Cathodic-leading pulses are more effective than anodic-leading pulses in intracortical microstimulation of the auditory cortex

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Abstract

Objective. Intracortical microstimulation (ICMS) is widely used in neuroscientific research. Earlier work from our lab showed the possibility to combine ICMS with neuronal recordings on the same shank of multi-electrode arrays and consequently inside the same cortical column in vivo. The standard stimulus pulse shape for ICMS is a symmetric, biphasic current pulse. Here, we investigated the role of the leading-phase polarity (cathodic- versus anodic-leading) of such single ICMS pulses on the activation of the cortical network. Approach. Local field potentials (LFPs) and multi-unit responses were recorded in the primary auditory cortex (A1) of adult guinea pigs \((n = 15)\) under ketamine/xylazine anesthesia using linear multi-electrode arrays. Physiological responses of A1 were recorded during acoustic stimulation and ICMS. For the ICMS, the leading-phase polarity, the stimulated electrode and the stimulation current were varied systematically on any one of the 16 electrodes while recording at the same time with the 15 remaining electrodes. Main results. Cathodic-leading ICMS consistently led to higher response amplitudes. In superficial cortical layers and for a given current amplitude, cathodic-leading and anodic-leading ICMS showed comparable activation patterns, while in deep layers only cathodic-leading ICMS reliably generated local neuronal activity. ICMS had a significantly smaller dynamic range than acoustic stimulation regardless of leading-phase polarity. Significance. The present study provides in vivo evidence for a differential neuronal activation mechanism of the different leading-phase polarities, with cathodic-leading stimulation being more effective, and suggests that the waveform of the stimulus should be considered systematically for cortical neuroprosthesis development.

Keywords: auditory, cortex, neuroprosthetic, cortical implant, hearing

Supplementary material for this article is available online
(Some figures may appear in colour only in the online journal)
Introduction

Electrical stimulation inside cortical tissue, i.e. intracortical microstimulation (ICMS), is a widely used stimulation method in neuroscientific research [1, 2]. It is a powerful tool in establishing the direct contribution of neuronal activity to perception and cognition, i.e. in forming a causal connection to the cortical network [3]. Methods of causal inference, like electrical stimulation, pharmacological intervention or lesioning, have been used to associate a specific function to a particular part of the cortical network. ICMS is especially powerful in this regard because it can be used to reversibly activate small parts of the network with high temporal and spatial precision. The precision as well as the overall efficacy of the activation achieved with ICMS depends on the exact stimulation parameters, like the intensity of the stimulation (including current and duration of a single pulse and number and inter-pulse interval of pulse trains) [4–7], or the position of the stimulating electrode [6, 8–12].

It is important to note up front the conceptual differences between ICMS and other electrical brain stimulation applications like cortical surface stimulation, stimulation of peripheral nerves or deep brain stimulation (DBS). The usual size of the stimulation electrodes and amplitude of current pulses differ on the order of magnitudes between ICMS and DBS/surface stimulation. A comprehensive analysis of the commonalities and differences between several electrical stimulation paradigms, however, is not in the scope of this report. The presented results are therefore specifically related to ICMS and not to other stimulation paradigms.

The standard pulse shape in ICMS is a biphasic, square-wave pulse [5, 13, 14]. While monophasic pulses allow for the most efficient and selective neuronal activation [15], they are most prone to damaging the tissue due to charge accumulation [13]. Therefore, in most cases electrical stimuli are designed with a second, charge-balancing phase of opposing polarity to the first phase, to ‘recover’ all reversible processes at the tissue-electrode interface and avoid damage to both the tissue and the stimulating electrode [13]. However, the second phase may also influence the neuronal response to the stimulus, as shown e.g. by including a brief interphase gap between the two stimulus phases [16].

The polarity of the first phase, the leading-phase polarity, is most often chosen to be cathodic (negative). This is based on theoretical considerations following the cable theory model of neuronal activation: applying current to neuronal tissue shifts the extracellular potential to a specific value proportional to the deposited charge. The influence of the extracellular potential on the neuronal activation is most often formulated using the ‘activating function’ proposed by Rattay [17]. Positive values of the activating function, the second spatial derivative of the extracellular potential in case of an unmyelinated fiber (figure 1(a)) and the second difference quotient of the extracellular potential for a myelinated axon, are thought to correspond to higher probabilities of neuronal excitation. Therefore, the activating function predicts the strongest neuronal excitation to occur during cathodic stimulation. However, the second spatial derivative of anodic stimulation also shows positive values, sometimes called ‘virtual cathodes’, but at some distance to the stimulating electrode (figure 1(a)). Based on these mathematical models, anodic stimulation is consequently also expected to activate excitable membranes, but with reduced efficacy.

