a problem during voiding with bilateral electric PN block. The onset response itself was well tolerated by the awake animals during bladder maintenance: no signs of discomfort were observed during sacral stimulation or the combined application of sacral stimulation and KHFAC PN block.
The voiding percentage during daily maintenance increased during the first two weeks of awake bladder voiding. This correlated with a gradual increase of sacrally evoked bladder pressures post SCI and a reduction in signs of incontinence in the animals. As the voiding percentage is a function of initial bladder volume and residual volume post voiding and the latter being relatively constant (2 to 5ml) throughout the study, resulted in the effect of voiding percentages increasing directly with the initial bladder volume over time. The effect was independent of the choice of electric (CSS/ISS/CSS+KHFAC/ISS+KHFAC) or manual bladder voiding. As voiding percentages stabilized at 80% and more using manual and KHFAC aided electric methods for voiding, the re-appeared bulbocavernosus, anal sphincter and lower urinary tract (LUT) reflex responses also were expressed stronger and more reliably indicating the full transition from spinal shock to chronic SCI stage.

Unilateral vs. bilateral PN block: KHFAC block thresholds were recorded under isoflurane and Propofol anesthesia during biweekly tests for both conditions. Unilateral sacral drive was used on the same side that PN block was applied as we did not see a difference in residual EUS pressures with bilateral PN block when unilateral sacral drive was applied. This indicates that there was no cross-activation of the EUS through the contralateral PN, if unilateral sacral stimulation was blocked with ipsilateral PN KHFAC block. All PN block aided bladder voiding on the other hand, were conducted with bilateral KHFAC PN block to ensure that urethral distention reflexes (due to urine flow) would not cause DSD and inhibit voiding.
II.5.3 Necessity for “clean KHFAC” waveforms without DC contamination

We noticed that the nerve activation thresholds increased over time in all of the five animals in the first part of the study, which eventually corresponded with a loss of PN conduction. Histological analysis showed chronic inflammation with macrophages in PN sections taken at the electrode location, indicating that the damage was likely not from one procedure at the end of the study, but instead due to repeated, unintended DC delivery to the nerve. Continuous deliver of as little as 10 µA can lead to changes in nerve conduction and eventually nerve damage. The unintentional DC current values measured in the electronic setup, prior to the addition of the LC(-DC) filter, were about three to four times the published safety limit for nerves [46, 49].

II.5.4 Nerve activation thresholds and nerve conduction

Both remained stable throughout the second phase of the study after the LC(-DC) filter circuit [117] had been added to both electrically floating KHFAC block channels. The LC(-DC) filter helped to prevent the accumulation of DC voltages across the PN block electrodes due to unintended DC contamination of the KHFAC waveforms. The post-mortem analysis of electrodes and histology confirmed that nerve tissue was not damaged after repeated KHFAC nerve block application in the animals of the second phase. The pudendal nerves of the animals in the first part of the study (prior to usage of LC(-DC) filters) showed chronic inflammation throughout the nerve, which coincided with loss of nerve conduction and substantial rise in activation thresholds (>10mA for 100 µs pulses).
The limitation of the present study is that the SCI model used was a complete spinal transection. This ensured that the research subjects would not be cognitively aware of any potential activation of sensory nerve fibers in the PNs during the KHFAC onset response. At the same time, however, activation of sensory fibers could prove to be a limiting factor for clinical implementation for partial SCI patients and should be addressed with other technologies, such as a combined charge-balanced DC and KHFAC nerve block as described by Franke et al. in [75].
II.6 Conclusion

This study demonstrated for the first time in an awake preclinical chronic SCI model that daily bladder voiding can be provided by a combination of electric sacral-root bladder drive and electric KHFAC pudendal nerve block. The KHFAC PN block was achieved reliably with chronically implanted, human-grade electrodes for up to four months post implant. Sacrally evoked $P_{EUS}$ was reliably reduced, while KHFAC block parameters remained stable. Bladder voiding volumes and percentages were increased significantly with KHFAC PN block under anesthesia as well as during daily awake bladder maintenance after chronic SCI, independent of choice between continuous or intermittent sacral drive. Residual $P_{EUS}$ were below average urethral opening pressures observed in terminal experiments during bladder voiding after PN neurotomy. Constant nerve recruitment and KHFAC block parameters indicate the feasibility of a reliable and healthy KHFAC PN block based bladder voiding prosthesis. In summary, KHFAC-aided, electric-only voiding likely offers a viable solution for bladder care with low residual volumes post chronic-SCI.

II.7 Acknowledgements

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III. Chapter III

Direct Current Contamination

of Kilohertz Frequency Alternating Current Waveforms

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III.1 Abstract

Kilohertz Frequency Alternating Current (KHFAC) waveforms are being evaluated in a variety of physiological settings because of their potential to modulate neural activity uniquely when compared to frequencies in the sub-kilohertz range. However, the use of waveforms in this frequency range presents some unique challenges regarding the generator output. In this study we explored the possibility of undesirable contamination of the KHFAC waveforms by direct current (DC). We evaluated current- and voltage-controlled KHFAC waveform generators in configurations that included a capacitive coupling between generator and electrode, a resistive coupling and combinations of capacitive with inductive coupling. Our results demonstrate that both voltage- and current-controlled signal generators can unintentionally add DC-contamination to a KHFAC signal, and that capacitive coupling is not always sufficient to eliminate this contamination. We furthermore demonstrated that high value inductors, placed in parallel with the electrode, can be effective in eliminating DC-contamination irrespective of the type of stimulator, reducing the DC contamination to less than 1 µA. This study highlights the importance of carefully designing the electronic setup used in KHFAC studies and suggests specific testing that should be performed and reported in all studies that assess the neural response to KHFAC waveforms.

Key words

Kilohertz High Frequency Alternating Current, Nerve Block, KHFAC, HFAC, Direct Current, DC in KHFAC
Highlights

- Unintentional DC contamination can be a problem in KHFAC waveforms used for nerve block applications.
- Current- and voltage controlled systems cause different amounts of unintended DC.
- DC-blocking inline-capacitors are not always sufficient to eliminate the resulting offset voltages.
- Large value inductors as DC-shunts can reduce DC offset to less than 1 µA.
III.2 Introduction

The use of charge-balancing in electrical stimulation has been well-established as a means of significantly increasing the charge per phase that can safely be delivered to living tissue [13, 127, 128]. Typical off-the-shelf neural stimulators generate charge-balanced waveforms and often rely on capacitors in series with the electrode to filter out DC components [129]. Any current passing to the capacitors passes as a displacement current causing the build-up of an electric field inside the capacitor. Ideally, in order for signals to be charge balanced, every electric pulse in one direction (e.g. cathodic) through the load (e.g. electrode) is followed by a pulse of equal amplitude and opposite charge (e.g. anodic). The second pulse is often referred to as the “recharge pulse” and is only used to establish safety of the stimulation. However, capacitors are not perfect charge storage elements, and this can produce a slight imbalance in charge between the two phases of the stimulus.

The resulting differential charge causes small residual electric fields after each pulse pair inside the dielectric of the DC-blocking capacitors [129, 130]. In conventional neural stimulation, these fields remain practically unnoticed as the residual charge can dissipate through the capacitor’s internal impedance or is shorted out by the signal generator during the interval following the current pulses. This is because stimulation waveforms typically have a relatively long “off” period between each stimulus pulse. For example, a motor neuroprosthesis using a 200 µs balanced biphasic pulse delivered at 20 Hz (typical parameters) will have a 400 µs “on” period (two balanced 200 µs phases) followed by 49.8 ms of “off” time, for an on:off ration of 0.008 [131]. The “off” period allows time for any small charge imbalance between the two stimulus phases to dissipate before the next stimulus pulse is delivered, preventing any significant
accumulation of charge. In general, the use of a capacitively-coupled stimulus output stage reduces the charge imbalance to negligible levels [129].

However, recent discoveries in the field of neuromodulation have resulted in the use of progressively higher frequencies of stimulation, such as deep brain stimulation (~130 to 300 Hz) [132], spinal cord stimulation (600 Hz – 10k Hz) [133], and peripheral nerve block (1 kHz-50 kHz) [46, 71, 84, 134]. Of particular interest is the kilohertz frequency range, where the biphasic pulses are often delivered continuously, i.e. there is no “off” time during delivery of the stimulus. In this manuscript, we evaluate whether a small charge imbalance in the output of these continuous waveforms might accumulate on the electrode (or charge-balancing capacitors) and possibly result in unintended electrical, electrochemical, and physiological effects.

Most reports in the literature regarding the use of continuous waveforms in the kilohertz range do not provide a detailed description of the hardware used to produce the waveform [4, 86, 133-135]. In particular, there have been no reports of direct measures of the magnitude of the DC offset during delivery of these waveforms, except when used in cochlear stimulator applications [129]. A significant DC offset in the signal could produce unexpected or unwanted effects in neural tissue, including nerve conduction block [49], and neural damage [129], both of which can occur below 10 µA. Therefore, it is important to make direct and accurate measurements of the magnitude of the DC offset during continuous waveforms at kilohertz frequencies. In this manuscript we present methods for measuring the DC offset and demonstrate that any offset can be significantly reduced in an experimental setting with the use of high value inductors.
III.3 Methods

Calibration and verification of the output of electrical stimulation generators is generally accomplished by substituting a fixed value resistor (commonly 1 or 10 kΩ) for the electrode and measuring the voltage across the resistor. However, in the case of continuous waveforms, we considered that it was important to evaluate the effect of charge imbalance using real electrodes in saline. Our evaluation proceeded in two stages, using two different experimental configurations, as detailed below. The first stage involved measuring the voltage directly across the electrode during delivery of 1 kHz continuous waveforms. This stage utilized a relatively simple measurement technique, and could be used for screening a variety of waveform generators, including those with voltage-controlled and current-controlled outputs. We also used this measurement technique to evaluate one current-controlled generator at 10 kHz and 40 kHz (both continuous waveforms) to determine if there were significant differences in the electrode voltage and voltage offset as a function of frequency. The second stage involved measuring the electrode potential of each contact against a standard reference electrode (Ag/AgCl) to evaluate whether the voltage offsets observed in the first stage were sufficient to reach or exceed the water electrolysis window for the electrode under test. Current flow through the electrodes during, and immediately after, delivery of the continuous high frequency waveforms was also measured in this second stage. The electrode contact potential and current flow through the electrode were measured using one representative current-controlled and one voltage-controlled waveform generator, first testing the generator directly connected to the electrode and then testing the generator with additional capacitive coupling to the electrode.
In both stages of our assessment, high value inductors, placed in parallel with the electrodes and/or the stimulator, were used as a final comparison to the results obtained with standard high frequency generator configurations. High value inductors serve to shunt direct current (DC) in parallel to the electrode, thereby preventing the accumulation of any significant charge-imbalance across the electrodes, while simultaneously providing a large impedance (>200 kΩ) to alternating currents in the kilohertz range, essentially forcing the kilohertz-waveform through the electrode. To our knowledge, inductors have not been commonly utilized in this manner with high frequency waveform generators when used for physiological purposes. We evaluated whether our circuit configuration with inductors included could be used to accurately assess essentially “DC-free” high frequency waveforms for future physiological experimentation.

### III.3.1 Stage 1a: Determining unintentional DC voltage offset: Experimental setup

Two current- and two voltage-controlled waveform generator configurations, listed in Table 1, were tested using the experimental setup shown in figure III-1. A 1 kHz sinusoidal signal, generated by the stimulator being tested, was applied across a bipolar spiral cuff electrode [136] suspended in 0.9% NaCl saline. The platinum contacts each had a 1mm$^2$ surface area. The impedance of the electrode ($Z_E$) was 1.28 kΩ at 1 kHz for sinusoidal signals, measured with an impedance meter (Protek Z580; Protek Test, Englewood, NJ). Two inline-capacitors $C_1$ and $C_2$ of 1 µF each were placed between the generator and the electrode. Two inductors (with switches) of 8.2 H at 1 kHz were placed on both the stimulator and the electrode side of the capacitors. The high value inductors were achieved using the primary side of signal transformers with an open
secondary side. These offered a frequency dependent impedance of $Z_{1kHz}=241\,k\Omega$ for KHFAC signals and a low DC-impedance ($Z_{DC}=418\,\Omega$), effectively acting as a shunt for DC-currents.

Figure III-1: Electronic setup for Stage 1, used to measure DC-offset voltages caused by KHFAC waveforms. Switches $S_1$ and $S_2$ allowed to selectively add DC-shunting inductors $L_1$ and $L_2$ to the filter circuit. Offset was first determined with electrically floating Agilent TDS2004c oscilloscopes 1 and 2, then acquired using a Solartron SI1280B (electrode side) and NI DAQ USB-6258 substituting the oscilloscopes.

Four capacitor/inductor combinations were evaluated for their ability to minimize offset voltages across the electrodes. Data were sampled at 20 kHz. On the electrode side, a Solartron SI1280B (input impedance $Z_{in}>10\,\Omega$) was used to measure the voltage between the two electrodes in saline, and on the stimulator side one channel of a USB DAQ-6258 (National Instruments, Austin, TX), captured the Solartron output on a second channel with both channels set as floating vs. earth ground. These measurements were further independently confirmed using two oscilloscopes (Agilent TDS2004c, Agilent Technologies, Santa Clara, CA) with 100 M\Omega probes, both on battery-powered floating power supplies and used to monitor the potential on the stimulator output (scope1 in figure III-1) before the two inline-capacitors $C_1$ and $C_2$ as well as between the capacitors and across the electrodes in saline (scope2 in figure III-1). Each element of the signal generation and the measuring setup was checked for impedance...
loops to other elements and to earth ground with DC, 1 kHz and 10 kHz signals (Fluke 87V, Protek Z580) to ensure the absence of unwanted DC or AC crosstalk.

Table III-1: Voltage- and Current-controlled Waveform Generator Configurations tested.

<table>
<thead>
<tr>
<th>Generator</th>
<th>Digitimer DS-5</th>
<th>Keithley 6221</th>
<th>Wavetek 395</th>
<th>Wavetek 395</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Digitimer Ltd. Hertfordshire, England</td>
<td>Keithley Instruments Cleveland, OH</td>
<td>Aeroflex Inc. Plainview, NY</td>
<td></td>
</tr>
<tr>
<td>Current ctrl. settings</td>
<td>1 kHz, 2 mAmp</td>
<td>1 kHz, 2 mAmp</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Voltage ctrl. settings</td>
<td>-</td>
<td>-</td>
<td>1 kHz, 2 Vpp</td>
<td></td>
</tr>
<tr>
<td>Offset</td>
<td>0 V</td>
<td>0 V</td>
<td>4 V</td>
<td>0 V</td>
</tr>
</tbody>
</table>
III.3.2 Stage 1b: Determining unintentional DC voltage offset: Signal definition and analysis

The initial test waveform for stage 1 was a 1 kHz sinusoidal signal at 2 mA peak-peak (pp) for current- and 2 Vpp for voltage-controlled stimulators. The current-controlled stimulators were both operated without any deliberate DC-offset. The voltage-controlled stimulator was tested in two configurations, one without an intentional voltage offset (0 Vdc condition) and secondly with an intentional 4 Vdc offset to allow demonstration of the ability of the inductors to shunt even intentional offsets. The calibration of each signal generator was measured before use following the manufacturer’s instructions and found to be within the specified limit. This procedure first involved measuring the average voltage across a test resistor (commonly 10 kΩ), while the output was set to “on” with an amplitude of zero mA/volts (Keithley, Wavetek) or a shorted input (Digitimer). Secondly, the procedure was repeated, while the device was providing a 1 kHz waveform with an amplitude of one mA/volt. The measured offset voltages were found to be of similar magnitude, given the background noise, and always below the limit set by the device’s specifications. The offset current limits according to the manufacturers for the current-controlled waveform generators were 6 µA (at 2 mApp AC) for the Keithley 6221 and 5 µA for the Digitimer DS-5. The offset voltage limit specified by the manufacturer for the voltage-controlled waveform generator for signals of 2 Vpp AC was 20 mV.

Each trial consisted of at least ten seconds of no waveform output to ensure a stable baseline measurement, 30 seconds of waveform output, followed by 60 seconds with the output off to allow any accumulated DC-offset on the stimulator and on the electrode side to dissipate before the end of the trial. To ensure that all capacitive charge storage in the circuit was completely depleted before initiating each new trial,
electrodes and capacitors were shorted between trials. Twelve trials were recorded for each stimulator configuration: a) with capacitors only (both inductors disconnected), representing the typical situation that KHFAC waveforms are applied in literature; b) with only the inductor on the stimulator side connected; c) with only the inductor on the electrode side connected; and d) with both inductors connected. Data were analyzed using custom Matlab routines. The baseline voltage ($V_{\text{Base}}$) was defined as the average voltage of the last second before KHFAC waveforms were initiated. The maximum offset voltage was defined as the maximum difference from $V_{\text{Base}}$ during the time of KHFAC application. The absolute value of the average change in potential, rounded to one millivolt, is reported here. The offset voltage measured with and without the two inductors added were compared using the paired t-test ($t(DF)$) for each waveform generator.
III.3.3 Stage 1c: Determining the unintentional DC voltage offset as a function of KHFAC frequency

To assess if the observed DC-offset voltages across the electrode were a function of the applied KHFAC frequency, we repeated the measurement with sinusoidal signals at 1, 10 and 40 kHz, acquiring eight measurements for each frequency in randomized order. The effect of frequency was measured for one current-controlled waveform generator (Keithley 6221). The 10 kHz to 40 kHz range is often used for KHFAC nerve conduction block [46]. We used the capacitors-only configuration of the filter-circuit (figure III-1 and figure III-2) as we would expect the typical experimental setup investigating effects of KHFAC to include capacitors to block unwanted DC [66, 71, 84, 137, 138]. A Pt electrode was used for this experiment with an impedance of 1.57 kΩ @ 1kHz, 1.48 kΩ @ 10 kHz and 1.45 kΩ @ 40 kHz. Data were pre-processed as described above. Paired t tests were done on the voltages measured at 1, 10 and 40 kHz stimulation frequencies with eight samples in each group. Three specific measures were compared: 1) the maximum difference between the average voltage during KHFAC and the average baseline voltage, 2) the average voltage offset after 30 seconds of KHFAC application (right before stopping KHFAC), and 3) the voltage offset 30 seconds after having stopped KHFAC. Values measured for these three locations were averaged for a duration of 0.5 seconds to compensate for noise.
III.3.4 Stage 2  Measuring the amplitude of DC through the electrodes and the potential for irreversible reactions

The measurements made in Stage 1 provided an initial screening to identify voltage offsets across the electrode. However, in order to determine if these voltages are indicative of contact potentials that might result in irreversible chemical reactions [139] and result in actual current flow through the electrode, a second experimental stage was devised, as shown in figure III-2. The potential of each Pt-electrode was measured against a Ag/AgCl reference electrode using a Solartron SI1280B (input impedance >10GΩ). This potential was measured at each electrode contact independently. Following each measurement, any remaining charge between the electrodes was allowed to dissipate using the 8H inductors. Charge balance was verified by ensuring that the potential of each electrode had returned to the initial potential vs. Ag/AgCl that was acquired at the beginning of the previous trial. In addition to the potential measurements at each electrode contact, the DC current through the electrode was measured as a voltage across a resistor $R_m=1kΩ$ in series between one electrode and the filter-circuit (figure III-1 and figure III-2).

One current-controlled waveform generator (Keithley 6221) and one voltage-controlled waveform generator (Wavetek 395) were used to provide a 10 kHz sinusoidal waveform at 2 mApp and 2Vpp, respectively. With the current-controlled device it was possible to set the voltage compliance at different levels, and we tested two levels of
voltage compliance (10 V and 100 V peak) to determine if this affected the electrode potential and current measurement. The voltage-controlled generator had a voltage compliance of 10V. The filter-circuit was used in three variations: (a) no filter circuit (device output directly connected to the electrodes), (b) two capacitors in series with the generator output, and (c) two capacitors with two inductors. Repeated application of KHFAC was tested by delivering the KHFAC waveform for 30 seconds, waiting 30 seconds (without shorting the electrodes) and then repeating another 30 second delivery of KHFAC. The saline was not nitrogen purged; the access resistance of the Pt electrode vs. reference was 544 Ω. To assess variability between trials, the coefficient of variation was calculated as the ratio of the standard deviation to the mean of the voltage measured at each electrode’s with reference to the Ag/AgCl reference electrode. The coefficient of variation was also calculated for the current passing through the two Pt electrodes.
Ill.4 Results

The results demonstrated that significant voltage offsets can be recorded across the stimulator’s output (measured before the capacitors, location of scope 1 in figure III-1) and the electrodes in saline (location of scope 2) when using continuous sinusoidal waveforms, even when the electrodes are capacitively coupled. Figure III-3 shows an example of the voltage drift as a function of time using a current-controlled generator (Keithley 6221) with two in-line capacitors, measured on the stimulator side of the in-line capacitors (scope 1, figure III-1). A “charge-balanced” sinusoid at 1 kHz was delivered to a bipolar Pt electrode in saline. The average voltage drifts away from the zero-potential over the course of less than two seconds, indicating an increasing offset voltage. This offset voltage continued to drift until the sum of KHFAC signal amplitude and offset voltage reached the voltage compliance of the stimulator (10 V in this example, figure III-3). In parallel, a voltage offset across the electrode was measured that was smaller in amplitude but of the same polarity as the voltage measured on the stimulator side of the capacitors, as long as the KHFAC waveform was delivered. This voltage offset on the electrode side could result in a net DC through the electrode during the application of KHFAC waveforms. Stage 1 and stage 2 of the experiment analyzed these findings more into detail.
III.4.1 Experimental Results of Stage 1: Inline-capacitors alone may not prevent an unintentional DC voltage build-up between the electrodes for KHFAC

The average voltages measured before and after the filter-circuit (figure III-1) differed significantly when inductors were used in addition to the inline-capacitors, as shown in figure III-4). The average voltages measured for both of the current-controlled devices increased immediately after KHFAC signals were initiated when the capacitors only condition was used (figure III-4, 1st row, left). The average voltage offset measured at the stimulator output $V_{\text{Sout}}$ for the Keithley (K.) was 8,259±26mV and for the DS5 was 6,584±499mV, while the average offset voltages across the electrode were $V_{\text{Ein}}(\text{K.})=619±36$mV and $V_{\text{Ein}}(\text{DS5})=912±58$mV (figure III-5). After adding one inductor to the stimulator side of the setup (figure III-4, 1st row, 2nd from left), $V_{\text{Sout}}(\text{K.})$ was 4±0mV and $V_{\text{Ein}}(\text{K.})$ at 34±7mV. The results for the DS5 (not shown in figure III-4) were similar: $V_{\text{Sout}}(\text{DS5})$ was 3±1mV and $V_{\text{Ein}}(\text{DS5})$ was 1±1mV. Inserting one the inductor on the electrode side of the setup affected primarily the voltages across the electrode (figure III-4, 1st row, 2nd from right) with $V_{\text{Sout}}(\text{K.})$ at 8,367±19mV and $V_{\text{Ein}}(\text{K.})$ at 4±0mV. The results for the DS5 were similar with $V_{\text{Sout}}(\text{DS5})$ at 6,559±997mV and $V_{\text{Ein}}(\text{DS5})$ at 3±0mV. Voltage offsets were further reduced with the two inductor configuration. In this latter case, $V_{\text{Sout}}(\text{K.})$ averaged 4±0mV and $V_{\text{Ein}}(\text{K.})$ averaged 0±0mV (Figure 4, 1st row, right). Comparable results were obtained for the two inductor configuration using the DS5 with $V_{\text{Sout}}(\text{DS5}) = 4±2$mV and $V_{\text{Ein}}(\text{DS5}) = 0±0$mV.

The average voltages measured for the voltage-controlled configuration with no intentional offset (“0V” condition) increased only minimally after KHFAC waveforms were initiated when capacitors only were used to isolate electrode and stimulator (figure III-4, 3rd row, left). The average stimulator output voltage $V_{\text{Sout}}$ proximal to the capacitors was 7±31mV, while the average electrode input voltage at the electrodes $V_{\text{Ein}}$
was at 3±19mV (figure III-5). After adding the inductor on the stimulator side of the setup, \( V_{Sout} \) was 29±0mV with \( V_{Ein} \) at 17±0mV. The added inductor on the electrode side changed \( V_{Sout} \) to 33±2mV and kept \( V_{Ein}=0±0mV \). Using the two inductor configuration, \( V_{Sout} \) averaged 28±1mV and \( V_{Ein} 0±0mV \).

Figure III-4: Raw data examples for three different stimulator configurations
Configurations (top to bottom) tested with capacitors only (left), additionally one DC-shunting inductor on the stimulator side (2\textsuperscript{nd} from left), capacitors and one DC-shunting inductor on the electrode side (2\textsuperscript{nd} from right), and DC-blocking capacitors with two DC-shunting inductor (right column). Light grey indicates voltages measured on the stimulator side of the DC-blocking capacitors, while dark grey shows voltages on the electrode side. Average DC-voltages are indicated with a white line in the middle of the waveform. Time scale in the lower left.
The average voltages measured for the voltage-controlled configuration with intentional 4V offset rose similarly after KHFAC waveforms were initiated when capacitors only were used to separate electrode and stimulator (figure III-4, 2nd row, left). The average stimulator output voltage $V_{\text{Sout}}$ (W.395_4V) proximal to the capacitors was 4,054 ±4mV, while the average electrode input voltage at the electrodes $V_{\text{Ein}}$ (W.395_4V) was 774±24mV (figure III-5). After adding an inductor on the stimulator side of the setup, $V_{\text{Sout}}$ fell to 3.826±1mV while $V_{\text{Ein}}$ rose to 676±44mV.

**Figure III-5:** Comparison of offset voltages across the stimulator output and the electrodes in saline
Average offset voltages (mean±1sd) measured for each signal generator setup (I-contr.1 = Digitimer DS5, I-contr.2 = Keithley 6221, Wavetek 395 +4V offset, and 0V offset). Each waveform generator was tested with capacitors only (left bar), capacitors plus one inductor on the stimulator side (2nd bar from the left), capacitors plus one inductor on the electrode side (2nd from the right), and capacitors with two inductors (right bar).
The added inductor on the electrode side of the setup again affected primarily the voltages on that side with $V_{\text{Sout}} = 4.107 \pm 0.007 \text{mV}$ and $V_{\text{Ein}} = 2.0 \pm 0.007 \text{mV}$. When two inductors were part of the configuration, $V_{\text{Sout}}$ averaged $3.875 \pm 1.0 \text{mV}$ and $V_{\text{Ein}} = 2.0 \pm 0.007 \text{mV}$, demonstrating that even with a significant intentional offset, the inductors were able to nearly eliminate the offset.

The offset voltage measured for each stimulator setup was significantly reduced, when two inductors were added to the two capacitors for three of the four cases: Digitimier (I-contr.1) ($912 \pm 17 \text{ mV}; t(11) = 54.9, p < 0.0001$), Keithley (I-contr.2) ($619 \pm 10 \text{ mV}; t(11) = -60.5, p < 0.0001$), Wavetek+4Voffset (V-contr.1) ($773 \pm 7 \text{ mV}; t(11) = -109.2, p < 0.0001$). The difference was not significant for the offset-free voltage controlled stimulator: Wavetek+0Voffset (V-contr.2) ($3 \pm 6 \text{ mV}; t(11) = -0.5, p = 0.66$). The numbers reported are mean and standard error of the difference.

We observed that immediately after the KHFAC waveforms were turned off following 30 seconds of continuous application, there was a slowly decaying offset voltage that took between a few seconds to tens of seconds until it reached the pre-

KHFAC equilibrium voltage. This effect was especially observable in current-controlled configurations using capacitors only (top left tile in figure III-4 and in figure III-6). When we tested if disconnecting the KHFAC generator at the end of the 30 second delivery would make this effect disappear, the same slow decay of offset voltages was observed upon disconnection of the generator, indicating that the effect was not caused by a discharge of parasitic capacitance inside the generator.
The comparison of KHFAC waveforms for 1, 10 and 40 kHz showed similar voltage offsets for all tested frequencies (figure III-6, upper section). In each case, the average voltage difference between electrodes rose until the stimulator indicated that the voltage compliance was reached (figure III-6, left bars correspond with the maximum difference from baseline in the example voltage trace in the lower section). Once the voltage compliance had been reached, the voltage difference slowly declined until the end of KHFAC application to values of about half (middle bars in top section, corresponding with voltage offset measured at the end of KHFAC delivery) of the initial maximum voltage measured. Right after KHFAC delivery ended, the offset voltage polarity reversed with a voltage spike (arrow in figure III-6) and began to decline from the opposite direction towards the initially measured baseline (figure III-6, example in lower section and “30 sec post”). The differential voltages measured 30 seconds after KHFAC application were found to all be above 200 mV, irrespective of frequency. Only the difference in measured voltage between 1 and 10 (0.097±0.018 V; \(t(7) = -15.325, p <0.001\)) and between 1 and 40 kHz (0.097±0.012 V; \(t(7) = -23.185, p <0.001\)) was statistically significant. The difference in voltage measured at 10 and 40 kHz was not statistically significant (0.013±0.004 V; \(t(7) = 0.002, p >0.998\)). The current
corresponding with the voltage spike (arrow), measured in separate trials under the same conditions, was passing though the electrode as what appeared to be a capacitive discharge, with typical amplitudes in the milli-ampere range and durations of 200 to 400 µs.
III.4.2 Experimental Results of Stage 2: KHFAC offset voltages indicate current flow in micro-ampere range; offset voltages remain after KHFAC stops

The case where the output of the generator was connected directly to the electrodes (no capacitors and no inductors), a continuous DC of 4 to 5 µA was measured for the entire duration that KHFAC waveforms were applied (figure III-7 C). The voltage potential for each contact compared to the Ag/AgCl reference electrode continued to drift further from the starting potential as long as the KHFAC was delivered. Further, 30 seconds of off time was not sufficient for the potentials to return to baseline levels and therefore the second session of 30 second KHFAC delivery resulting in even higher potential peaks relative to baseline. In this particular condition, the electrode potential was well outside of the water electrolysis window for Pt, and, during the second delivery of KHFAC was outside the water window for nearly the entire 30 second period of delivery.

For the capacitors only case, we observed that the majority of the unintentional DC was injected during the first few seconds after the initiation of the KHFAC waveform (figure III-7 A and B, lower current traces), until the stimulator’s voltage compliance was reached (based on the compliance indicator on the device). We measured DC amplitudes in the range of 4 to 5 µA for approximately two seconds when the voltage compliance was set to 10 V (figure III-7 B). This same current level was delivered for up to ~12 seconds when the voltage compliance was set to 100 V (figure III-7 A). The relationship between higher voltage compliance and longer DC injection was direct but non-linear, whereas the DC amplitude remained at the same value with different compliance settings. Once the voltage compliance of the stimulator had been reached, current flow through the electrode dropped to <1µA for the duration of the KHFAC delivery.
The electrode potential against the Ag/AgCl reference climbed during the first few seconds of KHFAC application and reached its peak when the stimulator’s voltage compliance was reached (figure III-7, upper voltage traces). The time between KHFAC start and reaching the maximum voltage against Ag/AgCl in figure III-7 corresponded with the time required for the initial rapid rise in voltage between electrodes in figure III-6. The open circuit potential (OCP) of the Pt electrode changed after the first application of 30 seconds of the KHFAC waveform. Repeated application of KHFAC further increased the OCP in a non-linear fashion (voltage traces in figure III-7). In contrast to the cases where no capacitors and no inductors were used (figure III-7 C) the offset voltage measured post KHFAC delivery was found to be of opposite polarity to the voltage measured during KHFAC application. A voltage spike, in polarity being the same direction as post-KHFAC offset, accompanied the end of KHFAC delivery, along with a small discharge current through the resistor in series with the electrodes (current trace in figure 7 III-A). The injected charge during the first few seconds of KHFAC delivery were orders of magnitude larger than the charge measured at the end of KHFAC delivery in the opposite polarity.

For the case with two capacitors with two inductors, we observed that the injected DC amplitude remained <1µA throughout the trials, even with repeated application (figure III-7, D). The voltage traces for current-controlled KHFAC delivery did not increase when KHFAC delivery started or stopped; the trace for voltage-controlled KHFAC had an increase of less than 0.1 V. No large voltage or current spikes were observed, the only change being a constant change in OCP over time, and this happened regardless of whether the KHFAC was delivered or not. The stimulator did not reach the voltage compliance. The coefficient of variation between trials (n=10 for each condition)
was 3.5% or less for all measurement variables across all generator and shunt conditions.

Figure III-7: Electrode potentials during KHFAC block measured against the Ag/AgCl reference

Potentials were measured against the Ag/AgCl reference in saline with current (A, B, C and D) and voltage (D) controlled stimulators. Shown is the low-pass filtered mean of the sinusoidal 10 kHz waveform (compare to lower tier of fig. 6). In each figure, the upper traces show the measured voltage of one of the electrodes for two times 30 sec. application of KHFAC followed by a 30 sec pause. The lower trace in each figure shows the direct current measured through a resistor $R_{m}$ in series with the electrodes. Inline-capacitors without inductors as filter-circuit (figure III-2) are used for acquisition of (A) and (B), difference there being the voltage compliance set at the current-controlled stimulator, 100 V for (A) and 10 V for (B). No inline-capacitors and no inductors were used to acquire (C) with a current-controlled setup and 10 V compliance. The combination of two inductors for a current-controlled setup is combined with a voltage-controlled setup only using capacitors in (D). Stimulators used were the Keithley 6221 for current and the Wavetek 395 for voltage-controlled experimentation. The saline was not nitrogen purged. Please note different voltage scales in A, B, C, and D chosen for visualization and how repeated application of KHFAC in A, B, and C increased the offset post application vs. Ag/AgCl. IR correction = 2.5mV = 4.5µA*544Ω.
III.5 Discussion

Our results demonstrate the importance of careful and accurate measurement of the output of KHFAC generators to be used in physiological applications. In particular, we demonstrated that commonly used generators produce a DC offset in the output that could have measurable effects on the physiology and on the electrode itself. Given the paucity of detailed descriptions of the measurement of the output of KHFAC waveform generators in the literature, the primary contribution of this manuscript is to present the experimental evidence that these measurements are critical to any interpretation of the neural response to KHFAC waveforms. As we saw unintentional DC contamination with both, current- and voltage-controlled systems, we suggest measuring the output of the experimental setup to ensure the absence of DC components. Although we only investigated KHFAC amplitudes of 1 mA and 1 V respectively, we did not investigate how higher or lower amplitudes of the KHFAC waveform impact the presence of unintended DC. The chosen amplitudes (with electrode impedances of ~1kΩ for the tested frequencies) allow the qualitative assessment of developing DC contamination in both, current- and voltage-controlled systems. We suggest that researchers investigate their specific setup at the intended KHFAC amplitudes and frequencies used for nerve block [46]. At a minimum, measurements such as those described in Stage 1 should be reported, and preferably direct measures of electrode potential and current flow, such as those described in Stage 2, should be performed and reported whenever KHFAC waveforms are used.

The two current-controlled waveform generators produced KHFAC signals with a DC-contamination of <5µA, which was within their stated specification. However, this current was sufficient to produce voltage potentials at the electrode contacts that,
when measured against an Ag/AgCl reference electrode, indicated levels that were outside the water electrolysis window and thus could potentially result in irreversible chemical reactions at one or both electrode contacts. Therefore we do not advise applying KHFAC waveforms without, at a minimum, capacitively de-coupling the electrodes from the waveform generator.

The addition of “charge-balancing” capacitors on the stimulator output limited the duration over which the electrode remained at these elevated potentials (figure III-7 A and B). We observed that the direct current was delivered with a preferential polarity and resulted in changing open-circuit potentials, which escalated with repeated delivery of the KHFAC. This probably indicates the occurrence of faradic reactions during or at the end of KHFAC delivery [139].

The amount of the initial DC delivery was influenced by the stimulator’s voltage compliance and whether KHFAC waveforms were applied repeatedly. Generally speaking, the higher the voltage compliance, the longer direct current was measured going through the electrodes. The increase in voltage potential between Pt electrodes and Ag/AgCl reference that results from repeated KHFAC application could not be due to the reversible charging of the Pt-electrolyte interface, but instead indicates the repeated occurrence of irreversible chemical reactions.

The current amplitude of the discharge spike measured when KHFAC application ended was generally in the range of 1 to 5 mA for a duration of ~200 µs. The size of the discharge spike was directly related to the duration over which the DC offset had been injected, and indirectly related to the time between reaching the stimulator’s voltage compliance and the end of KHFAC delivery. The off-spike itself may cause irreversible chemical reactions and is worthy of further investigation.
With the **application of two inductors**, one on each side of the DC-blocking capacitors, the measured DC-offset at the electrode remained well below 2 mV, independent of the choice between voltage- or current-controlled generators and even with an additional +4V offset using the voltage-controlled generator. The two inductors effectively provided a shunt for DC in parallel with the electrode as well as in parallel with the stimulator, while offering a large enough HF-impedance to minimize the impact on the amplitude of the KHFAC waveform. The application of one inductor on either side of the DC-blocking capacitors had an intermediate effect between using two or no inductors, usually lowering the measured offset voltage on the side to which it was applied. The specific DC-contamination of the any specific signal generator can help to decide if one inductor in addition to capacitors is sufficient or if two inductors and two capacitors should be utilized.

The **voltage-controlled** KHFAC waveforms showed significantly smaller DC-contamination as long as the generator was properly calibrated so that the DC offset was set to 0 V. We tested the configuration with an intentional 4 V offset to demonstrate that the increasing DC-voltage offset observed *over time* across the electrode is caused by a DC-offset on the stimulator side. This underscores the importance of careful testing of this equipment prior to use. Specifically, it should not be assumed that off-the-shelf voltage-controlled generators have a 0 V offset. Therefore, when these devices are used for KHFAC block, the calibration procedure and DC offset through a resistive load should also be reported in the experimental methods. We further advise the use of inline-capacitors to block unintentional DC. While inductors may not be required with voltage-controlled KHFAC generators, including inductors for testing purposes may offer a secondary level of safety by providing an additional passive DC shunt.
Inline-capacitors were unable to prevent rising DC-voltages between electrodes if an offset-voltage was present at the generator output. This effect was independent of the choice between voltage- or current-controlled stimulators and was observed at all tested frequencies. DC delivery occurred during the first few seconds following KHFAC initiation, and again with the opposite polarity at the end of KHFAC delivery when capacitors were used to de-couple the electrodes from the stimulators. Thus, most of the charge initially delivered was reversed fairly rapidly at the end of KHFAC delivery, and the entire period may be considered “charge-balanced”. However, if KHFAC is delivered continuously for many hours, this implies that the “recharge phase” is delayed for many hours. As we have demonstrated, even 30 seconds of KHFAC delivery resulted in reactions at the electrodes that could not be adequately reversed by the charge recovery, and, in fact, the charge recovery overcompensated for these effects, driving the electrode potential in the opposite direction (see figure III-7). It may be that the kinetics for the reactions at the electrode could not support complete reversibility for tens of seconds. The rise in offset voltages post KHFAC delivery, measured against a reference electrode after repeated KHFAC deliveries, indicate the occurrence of faradaic reactions. Rising offset voltages across the inline-capacitors indicated that the capacitors did provide DC-block once the voltage compliance of the generator was reached and, secondly, that all KHFAC generators produced small offset currents as contamination to the KHFAC waveforms.

The delivery of continuous DC to neural tissue can result in damage. Common damage thresholds are 2 µA for the cochlea [13, 140-143], 3 µA for the central nervous system [144, 145], and <10 µA for damage in the peripheral nervous system [49, 128]. These levels are primarily based on histological observation, which was not a part of this
study. Given the offset potentials reached during and remaining after KHFAC delivery that are reported in this manuscript, there remains the possibility that this offset could produce neural damage. However, we cannot infer damage directly based on the in-vitro studies reported here and further chronic in vivo study would need to be conducted in order to determine any deleterious effects.

It is important to note that unintended DC offsets delivered during KHFAC testing may have an unexpected effect on neural structures even if the electrode potential stays within the water window. Specifically, DC can reversibly block nerve conduction at current levels as low as 6 µA in some experimental conditions [49]. The unintended use of mixed-signal waveforms (AC + DC), particularly in nerve conduction block research, make it difficult to assess the effects of KHFAC as reported in the literature [46]. To achieve more clarity about the specific waveforms used in this area of research, we recommend that future publications should specify the methods used to measure the output of the signal generator. At a minimum, measurement of the average offset voltage between the two electrodes in-vitro or in-vivo with a floating oscilloscope provides an initial assessment of the magnitude of the unintended DC. If this measurement indicates that there is no offset voltage forming between the electrodes over the time of KHFAC application, then the risk of unintended DC is limited to ground loops between the stimulator and outside connections, a problem easily addressed using a stimulus isolator. However, it should be noted that a stimulus isolator alone, providing a current-controlled signal with or without capacitive de-coupling of the output, is no guarantee that DC is eliminated from the KHFAC signals, as we have shown. Ultimately, a more pragmatic approach may be preferable: the comparison of the output of the KHFAC generator under test with and without a large value inductor
placed across the electrodes. If the DC is negligible, there will be no difference in the neural response between these two conditions. Large value inductors for kHz AC signals can be obtained in a small package volume using iron-core transformers with an open secondary side. As the inductors used in our experiment came with a package volume of about 1 inch³, we are not proposing to use these for an implantable device, but a research tool. We are currently investigating the efficacy of inductors that provide about 1.3 Henry while needing a volume of about 1 cm³, in order to get closer to providing DC-free KHFAC waveforms with implantable waveform generators. A future publication will address if different inductance values and electrode impedances affect the efficacy of our approach to reduce unintended DC contamination with large value inductors that function as a DC-shunt.
III.6 Conclusion

Ensuring signal integrity is an essential step to evaluating the safety and efficacy of KHFAC waveforms used for neural stimulation or nerve block. We have demonstrated that unintentional DC-contamination is very likely for KHFAC signals generated by off-the-shelf stimulators and should be filtered accordingly to prevent unintended effects. The application of DC-blocking inline-capacitors alone is not always sufficient to prevent DC-contamination from delivery through electrodes and into neural tissue. DC-contamination can be virtually eliminated by adding two DC-shunting (high value) inductors to the DC-blocking capacitor circuit. This configuration was sufficient to reduce the voltage offset to less than 2 mV in all cases. This study highlights the importance of careful measurement of electrode potentials when using KHFAC. We recommend that specific measurement of DC-contamination be conducted and reported in all studies of the effect of KHFAC waveforms on neural structures. Such reporting will significantly improve the ability of the scientific community to assess the results that are being presented.

III.7 Acknowledgements

This work was supported by the NIH NINDS R01-NS-074149 and DK077089, NIBIB R01-EB-002091, the Department of Veteran’s Affairs VA RR&D B6685R, the Case Coulter Translation & Innovation Partnership and the Fulbright Foundation G-1-00001 Scholar Grant. The authors would like to thank Tina Vrabec and Jesse Wainright for their assistance.
IV. Chapter IV

Combined KHFAC+DC nerve block without onset
or reduced nerve conductivity post block

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IV.1 Abstract

**Background:** Kilohertz Frequency Alternating Current waveforms (KHFAC) have been shown to provide peripheral nerve conductivity block in many acute and chronic animal models. KHFAC nerve block could be used to address multiple disorders caused by neural over-activity, including blocking pain and spasticity. However, one drawback of KHFAC block is a transient activation of nerve fibers during the initiation of the nerve block, called the onset response. The objective of this study is to evaluate the feasibility of using charge balanced direct current (CBDC) waveforms to temporarily block motor nerve conductivity distally to the KHFAC electrodes to mitigate the block onset response.

**Methods:** A total of eight animals were used in this study. A set of four animals were used to assess feasibility and reproducibility of a combined KHFAC+CBDC block. A following randomized study, conducted on a second set of four animals, compared the onset response resulting from KHFAC alone and combined KHFAC+CBDC waveforms. To quantify the onset, peak forces and the force-time integral were measured during KHFAC block initiation. Nerve conductivity was monitored throughout the study by comparing muscle twitch forces evoked by supra-maximal stimulation proximal and distal to the block electrodes. Each animal of the randomized study received at least 300 seconds (range: 318 to 1563s) of cumulative DC to investigate the impact of combined KHFAC+CBDC on nerve viability.
**Results:** The peak onset force was reduced significantly from 21.9±4.7N to 0.7±1.1N with the combined block waveform (p<0.001). The area under the force curve was reduced from 13.3±12.9Ns to 0.6±0.4Ns (p<0.01). No change in nerve conductivity was observed post application of the combined KHFAC+CBDC block relative to KHFAC waveforms.

**Conclusion:** The distal application of CBDC can significantly reduce or even completely prevent the KHFAC onset response without a change in nerve conductivity.

**Key words:**

Kilohertz High Frequency Alternating Current; Nerve Block; Onset response; HFAC; KHFAC; Direct Current; charge balanced DC; CBDC
IV.2 Introduction

Electric nerve conduction block can provide a controlled interruption of motor and sensory nerve activity [46, 70, 91]. Applications of motor block include the reduction of spasticity in movement disorders, such as blocking urethral sphincter spasms for bladder control following spinal-cord injury or localized muscle spasms after stroke or dystonia [84, 146]. Applications of sensory nerve block include reduction of chronic pain and modulation of autonomic activity by blocking activity in sympathetic or parasympathetic fibers [91]. Kilohertz-frequency alternating current (KHFAC) waveforms can provide this form of electric nerve block on demand, but cause an undesirable transient burst of action potentials upon initiation of the block waveform called the onset response [147]. This onset response can lead to intense muscle contractions (see figure IV-1 A) and pain, limiting clinical applications of this technology. Various attempts have been made to eliminate the onset response, including electrode modifications, optimizing KHFAC waveform parameters as well as adding other forms of nerve block such as cooling or direct current. However, these techniques are either impractical for clinical implementation or were unable to completely suppress the onset response [51, 147-149].

Direct current (DC) waveforms, in contrast to KHFAC, can achieve an electric nerve block without an onset response (figure IV-1 B) [49]. This is accomplished by gradually ramping the amplitude of the DC waveform until a full nerve conduction block is achieved, a technique that was investigated for KHFAC waveforms as well, but without success [148, 150]. However, the disadvantage of DC nerve block is that unidirectional charge injection into the electrode-electrolyte interface will cause irreversible faradic reactions. These reactions could cause the dissolution of the
electrode, generation of charged free radicals or platinum salts, or the evolution of gaseous hydrogen or oxygen [139]. The byproducts of the faradic reactions can affect neural conductivity post block over short durations and nerve damage when applied for longer periods of [49, 69, 139, 151].

In this study, we tested the feasibility of using a charge balanced direct current (CBDC) waveform that incorporates a first (e.g. cathodic) phase to provide DC nerve block and a secondary (e.g. anodic) phase to achieve charge balance. The conceptual basis for the CBDC block is that the charge delivered during the block phase is stored in the double-layer capacitance of the electrode-electrolyte-interface. If the injected charge is limited to less than 90% of the maximum that could be stored in the double-layer, all the current injected in the several seconds of the nerve block phase would be recovered over the duration of the charge balancing phase. By using a high surface area platinum-black (platinized platinum) electrode, this CBDC waveform can provide a short-term (<30 seconds) nerve block without resulting in changes in nerve conductivity following block [152]. Potentials measured between the block electrode and a Ag/AgCl reference electrode during the application of the CBDC-block did not reach or exceed the limits of the water window. As a result, it can be concluded that oxygen evolution and hydrogen evolution are not occurring at the DC block electrode. Typical Pt-black electrodes of 3 to 9 mm² active surface area were fabricated to have charge injection capacities of tens of milli-coulombs. In order to provide DC nerve block, cathodic current in the range of 1 to 2 mA was applied. Using these values, the DC could be delivered for tens of seconds while maintaining the total charge within 90% of the water window. Complete DC nerve block was achieved with this approach and the onset response that can result from the initiation of the CBDC was avoided by slowly ramping the DC to the
blocking value [153]. Unfortunately, because of the necessity for a recharge phase to balance the charge injected during the nerve block phase, the CBDC nerve block alone did not allow for a continuous nerve block beyond a few tens of seconds.

Ackermann et al. demonstrated that it is possible to block the motor onset activity with the use of a second platinum electrode, distal to the KHFAC block electrode, delivering a brief DC block [69]. However, after only a few repetitions, the DC electrode caused a permanent reduction in the conductivity of the nerve. The use of high charge capacity electrodes in combination with the CBDC waveform allow for a longer DC delivery without causing a reduction in the conductivity of the nerve. Using this approach, the CBDC waveforms could provide a nerve conduction block for a

Figure IV-1: Comparison of onset forces from KHFAC and from CBDC block alone
Nerve conduction block was achieved with KHFAC (A) and charge balanced DC (B) waveforms. While an intense neural firing causing a strong muscle contraction (onset response) is characteristic for nerve block achieved with KHFAC waveforms, ramped DC waveforms allow for a nerve conduction block without resulting in any additional activity when appropriately ramped. Note that the recharge phase (seconds 20 to 70 in B) does not block nerve conduction but dominates the overall duration of the CBDC block trial. PS and DS indicate force comparison from proximal and distal stimulation to assess nerve conductivity post block. Both block waveforms do not change the nerve conductivity post block.
duration long enough to capture the KHFAC onset response, combined KHFAC+mpDC nerve block would be possible: by positioning the mpDC block electrode distally to the KHFAC electrode, the motor component of the KHFAC onset response could be captured and nerve block would become possible, that could be initiated without an onset-response, while offering the potential to be maintained for hours by relying on the KHFAC waveform. The objective of this study was to explore the feasibility of using this combined KHFAC+CBDC approach to provide a nerve block that minimizes the onset response and can be maintained for long periods of time without affecting nerve conductivity post block. We specifically wanted to address the following questions: (1) To what extent can the onset-response be reduced with the combined block waveform? (2) How stable is the block? (3) Does the application of the combined block waveform have an effect on nerve conductivity following hundreds of seconds of cumulative DC delivery? (4) Could the secondary recharge phase cause a break/reversal of the block, e.g. due to cross-talk between the two different waveforms?
IV.3 Methods

The complete study was conducted on a total of eight, adult, Sprague-Dawley rats. An initial feasibility study, conducted on four of the animals, was used to assess whether a combined KHFAC + CBDC nerve block could be achieved repeatedly and allowed the optimization of the electronic setup. Based on a power analysis ($\alpha=0.05$), done using the data acquired during the initial feasibility study, a sample size of four was chosen for the experiment described at hand with the goal to demonstrate a significant reduction of the KHFAC induced onset response using CBDC block, while retaining the conductivity of the electrically blocked nerves post block. All procedures were conducted following prior approval by the institutional animal care and use committee at Case Western Reserve University, Cleveland, Ohio, USA.

IV.3.1 Surgery

The surgical procedure has been described in detail previously [47, 69, 154]. Briefly, the animals were anesthetized with intra-peritoneal injections of Nembutal (phenobarbital sodium). One of the hind legs was shaved and an incision made along the lateral aspect of the leg and thigh. The biceps femoris was reflected, and the sciatic nerve exposed from 1 cm lateral to the spine to its distal branching into the tibial and common peroneal nerves. The tibial nerve was exposed to its branches to the gastrocnemius muscle. The sural and common peroneal nerves were crushed using a hemostat to prevent motion artifacts from the tibialis anterior muscle. The gastrocnemius-soleus muscle complex was dissected, and the calcaneal (Achilles) tendon severed from its distal attachment. The tibia was fixed to the table using a clamp, and the calcaneal tendon attached to a force transducer (Entran, Fairfield,NJ; resolution 0.005 N) with 1-2 newton of passive tension using a toothed clamp.
IV.3.2 Electrodes

Four electrodes were placed on the sciatic nerve, two each for stimulation and block (figure IV-2). The proximal (PS) and distal (DS) stimulation, as well as the KHFAC waveforms, (figure IV-3) were delivered using bipolar platinum J-cuff electrodes as described in Foldes et al. [155]. The CBDC block was applied using a monopolar electrode of high charge injection capacity with a return path through a needle electrode, placed sub-cutaneously in the animal’s back. The CBDC block electrode was manufactured by electrochemical platinum deposition on a platinum foil substrate (Pt-black) of 3*3 mm$^2$ surface area. The charge injection capacities of the electrodes used for the experiments were 27mC, 41 mC, 21 mC and 20 mC for subjects 1-4, respectively. The charge injection capacities were measured prior to the experiments as described in Vrabec et al. [156, 157], and were high enough to allow CBDC-block for up to a few tens of seconds [153]. The PS electrode was positioned on the most proximally exposed part of the nerve, and the DS electrode close to the gastrocnemius muscle. Both of the block electrodes were placed in between the PS and DS cuffs, with the KHFAC electrode proximal to the CBDC electrode.

![Figure IV-2: Electronic setup and animal preparation](image)

Four electrodes were acutely implanted on the rat’s sciatic nerve. These electrodes were, from left to right, proximal stimulation (PS), KHFAC block, DC block and distal stimulation (DS). The forces evoked by the gastrocnemius muscle were measured to determine nerve activation and block. The waveform supplied to each of the electrodes was provided by a stimulator with its own dedicated power supply providing a completely floating setup. The LC(DC) filter circuit, two capacitors with DC-shunting inductors, between the KHFAC generator and HF electrode ensured that only KHFAC waveforms without unintended DC contamination could reach the HF electrode.
IV.3.3 Electrical Waveforms

A Grass S88 stimulator (Grass Technologies, West Warwick, RI) was used to deliver PS at 2 Hz and, on a second isolated channel, DS at 1 Hz (figure IV-3). Both the PS and DS waveforms were current-controlled, 20µs long pulses that caused supramaximal twitches. The KHFAC block waveform was a charge-balanced, voltage controlled, sinusoidal, 20 kHz signal generated by a Wavetech 395 (Fluke, Everett, WA). A DC filter circuit [117] was placed between the KHFAC generator and electrode to prevent the accumulation of unintended DC charges in the electrode-electrolyte interface (figure IV-2). This DC filter circuit consisted of two capacitors of 1 µF each and two inductors of 8.2 H each. The amplitude of the KHFAC waveform was chosen to be about 125% of the block threshold in order to achieve KHFAC-only onset-response durations of five seconds or less [48].

Figure IV-3: Stimulation sequence and expected force measurement over time
The 2Hz PS (grey) and 1Hz DS (black) stimulation signals were easily differentiated from each other and provide information on the nerve conductivity before, during and after the combined KHFAC+DC block. The DC block amplitude was ramped cathodic (more negative) at the beginning for 2s, followed by a plateau or variable duration (2-10s) and subsequently a down ramp back to zero. The cathodic DC block was followed by the anodic recharge of the electrode-electrolyte-interface providing charge-balanced DC block over time. Note that the ramped cathodic DC is expected to initially cause partial block (as indicated here by partially reduced PS twitch forces).
The current-controlled, cathodic block waveform for CBDC block was provided by a custom-built, battery-powered, voltage-to-current converter (DC-offset <1µA) that received a voltage-controlled signal from a USB DAQ-6258 (National Instruments, Austin, TX). This voltage-controlled signal was designed before each trial on a custom-built program (Labview, Austin, TX), allowing independent customization of the shape of the cathodic block phase and the anodic charge-balancing phase. Both phases of the signal had adjustable on- and off-ramps with durations of up to several seconds in order to achieve the DC-block without nerve activity caused by the block onset or an anodic break at the end [49]. In some cases, an extra inflection point was used in the middle of a ramp (figure IV-4, figure IV-5) to achieve a full DC block without onset-activity. The amplitude of the plateau of the cathodic phase of the DC block was chosen as the current needed to reduce the maximum KHFAC onset-response force to levels below the forces evoked by PS. Once this amplitude had been reached at the end of the DC ramp, it was held constant as a plateau current for 2-10s in order to block the KHFAC onset activity. The CBDC waveform timing parameters were chosen to ensure that the net charge injected into the electrode-electrolyte interface was below 90% of the charge injection capacity of the DC block electrode. Once the cathodic block had been successfully established, the anodic amplitude of the recharge-phase was chosen to be 10% of the cathodic plateau current or a maximum of 0.2 mA to avoid anodic activation of the nerve. This low current level in combination with on- and off-ramp durations of 2 to 8 seconds ensured the absence of new stimulation effects during the recharge phase, caused by either virtual cathodes, anodic break or waveform mixing due to potential KHFAC crosstalk. The charge injected during the anodic phase was set to 100% of the formerly injected cathodic charge. A combination of voltage controlled KHFAC and current-controlled CBDC block was chosen because it resulted in the least amount of
crosstalk between the electronic waveform generator circuits during the initial feasibility studies.

The maximum duration of the cathodic DC block phase was limited by the electrode’s charge-injection capacity, and the current needed to achieve a successful mitigation of the KHFAC onset response. We tried to minimize the DC block duration to (1) shorten the DC recharge time (which could exceed 100 seconds, taking up the majority of the block trials duration) and to (2) remain as far from the maximum injectable DC charge limit (<=90% of max). We further minimized the required duration of the cathodic-DC phase by selecting KHFAC block parameters (frequency, amplitude, electrode contact spacing etc.) such that the onset-response would be no more than five seconds long. We knew from prior experiments that the charge-injection capacity of the DC electrode declined over the duration of the experiment. This is mostly due to bending during implantation and explantation, followed by careful mechanical cleaning under running water, as the Pt-black has less than ideal mechanical properties.
Figure IV-4: Comparison of onset forces with KHFAC vs. combined KHFAC+CBDC block
Forces caused by the onset response at the initiation of KHFAC-only nerve block (A) can be significantly reduced with an additional, distally placed, CBDC block (B). Proximal stimulation (PS) was completely blocked by both forms of nerve block. The arrow indicates where the on-ramp rate is increased to reach the plateau current faster.
IV.3.4 Data acquisition, reduction and analysis

All data were sampled at 1 kHz using a custom designed software interface running a data acquisition card (NI6258, National Instruments, Austin, TX) [47, 49, 69]. Three waveforms were acquired: (1) the force due to the contraction of the gastrocnemius muscle evoked by electrical stimulation, and the voltage signals corresponding to the (2) KHFAC and (3) DC block waveforms.

Activation and saturation thresholds for the PS and DS electrodes were acquired at the beginning of the experiment and verified every half hour to ensure complete activation of the nerve with the supramaximal PS and DS twitch stimulation. With both stimulation electrodes fully activating the nerve, any difference between forces resulting from PS and DS could be related to either residual conduction block or after all block waveforms had ended, to changes in nerve-conductivity [46]. Thus, the PS/DS-ratio provides one method to assess acute changes in nerve viability for the duration of the experiment. Recording the PS/DS-ratio at the end of every trial provided a record of nerve viability over time.

Block thresholds were determined individually for the CBDC and KHFAC waveforms using suppression of twitches evoked by PS as the measure of block outcome. The optimal parameters for the combined KHFAC+CBDC block waveform were determined as follows: the CBDC block was first initiated with a slow ramp to prevent onset activity, then the slope of the ramp was increased to reach the CBDC block plateau (= DC block threshold) as quickly as possible without causing onset activity. The plateau was held for at least 0.5 seconds prior to the initiation of the KHFAC waveform to stabilize the DC block. The KHFAC waveform was not ramped, but immediately initiated at the previously determined threshold amplitude. The first combined block
trial where the peak force at KHFAC onset was smaller than the PS twitch forces measured in the same trial was considered the first successful trial. To determine the optimal CBDC-block plateau amplitude, the value was then increased in subsequent trials, until the peak-onset force could not be distinguished from noise, or until the electrode’s charge injection capacity was reached. When the amplitude was limited by the charge injection capacity, the onset forces were above the noise threshold and therefore appear as outliers in the combined-block data (figure IV-6).

Figure IV-5: Example of three side effects seen with sub-optimal waveform parameters. Black arrow indicates KHFAC onset peak force being just above the force for a PS twitch because the DC plateau was not high enough. Grey arrow indicates part of the phase II onset not being blocked because the DC block was not long enough and the overall KHFAC onset was longer than five seconds. Dashed black arrow indicates small onset firing caused by the first part of the CBDC on-ramp being too steep.
Each block of trials consisted of 5 combined KHFAC+CBDC trials and one KHFAC-only trial. Within each block, the trials were randomized in a pre-determined fashion, that was different for each animal. The order in which KHFAC and the combined block waveforms were applied was randomized, providing a unique sequence set for each subject prior to the experiment. If the health of the animal deteriorated, requiring an early termination, a ratio of three combined block trials to one KHFAC trial was performed. The ratio of five to one was chosen as an optimum between maximizing the number of combined block trials, while collecting sufficient KHFAC trials, within the time available (duration per animal: 5.5, 6.5, 6, and 8.5 hrs). The end-of-experiment criteria for the first three animals was the acquisition of at least six KHFAC only (control) and 20 to 30 combined block (KHFAC+CBDC) trials, while having applied the CBDC waveform for at least 300 seconds of cumulative DC delivery time during the KHFAC+DC block trials. We chose the value of 300 seconds because the PS/DS ratio in the majority of the animals tested by Ackermann et al. had dropped to 50% after 30 seconds of cumulative unbalanced DC indicating an acute nerve damage [69].

Data reduction was done offline using custom-written Matlab routines. For each application of the block waveform the following data were computed: peak twitch force both before and after block application with PS and DS (only after block) stimulation; peak force and area under the onset-response curve; ratio of twitch force evoked by PS and DS (PS/DS); cumulative time of cathodic DC delivery; and the total charge delivered. Statistical analysis was performed using JMP (SAS, Cary, NC). Normality of data was verified using the Kolmogorov Smirnov test. Since the measures of onset response were not normally distributed, the Mann-Whitney U test (U(DoF)), this test is equivalent to a one way ANOVA on ranks) was used to assess the effect of block type and subject on
peak force and area under the force-time curve during the onset response. Bonferroni correction of the p value was used to avoid an increase in type I errors due to the multiple comparisons. Additionally, a paired t-test was used to compare the onset response during the combined block trials to the average twitch force evoked by PS forces recorded in the same trial pre-block.

To assess the effects of repeated DC stimulation on nerve conductivity, a regression model was built with subject, trial number, cumulative charge injected and cumulative time of DC application as predictor variables; the PS/DS ratios during the combined-block trials were determined to be normally distributed and hence, the least squares regression method was used. The following statistics are reported for the model in the results section: the total variance in the data explained by the model (R2), the F statistic and associated p value for the model, the coefficient estimate for each predictor variable and the corresponding p value. The two sample t test(t(DoF)) was used to compare the PS/DS ratio during the KHFAC and combined block trials.
IV.4 Results

All three forms of electric nerve block, CBDC and KHFAC alone as well as the combined CBDC+KHFAC block were successfully achieved in every animal of the initial feasibility group of four and the secondary group of four subjects (figure IV-4) we conducted the randomized comparison study in. The combined KHFAC and CBDC block significantly reduced the onset response resulting from the KHFAC block alone (figure IV-6 and figure IV-7). Neither the application of CBDC nor the combination of CBDC and KHFAC waveform significantly affected nerve conductivity, even after several hundred seconds of block application (figure IV-8).

IV.4.1 The onset response was significantly reduced with combined block waveforms

The choice of block type had a significant effect on peak-onset force (Mann-Whitney U(1)=10.8; p<0.0001) and the area under the curve (U(1)=10.6; p<0.0001) (figure IV-6). Peak-onset force reduced from 6.8 N (range: 3.5-21.9 N) with KHFAC alone to 0.54 N (range: 0.18-0.86N) with combined DC and KHFAC, and the mean area under the curve reduced from 20.73 Ns (range: 18.6-26.5 Ns) to 0.45 Ns (range: 0.2-0.7 Ns) (Fig 6). Both the peak-onset force (U(3)=17.1; p=0.0007) and the area under the curve (U(3)=19.5;p=0.0002) varied between the subjects as well.
Figure IV-6: Reduction of the onset force across all animals comparison to a PS twitch force (far right). A statistically significant reduction was achieved in each one of the animals as well as across all animals combined (* p<0.001).

Figure IV-7: Reduction of the area under the onset-force-over-time graph. Comparison across animals and to PS twitch (far right). A statistically significant reduction was achieved in each animal as well as across all animals combined (* p<0.01, ** p<0.05).
The peak-onset force during the combined-block trials was also significantly smaller than a muscle twitch resulting from supramaximal sciatic activation (5.1±1.0 N vs. 0.7±1.1 N; t(163) =49.8; p<0.0001; figure IV-6 far right). However, in comparison to the peak onset force, the area under the force curve for a muscle twitch resulting from supramaximal sciatic activation was slightly but significantly larger than the onset response during the combined block (0.6±0.4 Ns vs. 0.2±0.1 Ns; t(163) = -10.6; p<0.0001; figure IV-7 far right). The results from a paired comparison of the means can be seen in Table 1. Same DC and KHFAC amplitudes led to reproducible and stable reduction of the onset during the experiment. Furthermore, we were able to achieve a complete reduction of the onset force in each one of the eight animals using the combined CBDC+KHFAC nerve block.

Table IV-1: CBDC significantly reduced onset peak force and onset area.

<table>
<thead>
<tr>
<th>animal</th>
<th>Onset peak Force (N)</th>
<th>Onset Area (Ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KHFAC alone</td>
<td>KHFAC +CBDC</td>
</tr>
<tr>
<td>1</td>
<td>25.3±6.7</td>
<td>1.6±2.0</td>
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<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.01</td>
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<td>23.6±4.5</td>
<td>0.7±0.7</td>
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<td></td>
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<td>p&lt;0.01</td>
</tr>
<tr>
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<td>0.4±0.4</td>
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<tr>
<td></td>
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<td>p&lt;0.05</td>
</tr>
<tr>
<td>4</td>
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<td>0.4±0.7</td>
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<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>all</td>
<td>21.9±4.7</td>
<td>0.7±1.1</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>
IV.4.2 Application of the block waveforms did not affect nerve conductivity

The PS/DS-ratio was not statistically different between the combined (0.99±0.03) and the KHFAC (0.99±0.04) nerve block (t(1)=-0.52; p=0.6). Regression analysis indicated that the four predictors explained 53% of the variance ($R^2 = 0.53$, $F(6,157)=29.6$, p<0.0001). It was found that only the subject variable ($\beta_1 = 0.027$, $\beta_2 = 0.017$, $\beta_3 = 0.03$ and $\beta_4 = 0.01$ p<0.0001) significantly contributed to the variability in the PS/DS ratio.

![Graph A](image1.png)

![Graph B](image2.png)

Figure IV-8: Nerve conductivity did not decrease during the combined block application
Nerve conductivity for all animals shown vs. cumulative time spent in the cathodic portion of the CBDC waveform (A) and shown vs. cumulatively injected cathodic DC charge during combined KHFAC & CBDC block (B). The PS/DS-ratio, describing the nerve conductivity post block between proximal and distal stimulation electrode, did not significantly change from 1 or between animals, indicating no undesirable effects for nerve viability. Underlying PS/DS-data is the same in A and B, difference is depiction vs. DC block time and depiction vs. injected DC charge. The grey area in (A) depicts the duration of one minute, which is the time after which the nerve conductivity all animals in Ackermann et al.’s study had stopped (PS/DS-ratio = 0) [13].
whereas trial number ($\beta = 0.0004$, $p<0.74$), cumulative DC charge ($\beta = 0.0002$, $p<0.14$) and time ($\beta = 0.000$, $p<0.06$) did not. The average cathodic DC plateau time without ramps was $2.5\pm1.5$ s (range 1 to 7 s, median 2.6 s). The overall average cathodic DC delivery time (table IV-2), including ramps, was $16.5\pm9.7$ s (range 6 to 33.3 s, median 11.5 s). The injected charge during cathodic DC block for all combined block trials was $11.5\pm2.5$ mC (range 6.4 to 18.8 mC, median 11.3 mC, n=163, N=4).

IV.4.3 Stability of block parameters

The CBDC average plateau amplitude during the combined waveform block trials was $2.3\pm0.4$ mA (n=164). The average KHFAC amplitude, used for both, the KHFAC-only and the combined KHFAC+CBDC trials, was $6.8\pm1.6$ V (n=211). The average parameters across all successful combined block trials for each animal can be found in table IV-2. The optimal parameters, found after an average of twelve initial trials per subject, are listed in table IV-3.

Table IV-2: Average parameters across all successful combined CBDC+KHFAC block trials and cumulative over time.

<table>
<thead>
<tr>
<th>animal</th>
<th>CBDC block parameters (per trial)</th>
<th>Values for the duration of the experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amplitude of cathodic plateau (mA)</td>
<td>Duration of entire cath. phase (s)</td>
</tr>
<tr>
<td>1</td>
<td>1.7±0.3</td>
<td>10.9±1.0</td>
</tr>
<tr>
<td>2</td>
<td>2.5±0.1</td>
<td>6.7±0.9</td>
</tr>
<tr>
<td>3</td>
<td>1.8±0.3</td>
<td>24.3±0.3</td>
</tr>
<tr>
<td>4</td>
<td>2.4±0.1</td>
<td>22.2±4.3</td>
</tr>
</tbody>
</table>
**IV.4.4 Optimal waveform parameters**

The main difference between the parameters for each research subject were the ramp and plateau durations for the CBDC block. In order to prevent an onset during the initiation of the CBDC block for subjects three and four, the cathodic ramp required an additional inflection point (table IV-3). This provided a ramp with two inclines: A longer first, more shallow incline to reach a current value of -0.8mA (subject 3) and -0.4mA (subject 4), at which point the nerve was partially blocked, was followed by a shorter and much steeper secondary incline to quickly reach the complete cathodic CBDC block plateau. Similarly, subject 4, required a two-inline off-ramp of the cathodic phase to prevent onset-forces during combined CBDC and KHFAC application (table IV-3).

**Table IV-3: Optimal parameters defining the KHFAC and CBDC waveform for each animal.**

<table>
<thead>
<tr>
<th>animal</th>
<th>Amplitude (V)</th>
<th>Plateau amplitude (mA)</th>
<th>Duration cathodic on-ramp (s)</th>
<th>Duration cathodic plateau (s)</th>
<th>Duration cathodic off-ramp (s)</th>
<th>Duration Recharge on-ramp (s)</th>
<th>Duration Recharge anodic plateau (s)</th>
<th>Duration Recharge off-ramp (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0</td>
<td>-1.8</td>
<td>6.0</td>
<td>2.6</td>
<td>2.0</td>
<td>1.0</td>
<td>47.5</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>8.0</td>
<td>-2.5</td>
<td>2.0</td>
<td>3.0</td>
<td>1.0</td>
<td>1.0</td>
<td>44.3</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>-1.5</td>
<td>20.0 to -8 mA</td>
<td>2.0</td>
<td>2.0</td>
<td>4.0</td>
<td>85.2</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>8.0</td>
<td>-2.4</td>
<td>16.0 to -4 mA</td>
<td>1.0</td>
<td>3.0 to -6 mA</td>
<td>11.3 to 0 mA</td>
<td>4.0</td>
<td>63.5</td>
</tr>
</tbody>
</table>

**IV.4.5 Reduction in charge injection capacity**

We observed a reduction in the charge injection capacity in every animal of the randomized study: The value of the electrode used in animal 1 changed from 27 mC before the experiment to 3.7 mC after the experiment. The reduction in animal 2 was less severe, from 41 mC to 36 mC; the value for animal 3 post-experiment is unknown as the electrode broke during or after explantation, before it could be cleaned. The change observed in animal 4 was from 20 mC prior to 3 mC post experiment.
IV.5 Discussion

This study demonstrated the feasibility of an electric nerve block that combines the strengths of KHFAC and charge balanced DC waveforms. We were able to initiate a combined KHFAC+CBDC nerve block in all eight animals without an onset response and showed in four animals that a statistically significant reduction of the onset force and area can be achieved. Once initiated, the electric nerve block can maintained for minutes, if not hours, without a reduction in nerve conductivity following the block. From a clinical standpoint of view, a complete reduction of the KHFAC onset is optimal, which was achieved in every animal once proper block parameters were found. A reduction of the onset peak force to levels below a muscle twitch marked a major improvement in comparison to the normal forces measured during KHFAC block onset. In comparison, muscle twitch stimulation is routinely done in the clinic to measure nerve conductivity velocities and is well tolerated by the subjects. Thus, a combined nerve block approach could provide one path to translate nerve block technology from bench to bedside, providing a tool in addition to neuro-stimulation to interface with the nervous system and modulate activity for better patient benefit.

We used an approach similar to Ackermann et al. [69], who attempted to reduce or completely eliminate the KHFAC onset response with a distally applied, conventional DC block. The main differences between the approach used in this study and the one tested by Ackerman et al are: the charge balanced DC waveform, the Pt-black electrode of high charge injection capacity used to provide the CBDC block, and the use of an LC(-DC) circuit to limit unintentional DC contamination of the KHFAC waveform and to prevent crosstalk between the KHFAC and DC-electrode [117]. The combination of the CBDC waveform and the Pt-black electrode, unable to provide a continuous nerve block
longer than a few tens of seconds, did allow flexibility in the design of the CBDC waveform. If necessary, the current ramps at the initiation of the CBDC waveform could be over ten seconds long, until the current amplitude for the comparatively short DC plateau was reached. To block the first phase of the KHFAC onset response [147], cathodic DC plateaus as short as two seconds were sufficient. In order to block the second phase of the onset response, the ramped reduction of the cathodic block phase was chosen to be longer than the combined phase I and II onset response. If the CBDC block duration was too short, and the KHFAC onset response had not ended, we observed a transient muscle contraction of approximately twitch force amplitude that began as the cathodic phase of the CBDC block was being decreased to zero. The transient forces could be eliminated by increasing the CBDC block plateau or ramp duration by a few (2-5) seconds. We did not note a reduction in the forces evoked by PS post-block relative to the pre-block condition.

The PS/DS-ratio was used as a measure of any change in nerve conduction that persisted post block. In the study done by Ackermann et al. [69] the PS/DS ratio, in 3/5 animals, had reduced by 50% after 30 seconds and the nerve was completely damaged in all animals after 60 seconds of cumulative DC delivery. The nerve conduction did not recover even after 30 minutes post DC block application [69]. In the present study, the PS/DS ratio remained consistent and no trends were observed in the PS/DS ratios over the duration of the experiment. Across all animals, the coefficient of variation for the PS/DS ratios was approximately 3%.

One of the concerns of using the anodic phase to balance the cathodic charge in the electrode-electrolyte interface was that crosstalk from the KHFAC to the DC-block electrode could cause the block to fail at the recharging DC-electrode. We did observe
this effect during the preliminary phase of the experiment when we used two current-controlled waveform generators, one for the CBDC and the other for the KHFAC block. This effect was eliminated by using voltage-controlled KHFAC waveforms. Additionally we used the DC shunt circuit to minimize unintended DC contamination of the KHFAC electrode [117]. An added benefit of using the DC shunt circuit was that the high frequency contamination of the CBDC electrode was minimized.

It was important to keep the anodic current below 0.2 mA. While higher currents allowed shorter anodic recharge phases, the limit of 0.2 mA was determined in preliminary experiments as a value that limited stimulation during the block application, either being caused by virtual cathodes distally to the CBDC electrode, anodic break for off-ramps of too-short durations or waveform mixing due to potential KHFAC crosstalk. The crosstalk was further minimized by ensuring that each generator was electrically floating, resulting electrically in a star connection at the acute nerve preparation, the only point where all four electrodes came together. Although the DC filter circuit helped to minimize HF crosstalk, there was still the potential for capacitive and inductive coupling between cables connecting the electrodes to the waveform generators. Keeping the anodic recharge current at the said levels allowed a reasonable short recharge phase without nerve activation during the application of both block waveforms.

There was no need to change the DC block amplitude once the optimal current for blocking the onset response had been identified. Once set, optimal block parameters were generally kept unaltered for the remainder of the experiment, resulting in combined onset forces within the formerly mentioned median and confidence intervals. The only change we investigated briefly in subject 4 was to shorten the on- and off-
ramps of the cathodic phase in order to achieve the block plateau faster. After seeing that this caused a small force-onset again, we went back to the original parameters. We did furthermore strive for 100% charge balance by injecting the same amount of charge anodically that had formerly been injected cathodically for the onset-block. We did not investigate how an incomplete charge-balancing would affect the nerve conduction post block and see this as an important point of interest for future studies with the goal to minimize the time necessary for the anodic recharge period.

We do not know what caused the loss in charge injection capacity for the Pt-black CBDC block electrodes and assume that the loss was a cumulative effect due to bending of the electrode during implantation and explantation, deposition of proteins on the electrode surface, and the rinsing of the electrodes under water. We do know that Pt-B is not mechanically stable enough for chronic use. A future chronic study would require the use of a more durable material.

We are also aware that these results need to be reproduced in a study with chronically implanted electrodes for KHFAC and for charge balanced DC block, which would have to be repeatedly applied and followed up with a histological analysis of nerve tissue at and adjacent to the nerve block electrode sites, in order to provide strong evidence that this combined nerve block approach does indeed not negatively impact nerve viability. Nevertheless, our goal was to provide evidence that a subsequent chronic study using a similar approach would have a high probability for success with regard to efficacy (electric nerve block without an onset response) and safety (no change in nerve conduction in an acute setting), which could then be confirmed histologically.
IV.6 Conclusions

This study demonstrated that an electric nerve block can be initiated without an onset response and maintained for minutes, if not hours, without a decrease in nerve response following block. We were able to achieve a reduction of the KHFAC onset response using a distally placed charge balanced DC (motor) block in eight out of eight animals. A randomized control study, involving four of these eight animals, showed statistically significant differences for both onset force height and area, for each animal as well as across the four animals as a group. The success of this proof-of-concept study promotes that a chronic implementation should be conducted. The goal of this chronic study would be to replicate these feasibility results and to gather safety data with the aim to verify that neither the CBDC block alone nor the combination of KHFAC+CBDC waveforms causes a reduction in nerve viability or results in histological changes.

IV.7 Acknowledgements

This work was supported by the NIH NINDS R01-NS-074149 and the Fulbright Foundation G-1-00001 Scholar Grant.
V. Chapter V

Summary Conclusion
V.I Revisiting the Specific Aims

Each project had two specific aims. These were chosen to direct the functional outcome of each study. An evaluation thereof will help to assess the impact of the work done.

Chapter II

Electric Bladder Voiding using KHFAC Pudendal Nerve Block in an awake chronic SCI model

Central Hypothesis:

Bilateral KHFAC pudendal nerve (PN) block can significantly increase bladder voiding in awake animals in the chronic stage of SCI compared to sacral bladder drive alone.

We achieved bladder voiding percentages that were significantly higher with bilateral PN block for awake, chronic stage SCI preclinical subjects with chronically implanted and utilized electrodes for KHFAC block delivery. Three animals were maintained for up to 12 consecutive days on low residual bladder volumes relying only on KHFAC-aided, sacrally driven bladder voiding. Voiding volumes achieved with the aid of KHFAC were stable and significantly larger than volumes achieved with any of the controls (manual expression or sacral drive alone).
**Aim 1:** Demonstrate that KHFAC PN block can provide reproducible reduction in sacrally evoked EUS spasms with stable block current thresholds.

We were able to show that sacrally evoked EUS pressures were significantly reduced throughout the study and that the reduction was achieved reliably over time (figure II-2). The high flow rate measured during anesthetized and awake bladder voiding confirmed the unimpeded voiding from a secondary perspective and thereby the complete bilateral PN block. The block current thresholds remained stable over time (figure II-3) with current amplitudes needed for 25 kHz waveforms being significantly higher than the currents from 12, 8 and 6 kHz. The frequencies utilized during awake bladder maintenance (8 and 10 kHz) were chosen for (1) providing a short KHFAC onset response to prevent fatigue of the EUS muscle and (2) requiring minimal current amplitudes. It is likely that the choice for current-controlled KHFAC waveforms contributed to the stability and reproducibility of complete block across animals and experiments of the study, even though the electrode impedances changed from experiment to experiment, within the experiment of one day as well as between animals. As bladder pressures were not influenced by the addition of bilateral KHFAC nerve block (table II-1), increase in voiding volume must be the result of reduced outlet resistance due to reduction of sacrally evoked EUS spasms.
Aim 2: Provide daily bladder voiding using only a combined KHFAC PN block and sacral bladder drive for three or more consecutive days.

We were able to achieve this aim in three out of five animals of the second stage of the study. These three animals showed sufficiently high evoked bladder pressures to allow undergoing SCI. Each of the three subjects that underwent SCI and subsequent daily electric bladder maintenance was maintained for at least three days, a time period that would be fatal, if no or insufficient bladder voiding would have been provided. One of the animals was maintained for 3.5 days, a second one for 8 days and a third one for 12 days (figure II-5). Without the addition of KHFAC PN block, electric (control) bladder voiding provided voiding percentages of about 40%, compared to 80 to 90% and more with the aid of KHFAC nerve block. The latter was independent of the type of bladder stimulation (intermittent or continuous), whereas the control voiding percentages differed significantly, showing the continuous bladder drive was inferior to the Brindley-approach of 2 seconds on, 2 seconds off (figure II-4) which provided higher voiding volumes due to the temporarily low EUS pressures during the stimulation pauses of the intermittent sacral drive.
Chapter III

Direct Current Contamination of Kilohertz Frequency Alternating Current Waveforms

Central Hypothesis:

Off-the-shelf waveform generators producing KHFAC signals for nerve block supply waveforms with a KHFAC and a direct current (DC) component, the latter being able to charge the electrode/electrolyte surface boundary capacitance to values outside the water window.

We were able to show that the potential of an electrode relative to a Ag/AgCl reference can reach to levels of 0.8 V and beyond both, with and without the application of inline-capacitors meant to prevent the passage of harmful DC currents. We were able to show that the tendency of the offset-voltage to reach these levels increases with higher voltage compliance and that the actual value is specific to the waveform generator.

Aim 1: Measure amplitudes of unintended DC offset caused by KHFAC application with different filters between signal generator and electrodes.

We investigated the effect of four different passive filter combinations, defined by capacitive and inductive components, on the measured offset voltage at both, the stimulator output and across the electrodes, in saline. We were able to show that inline capacitors were in general able to reduce unintended DC contamination of the signal. However, the capacitors alone were insufficient to prevent voltages at the electrode leaving the water window.
Aim 2: Demonstrate a filter that reduces the unintended DC below levels considered safe for chronic implementations.

We were able to show that a filter built of capacitors and large value inductors can reduce the DC current through the electrode to levels below 1uA. At the same time, offset voltages measured between different pairs of work electrodes or the work and AgAg/Cl counter electrode showed the positive impact that this technology could have in the future.
Chapter IV

Combined KHFAC+DC nerve block without onset or reduced nerve conductivity post block

Central Hypothesis:

A combined KHFAC+CBDC waveform approach can provide an electric nerve block with a significantly reduced onset response, while showing no significant effect on the nerve conductivity post block in three or more animals.

We were able to show that the KHFAC onset response could be reduced significantly with a distally placed CBDC block (figures IV-4, IV-6 and IV-7). Nerve conductivity post block remained stable throughout the study (table IV-2). A randomized trial study was conducted successfully in four preclinical subjects. Complete reduction of the onset response was achieved in both, the four animals of the randomized trial study as well as the four animals of the initial feasibility stage for a total of eight out of eight animals.

Aim 1: Demonstrate the feasibility for an electric no-onset nerve block using the combined CBDC+KHFAC approach with Hi-Q electrodes.

High-charge-injection-capacity electrodes, made from a material of high surface roughness, allowed us to use charge-balanced (multi-phased) direct current (CBDC) nerve block to completely inhibit action potential conduction of the sciatic nerve. This CBDC block was used successfully in eight out of eight preclinical subjects to demonstrate an electric nerve block without an onset response that could be sustained for minutes or hours by relying on the KHFAC component for continuous long-term
application. The reduction in maximum onset force and onset-force-area-over-time using the two waveforms was shown in four animals (table IV-1 and figures IV-4, IV-6 and IV-7). Block parameters were stable over the duration of the study (table IV-2 and IV-3).

**Aim 2:** Show that repeated CBDC+KHFAC block does not decrease nerve conductivity post block.

The combined CBDC+KHFAC block included a cathodic block component, provided by the CBDC electrodes, for the duration of greater than six (and up to 24) seconds for each combined block trial. A cumulative cathodic block duration of greater than 300 seconds was achieved in each of the four subjects of the randomized control study, the longest duration being 1563 seconds, or 26 minutes (table IV-2 and figure IV-8). There was no significant change in nerve conductivity over time or across subjects, as functionally captured by the stable PS/DS ratio (figure IV-8).
V.2 Lessons learned

The three projects presented in the document at hand lead to a number of “take-away messages” which are especially of interest from a translational standpoint of view.

Chapter II

Bladder management post chronic-stage SCI using KHFAC pudendal nerve block

1. Kilohertz frequency alternating current (KHFAC) nerve block can be utilized regularly and repeatedly with chronically implanted electrodes to provide a nerve conduction block with stable parameters and without changes to nerve conduction post block.

2. Bilateral application of KHFAC nerve block to the pudendal nerve can prevent spasms of the external urethral sphincter, thus facilitating bladder voiding in sedated subjects with chronic stage spinal cord injury without the need for dorsal rhizotomy.

3. Bilateral application of pudendal KHFAC nerve block and bilateral sacral drive can provide means for a proof-of-concept bladder management device in awake animals post chronic stage SCI without the need for dorsal rhizotomy.

4. Pure KHFAC nerve block without DC contamination does not cause an acute depression in nerve conduction post block in the same form that pure DC or mixed DC and AC block waveforms can result in.

5. Filtering unintended DC components using large-value inductors (secondary-side open transformers) was essential to long-term stability of PN activation thresholds and post-block neural conductivity.
6. Preventing crosstalk between channels providing waveforms for neural stimulation and block is essential for successful PN block and KHFAC-aided bladder voiding. Crosstalk resulted primarily from inductive and capacitive coupling between either the waveform generators or the electrode leads due to the nature of kilohertz-frequency waveforms being used (“radio-frequency”). Most signal generators only specify the output vs. ground impedance for direct-current signals (aka DC impedance vs. GND) in the MΩ-range, while their kilohertz-frequency impedance can be below 10 kΩ. Frequency-selective LCR-meters measuring with frequencies used for nerve stimulation (a 100µs pulse-width = 5kHz pulse) and block (5 to 25 kHz) allow the correct determination of the stimulator’s output impedance vs. ground (e.g. Protek Z-580, Agilent U1733C). Output impedances in the lower kΩ range allow a partial KHFAC waveform crosstalk that can evoke unintended neural excitation on block and stimulation channels, interfering with the intended objective.

7. Similar to acute findings, the combination of high frequencies and high waveform amplitudes are more likely to achieve short-onset KHFAC nerve block with chronically implanted electrodes.

8. Anecdotal: Monopolar KHFAC nerve block is possible with chronically implanted electrodes (unpublished data). Block amplitudes were higher than those for bipolar and tripolar approaches. We achieved unidirectional PN activation by stimulating one of the outside contacts of a cuff electrode and providing a bipolar KHFAC block on the other two contacts within the same electrode. This information might be of interest for judging the localized specificity of the KHFAC nerve block, as the distance between each of the three contacts inside the 1 mm diameter cuff were only 2 mm.
Chapter III - Preventing unintended DC in KHFAC waveforms for neural stimulation and block

9. Off-the-shelf kilohertz frequency waveform generators are likely to provide signals with unintended DC contamination that can lead to significant voltage offsets during and post KHFAC application, regardless if a capacitive isolation is used between electrodes and waveform generator.

10. Large inductors can function as DC shunts and prevent the build-up of DC offset voltages.

11. The potentials measured from KHFAC Pt electrodes vs. Ag/AgCl references were able to reach (beyond) the limits of the water window during application of KHFAC nerve block when no DC-shunting inductors were present.

12. Preliminary, unpublished acute data: Applying current-controlled KHFAC using only capacitors proved to affect nerve recovery directly after the end of KHFAC waveform application (unpublished data; more: see appendix). Muscle-activity at the end of KHFAC delivery was atypical (off-spike), see Tai [86], and nerve conductivity later often reduced, first seconds, later minutes (see [4]).

13. Anecdotal: We were able to use the LC(ΔC) circuit to achieve charge-balanced waveforms from monophasic kHz-waveforms. Using the monophasic GRASS S88 output from each of the two (monophasic) output channels, we were able to provide two biphasic, charge-balanced KHFAC waveform channels.

14. Anecdotal: The same LC(ΔC) circuit approach was used to achieve quasi-sinusoidal, charge-balanced KHFAC waveforms from a Joule-Thief circuit (see fly-feedback circuit), allowing for low-supply-voltage, high-output-voltage-kHFAC-waveforms. The circuit diagram is explained in the appendix.
Chapter III - Combined KHFAC+DC nerve block without onset or reduction in nerve conduction post block

15. The KHFAC onset-response can be mitigated or even completely eliminated with the aid of a new form of multi-phased DC nerve block that does not cause a change of the nerve conduction post block in an acute setting.

16. Preventing crosstalk between both nerve block channels is essential to successfully achieve onset-free combined CBDC+KHFAC nerve block. Easiest way to prevent crosstalk was to use current-controlled CBDC and voltage controlled KHFAC nerve block. Battery-powered KHFAC via Joule-Thief circuit worked too.

17. Anecdotal: Although we were able to achieve anodic and cathodic CBDC block alone, we noticed that the CBDC block was more stable with a cathodic block phase and anodic charge balancing phase (unpublished data); hence all the combined CBDC+KHFAC nerve block trials were acquired with this setting.

18. Anecdotal: Tightly fitted KHFAC cuff electrodes did provide shorter KHFAC onset responses than cuff electrodes that were not as tightly fitted. Might be related to the distribution of the electric field during application of block waveforms and that a tight fit provided a more homogenous field. In fact, loosely fit KHFAC cuff electrodes did repeatedly lead to onset responses with a typical phase I (high, sharp), followed by an atypical phase II that was not just very long and wide but also continued at force amplitudes of a nerve twitch for as long as KHFAC was on.

19. Anecdotal: Adding saline to the nerve preparation or inside the KHFAC block electrode can result in increasing (doubling!) of the KHFAC block threshold. Likely a result of good conductivity of saline, providing a parallel current path for KHFAC waveforms outside the nerve (hence not aiding in the block effect).
V.3  Impact on the field of neural engineering and nerve block

There are a number of neurological disorders that result in localized dysfunction of neural circuits, causing chronic pain, motor impairments or abnormal postures of limb and torso. Some examples include equinovarus foot deformations in cerebral palsy and post stroke [32, 158-161], increased flexor muscle tone in stroke [160, 161] and dystonia [35, 162-165], and bladder dysfunction in SCI patients [118, 166-168]. Common to these impairments is a lesion in the central nervous system (CNS), which causes an imbalance in the activity of the musculoskeletal system. When the cause for imbalanced muscle activity cannot be treated by restoring circuitry inside the CNS, minimization of symptoms becomes the primary mode of reestablishing muscle control and improving quality of life [33, 99, 125, 168, 169]. Often, this is achieved by blocking communication between the CNS and the target organ in the periphery. This conduction block for the peripheral nervous system (PNS) can be realized by surgical [170], chemical [34, 171-173], or electrical intervention [3, 69, 82, 150].
V.3.1 Implications for research and the development of electric nerve block

The presented work has implications for the development of nerve block applications and the way nerve block studies are conducted. The most important contribution is the realization of potential unintended DC components in KHFAC (and potentially other) waveforms used for neural stimulation, modulation and block. Every electric device operates within a certain range of tolerance with typical tolerances for off-the-shelf stimulators being around 0.2%. The problem with this tolerance is that 0.2% of 5 mA is 100µA. Five milli-Amperes is a typical current amplitude for KHFAC nerve block as observed in the chronic PN block study. If 100µA are delivered continuously to an electrode-nerve interface until the stimulator’s voltage compliance is reached, then neural depression or damage will likely result and become especially obvious in a chronic study. While this might not be observed in acute experiments, it is impossible to predict if repeated delivery of small levels of DC currents in the 100µA range will cause long-term neural damage as it did in the first five preclinical subjects of the PN study.

The second most important contribution was to find a simple solution to counter the accumulation of DC offset voltages by using large inductances as a DC shunt in addition to the inline capacitors. Using the primary side of instrumentation transformers with ferrite core and open secondary side allowed for these high-value inductances (from 1 to 8 Henry) while requiring a comparatively small footprint and volume (from 1cm³ to 1in³) for an implantable stimulator in the future. Adding the DC-shunting inductors allowed us to proceed from the first initial stage of the chronic PN study to the secondary stage as activation thresholds remained stable and, later, histology confirmed
the absence of tissue and electrode discolorations seen in feasibility studies without the use of DC-shunting inductors.

The realization that the combination of DC-blocking capacitors and DC-shunting inductors (short: LC(DC) filter) can provide charge balanced waveforms on the output side when receiving monophasic waveforms on the input side might prove valuable for more future applications. Detailed information can be found in the appendix.

Further contributions were a battery-powered, low-supply-voltage KHFAC waveform generator, detailed in the appendix, as well as other means to reduce or prevent effects of unintended DC in KHFAC waveforms. A list of filed invention disclosures and IP can be found in the appendix.
V.3.2 Necessity for clean KHFAC waveforms

With a better understanding of unintended DC effects caused by neural stimulation and block waveforms, scientific literature can be interpreted from a different perspective. Recent publications show contradicting results regarding effects directly following the application of KHFAC block waveforms. Liu et al. [174] report results from an acute frog sciatic nerve study, showing that neural conduction post block was depressed and that it took about 60 seconds post KHFAC application for compound action potentials (CAP) to return to similar values as before the application of block waveforms. They used stimulation isolators, current-controlled waveforms and no capacitors (as well as no DC-shunting inductors).

Ravid and Prochazka used direct current for controlled nerve ablation and showed that the persistent block effect of DC is likely due to a change in pH and not sodium channel over-activation [74]. As their goal was to achieve DC nerve block with lasting effects post block, relatively high DC amplitudes of 3 mA were used. Several groups have demonstrated DC block using currents as low as tens of micro-amperes, Bhadra et al [49], Shepherd [13, 140], Petruska [175], Hurlbert [144] and others. The chronic studies showed that persistent block effects were found with waveforms that did not cause a persisting nerve block in acute experiments. These findings correlate with our observation that a study setup used in acute studies by Boger et al. [84] did lead to a reduction in sacrally and pudendally evoked EUS pressures after four or more hours of experimenting with KHFAC pudendal nerve block (having later measured unintended DC contamination), while additional LC(DC) filter circuits using capacitors and inductors were needed to prevent nerve conduction failure in the chronic implementation.
Similarly, Waataja et al. reported in a study aimed at blocking the vagal nerve for
the treatment of obesity that nerve block persisted for tens of seconds after KHFAC
nerve block application. Considering that there is no mention of capacitive decoupling
between stimulator and electrode, and that no inductors were used to shunt DC, it is
likely that unintended DC components accumulated at the electrode-to-electrolyte
surface boundary and repeatedly changed the pH near the vagal nerve during
experimentation. Neural depression, a sign of beginning DC-induced damage, as
previously reported by Ackermann et al. [176], was observed as a temporary condition.
As all of our KHFAC studies indicate that nerve conduction returns immediately (<1 sec)
after the application of KHFAC nerve block without any additional (intended or
unintended) DC components, Waataja et al might have experienced a combination of
DC and KHFAC nerve block. Waataja’s work coincided with Enteromedics’ research on
VBLOCK technology, which was followed up by one (!) scientific abstract by Tweden et
al. [91], reporting data from a study involving seven subjects (5 active and 2 control) to
show that eight weeks of implantation did not change nerve morphology (unclear if with
or without continuous KHFAC “stimulation”). Clinical studies using VBLOC technology
were able to show effects that correlate with blocking neural activity on the vagal nerve
[6, 89] but it is unclear if transection of the nerve would have provided the same results
as repeated application with DC-contaminated KHFAC waveforms is likely to reduce
neural communication post block application as seen by Waataja.

Spinal cord stimulation technology, developed by Nevro Medical in collaboration
with Tweden et al, has more recently started to apply waveforms at 10 kHz (called “HF-
10”) to reduce lower back pain. It is so far unclear, if the reduction of pain is an effect of
sub-block threshold high-frequency stimulation of dorsal spinal roots or if unintended
DC components help to reduce neural activity and aid in the reduction of pain perception. Comparing pain relief with an additional LC(DC) filter circuit between the signal generator output and the electrode connected to the patient would likely aid in the assessment of effects contributing to the observed treatment.

In summary, if DC-contaminated KHFAC waveforms are applied and the resulting nerve block is a combination of two independent block effects, then the acutely determined KHFAC block thresholds are unlikely to translate into a chronic implementation: instead, the block thresholds with DC-contaminated waveforms will likely be lower than those of “clean” KHFAC, potentially impacting the successful outcome of a chronic study. All currently available publications reporting chronic implementation of KHFAC waveforms are in abstract form [90-92, 177] and none present clear information on histological analysis. Techniques to measure electrode offset potential and small DC currents have been presented in this publication and can be applied to ensure DC-free “clean” KHFAC waveforms for the reproducible, temporary application of electric nerve block.
V.4 Future Outlook

Future treatments of neurological diseases or injuries will likely be aided by the addition of either temporary or continuous application of different nerve block technologies. The specific strength of KHFAC nerve block is the immediate initiation of and recovery from the nerve block as well as the ability to provide this form of block for minutes and hours at a time; the main weakness being the onset response and the required voltage compliance to drive e.g. 5mA HF signals through kΩ electrode impedances near the nerve. The strength of CBDC nerve block is the initiation without an onset response, the main weakness being so far that a complete block can only be provided for a few seconds until a recharge phase is needed to prevent potentials at the electrode/electrolyte interface from drifting outside the water window. Intentional lesions on the other hand can be placed repeatedly with (normal) DC waveforms to provide a medical alternative for repeated injections of chemical nerve block agents such as botulinum toxin. These are only three forms of electric nerve block in preclinical and clinical testing today. As more applications using these and other forms of electric nerve block (e.g. Wedensky synaptic junction block) are implemented, more data regarding application-specific chronic nerve viability and treatment efficacy will become available.
To ensure a better understanding of the chronic effects of various nerve block technologies, researchers are advised to

(1) Correctly measure the electric waveforms in-vitro and **vivo** to ensure waveform integrity.

(2) Conduct nerve and/or application specific *chronic* studies with the same stimulation protocol as intended with human implementation and include measurements for nerve conductivity post-block.

(3) Ensure that their waveform generators do not influence each other if more than one pair of electrodes are electrically activated inside a subject (e.g. one for stimulation and one for block, or two for block). Using two or more waveform generators can easily result in crosstalk between stimulation and block channels, resulting in impaired stability of the block. This is especially true for chronic implementations.

The development of applications using nerve block might be challenging, yet the possibilities of treatment that this technology offers are vast. A list of potential implementations follows below.
Potential applications for electric nerve block include in the future:

**PNS Block**

- Reduction of peripheral nerve pain (KHFAC or CBDC or (destructive) DC block)
- Reducing over-activity of various inner organs (pancreas, kidney, etc.)
- Asthma and chronic obstructive pulmonary disease - bronchial innervation
- Block of unintended activation caused by electrical stimulation on the same nerve trunk. Block of AP traveling retrograde or on a secondary branch.
- Increase of stimulation selectivity (e.g. fiber size) and specificity (e.g. target location).
- Partial CBDC and KHFAC nerve block; see [51] – starting KHFAC above block threshold, then lowering it to e.g. 70% block, later increase without onset.
- Block sensory over-activity leading to reflexive movements (see afferent input)
- Partial or full block of the occipital nerve might provide yet another approach to migraine treatment; reducing seizures by blocking various cranial nerves.
- Partial block of central over activity on peripheral nerves to allow the patient regain a larger field of movement (not having to fight the full amount of force caused by e.g. spasticity) by blocking big nerve fibers (selective block!), thus allowing muscle drive using the smaller axons innervating smaller muscle fibers.
- Using the KHFAC onset response or KHFAC waveforms run at sub-block threshold amplitudes can provide de-synchronized activation of nerves. This “random” drive of PNS nerves can modulate organ activity in the periphery as well as provide reduction of sensory information such as pain, tingle, itching etc. and could be implemented with electrodes and electronic both being placed more distally than e.g. current SCS approaches. This could mean less invasive procedures and quicker patient recovery; potentially even providing a transcutaneous solution for short-term treatment.
CNS Block

- Pain reduction - CBDC block, KHFAC-sub-block-threshold-drive used for neuro-modulation in e.g. the dorsal spinal cord rootlets or entire column
- Mitigation of spasms – similar approaches as listed in the PNS approach above, yet by relying on reduction of afferent feedback to the CNS and thereby changing the processing of information in the CNS (i.e. gate control theory of pain etc.)
- Seizure mitigation by “capturing neural over-activity wave-fronts” with e.g. CBDC block – a seizure running as wave-front through the cortex could relatively easily be captured with a partial or full CBDC block that is provided with an (micro-) EEG electrode matrix made of a HiQ material providing the necessary large charge injection capacity.
- Reduction of rhythmic activities such as clonus, reduction of over-activity in central pattern generators

ANS Block – various locations in the autonomic nervous system via PNS and CNS block

- Specific increase and/or reduction of sympathetic or parasympathetic tone.
- Obesity and satiety regulation
- Blood pressure reduction
- Heart rate regulation
- Headache reduction
- Reduction of over-activity of sweat glands
- Reducing the perception of stress levels in patients with abnormally high sympathetic tone
V.5 Summary statement

Electric nerve block can be achieved with a variety of waveforms, each of them providing specific advantages and disadvantages. In order to provide the patient with the most optimal solution by using the right form of electric nerve block, detailed knowledge of the specific waveform components and their effects on neural tissue and electrode materials is essential. Filtering of unwanted (or unintended) waveforms components can provide clean signals that can result in reproducible results with chronically implanted neuro-prosthetics.

The document at hand described methods to ensure proper waveform integrity for KHFAC signals, an alternative to significantly reduce the KHFAC onset response and a chronic implementation of said nerve block to provide a bladder voiding prosthesis for preclinical subjects with chronic-stage SCI that operated at the push of a button and delivered a significant improvement in voiding outcome.

Electric nerve block has a variety of potential applications and proper implementation will likely lead to improved patient acceptance with fewer side effects than current medical treatments involving chemical, surgical or otherwise physical approaches to reduce or prevent neural activity.
Appendix

A.1 Comparison of healthy bladder system and system post SCI; treatment approaches

Urethral Sphincter reflexes prevent bladder voiding post SCI

Healthy bladder system

Post SCI

Continence
Bladder Emptying (Voiding)

Sphincter ON

Bladder OFF

Sphincter OFF

PN

PN

Spasms: Sphincter ON
Reflexes prevent voiding!

Synchronized activation of bladder and sphincter muscle

The Standard of Care is insufficient and has strong side effects.
Numbers 1-5 increase in invasiveness.

1. Catheterization
   (Clean intermittent, Suprapubic, Foley)
   (Clinical standard)

2. Pharmacologic

3. Sphincterotomy or Botulinum Toxin
   EUS = External Urethral Sphincter

4. Neurotomy of PN

5. Brindley Vocare System
   (requires dorsal rhizotomy)
   (Standard Neuro-prosthesis)

Stimulate

Cut

Efferent

Afferent

Cut
A.2 Background on the solution of KHFAC nerve block for bladder voiding

Approach: Instead of cutting permanently, why not block temporarily?

- **Irreversible approach**
  - Bladder ON
  - Neurotomy of the PN?
  - Sphincter Off Forever.
  - Achieved: Voiding.
  - Side effect: Incontinence.

- **Selective neural block**
  - Stimulate extradural Sacral root
  - KHFAC Block
  - Sphincter OFF
  - Voids
  - No neurotomy / rhizotomy!

Background: **Selective electric KHFAC nerve conduction block**

- **Electric difference**
  - Kilo-Hertz Frequency Alternating Current
  - “Typical” stim waveform

- **Physiologic effects**
  - Specific Characteristics of KHFAC block
    - “electric lidocaine”
  - Immediate
    - Full nerve conduction block in 7.5ms
  - Complete
    - No (sphincter) muscle activity
  - Reversible
    - Nerve recovers right away, muscle force comes back & function resumes
  - Conduction block in several species
    - Sea slug, frog, rat, cat, dog, non-human primate (monkey), and human
    - Motor and sensory nerve fibers

References:
A.3 Background on the formerly conducted work leading up to the current solution

**Background:** Complete electric Pudendal Nerve Block

kHz-Frequency (2 - 40 kHz) AC waveforms produce complete motor nerve block.

**Challenge of the Onset-Response**
- Application is intended for complete SCI (Potential pain response = no concern)

Acute study showed:

**Improving bladder voiding via HFAC PN block and sacral bladder drive**

Pudendal nerve block may provide a valuable tool to produce voiding in individuals with bladder sphincter dyssynergia.
A.4 Study design

Study performed in a chronic-SCI feline model with chronically-implanted electrodes.

Input $\rightarrow$ System $\rightarrow$ Output
A.5 Electronic setup used for anesthetized experiments

**Electronic setup**
(used in bi-weekly experiments)

Details:

![Diagram of electronic setup](image-url)
A.6 Removing one major source of crosstalk: GND connection via C43 in output amplifiers

Crosstalk – Details 1/2

Crosstalk – Details 2/2
A.7 Comparison of voiding results: continuous control sacral drive vs. KHFAC nerve block and lidocaine nerve block;

Flow & Pressure analysis - Preliminary Results

Raw Data from Terminal experiment
- Volume (& flow)
- Bladder pressure

KHFAC Pudendal Nerve Block improves Bladder Voiding (post chronic stage SCI)

No block:
- A
  - Blue: flow
  - Voids only post-stim.

With block:
- C
  - Blue: flow
  - Continuous Voiding.

Comparison of awake bladder voiding

A B C D
Control Continuous Control Interstim Continuous KHFAC Interstim KHFAC Interstim Manual Expression

Clinical Treatment

Continuous Voiding.
A.8 Preliminary characterization of the LC(-DC) circuit

What is **DC-Offset during HFAC** and why is it a problem?

Measuring setup:

![Diagram](image1)

What one expects to see: ![Waveform Image 1](image2)  
What one does see: ![Waveform Image 2](image3)

Solution

**The LC(DC) Circuit**

- **Job description:**
  - AC passes
  - DC is shorted

**LC(DC)**

- only Capacitor bad DC clearance
- Inductor Shortens DC
Transfer Functions of the Inductor alone:

Capacitors alone

Effect
Two capacitors (1uf) and two inductors (8H) providing the LC(DC) filter circuit

Effect:
A.9 Low-supply-voltage KHFAC waveform generator using Joule-Thief approach

Block parameters / details

“Input”
- Battery voltage: 1.5 V (one AAA)
- Battery drain: 76 mA

“Output”
- HFAC current controlled
- HFAC @ 14.8 kHz
- Vpp = 5.2 V
- Waveform “near-sinusoidal”
- Very short HFAC onset
- No HFAC to DC electrode crosstalk

Location of the oscilloscope

Circuit - Overview
Circuit – Center: “Joule-Thief” or “Joule-Ringer”

Circuit – Part 2: Resonator to “round the waveform”

Circuit – Part 3: De-coupling the DC with optionally added $C_{\text{inline}}$
A.10 Presence of unintended DC can affect the KHFAC onset (completely abolish it!)

Summary:

- Using KHFAC waveforms to provide a sub-block-threshold activation to the nerve (e.g. sciatic) should lead to a continuous muscle force measured at e.g. the gastrocnemius muscle of a rat.
- If unintended DC is present in the KHFAC waveform due to using only capacitors to filter DC between signal generator and electrodes, then the DC can depress the activation.
- We saw KHFAC onset responses that looked like a “perfect” choice of KHFAC parameters after a few KHFAC onset trials as DC onset had accumulated on the electrode.
- This was not the case when inductors were used, shunting the DC and causing a continuous nerve (and muscle) activation with sub-block-threshold KHFAC waveforms.
- This is only “proof of concept” data that DC can even affect outcome of the nerve block in an acute experiment, meaning that DC can cause faster/stronger/better nerve block if it’s added to the KHFAC waveform unintentionally. This will also likely lead to false results in nerve block experiments as the researcher does not know if he/she applies only KHFAC or mixed KHFAC and DC waveforms that might block different fibers at different amplitudes. Furthermore, depressed nerve conductivity post block might result from wrongful application of combined DC+KHFAC waveforms if not handled correctly.
- Setup similar to that of KHFAC+CBDC Project (Chapter IV), but without the HiQ-electrode and the DC-generator. Electrodes used were Pt-electrodes (not Hi-Q). Stimulator used was the Keithley 6221 at 20 kHz, calibrated to zero offset. The unintended DC was observed when NO inductors were present. The amplitude of the KHFAC waveform was intentionally set to be BELOW the KHFAC block threshold, to achieve continuous onset-activation (see Wodlinger [77, 78]).
   **This onset was then depressed using the unintentional DC to show the effect.**
- Inductors were then added bilaterally (on electrode and on stimulator side of the capacitors) to provide “DC-free KHFAC continuous onset”.
- Four (4) sections of 10 sec KHFAC plus 15 sec pause were applied to show the effect of repeated accumulation of unintended-DC contamination induced onset-block.

*Raw data in figures attached below.*
Raw data examples:

Explanation of figure A10-1:

- **KHFAC** is applied with only capacitors (no inductors)
- All values shown are on the same Y-scale in form of voltage measurement, even though they might be current or force measurement.
- **Red**: voltage of one Pt electrode in saline vs. Ag/AgCl reference
  → unintended DC is shown in differential voltage values; baseline, minimum and the maximum of the charge-balancing spike at KHFAC end is indicated.
- **Black**: Onset-force measurement over time.
- **Blue**: current measurement through the electrode (floating, with some crosstalk) and added here only to provide the qualitative information of direction and general magnitude.

![Graph](image)

**Fig. A10-1** – continuous KHFAC onset for 10 sec each with 15 sec break – notice depression of onset-force (black) with especially the 3\(^{rd}\) and 4\(^{th}\) repeat.
Explanation of figure A10-2:

- KHFAC is applied with only capacitors (no inductors)
- All values shown are on the same Y-scale in form of voltage measurement, even though they might be current or force measurement.
- Note how onset-force (black) does not diminish now / is not depressed.
- Note how there is no change in offset-voltage (red) of one of the electrode contacts (it’s a bipolar measured vs. Ag/AgCl)
- Repeats of the setup with vs. without inductors showed remarkably similar results, over and over again.
- This is preliminary data to show that even in an acute experiment unintended DC can result in a depression of neural activity post application of KHFAC nerve block. When reproduced, please allow for a few repeats without using the inductors first in order to experience the effect of neural depression, same applies to the approach in A11.

Fig. A10-2 – continuous KHFAC onset for 10 sec each with 15 sec break – notice depression of onset-force (black) with especially the 3\textsuperscript{rd} and 4\textsuperscript{th} repeat.
A.11 Presence of unintended DC can affect the return of PS post block

Summary:

- Block post application of full KHFAC nerve block (amplitude above block threshold) is shown after current-controlled KHFAC was applied with capacitors only (1) and with the combination of capacitors and inductors (2) as described before in the LC(BC) filter circuit design.

- KHFAC was started at above block threshold, then slowly reduced and eventually switched off. Setup used was the same as in A10 (but in a different preclinical subject, yet same rat model). Keithley 6221 (current-contr.) was used.

- Take-away:

  Depression of PS force post block takes a few seconds to allow full recovery of PS force when no inductors were used.

  Depression of PS is not observable when inductors are used.

- This is preliminary data to show that even in an acute experiment unintended DC can result in a depression of neural activity post application of KHFAC nerve block. When reproduced, please allow for a few repeats without using the inductors first in order to experience the effect of neural depression, same applies to the approach in A10 before.

*Raw data in figures attached below.*
Comparison between returning Proximal Stimulation (PS) post block, getting through a reduced KHFAC block, with a setup that used

- (A) only two capacitors (1μF) between the stimulator and the electrode or
- (B) two capacitors (1μF) and 2 inductors (8H @ 10kHz)

**Figure A11-1.** Data from acute rat experiment. Return of PS post block (spikes at 2 Hz) show depression of first few seconds after the end of KHFAC waveform application. The height of the PS evoked force twitches starts way below the DS height (twitches at 1Hz) at the very end.

The depression might be the first indication of a change in nerve conductivity post block due to injection of DC.

Blue indicates amplitude of KHFAC waveform.

Only capacitors were used to "filter DC" between stimulator and electrodes.

**Figure A11-2.** Data from acute rat experiment. Return of PS post block (spikes at 2 Hz) show no depression after the end of KHFAC waveform application. The height of the PS evoked force twitches starts immediately at the DS height (twitches at 1Hz) at the very end.

Blue indicates amplitude of KHFAC waveform.

Capacitors and inductors in LC(-DC) configuration were used to filter DC between stimulator and electrodes.
References


Franke, M., et al., KHFAC+DC nerve block without onset or damage to nerve conduction, in Society for Neuroscience SfN. 2013: San Diego, CA.


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KHFAC٤DC – Let there be Block!