The efficacy of ICMS to activate neuronal populations is usually measured in terms of the elicited cortical response. Often this was achieved indirectly by measuring a behavioral response of an animal following ICMS [1, 18–20], sometimes more directly by recording neuronal activity including intra- or extracellular electrical potentials [7, 11, 21] or optical surrogates of neuronal activity like fluorescence [22], voltage sensitive dye activity [23, 24] or intrinsic optical signals [9].

In previous work we have shown the possibility to measure the efficacy of ICMS in close proximity to the stimulation by combining ICMS with extracellular electrophysiological recordings on a single shank of a linear multi-electrode array in vivo (figure 1(a)) [6, 25]. In the present study we used this method to determine the influence of the leading-phase polarity, i.e. cathodic- versus anodic-leading (figure 1(b)), of single, charge-balanced, biphasic ICMS pulses on the activation of the cortical network. We recorded the neuronal activity inside the primary auditory cortex of guinea pigs during single pulses of ICMS with both leading-phase polarities in two sets of experiments (supplementary table S1 (stacks.iop.org/JNE/16/036002/mmedia)). In the first group of experiments (‘varying depth’), each of the 16 electrodes of the linear multi-electrode array was stimulated sequentially, with a fixed stimulation current of ~6 μA. In the second group of experiments (‘varying current’), we sequentially stimulated electrodes 1 (most superficial electrode), 9, and 16 (deepest electrode) with both leading-phase polarities while varying stimulation current. All other stimulus pulse parameters were kept the same throughout all experiments (charge-balanced, biphasic square waves, 200 μs/phase, figure 1(b)). The neuronal activation data were collected during single-pulse ICMS from the remaining 15 electrode contacts.

Materials and methods

Experimental animals

Data from a total of 15 experiments on Dunkin-Hartley guinea pigs (Crl:HA, Charles River Laboratories International Inc., France, 350–665 g, 13 male, 2 female) were used for the present experiments.

All experiments were conducted in accordance with EU Directive 2010/63/EU, the German law for the protection of animals, and were approved by the ethics committee of the government of the state of Lower Saxony, Germany (Lower Saxony state office for consumer protection and food safety, LAVES; approval no. 14/1548).

Experimental groups

The experiments were separated into two sets (supplementary table S1). The first set of animals (n = 7, ‘Varying depth’) was stimulated with both leading-phase polarities on all 16 electrodes
of the first shank of a $2 \times 16$ linear multi-electrode array, with
a fixed current. The second set of experiments ($n = 8$, ‘Varying
current’) was stimulated with both leading-phase polarities and
varying currents ($-0.1$–$37 \mu$A) on electrodes 1, 9, and 16 of a
$1 \times 16$ linear multi-electrode array. One animal was part of both
stimulation paradigms. The data presented here is a subset of
a larger stimulation paradigm presented to each animal. Some
data related to cathodic-leading ICMS and acoustic stimulation
has been published previously in [6, 25].

Anesthesia and preparations

The detailed procedures for animal handling and preparation
can be found in previous publications [6, 25] and are only
shortly recapitulated herein.

All animals were pre-medicated with 0.5 g Bene-Bac®
(Albrecht GmbH, Germany) and 0.3 mg diazepam (Ratiopharm
GmbH, Germany). Anesthesia was induced and maintained
by intramuscular injections of a ketamine/xylazine mixture
(50 mg kg$^{-1}$, 10% Ketamin, WDT, Germany, 10 mg kg$^{-1}$
induction and 5 mg kg$^{-1}$ maintenance, 2% Xylazin, Bernburg,
Germany). The induction mixture was supplemented with
atropine sulfate (0.1 mg kg$^{-1}$, B.Braun Melsungen AG,
Germany). Analgesia was provided by subcutaneous injection
of 0.05 ml carprofen (Rimadyl, Pfizer GmbH, Germany). To
control the physiological status during the experiment the ani-
mals were artificially ventilated and core body temperature,
ECG, expiratory CO$_2$ concentration and respiratory pressure
were constantly monitored.

Electrophysiological recordings were made in a sound-
proof, electrically shielded chamber with the animal fixed
inside a stereotaxic frame using a metal bolt cemented to
the frontal bones with dental cement (Paladur; Heraeus Kulzer
GmbH, Germany). In the second set of experiments a small
cranietomy was made at the vertex to place an Ag/AgCl refer-
ence electrode on top of the dura. In all experiments a broad
cranietomy exposed the right auditory cortex. The dura was
resected and the opening was filled with silicone oil. An Ag/
AgCl electrode in the neck served as common ground.

Cortical recordings and electrical microstimulation
were performed using an Alpha Omega electrophysiology system
(AlphaLab SnR, AlphaOmega LTD, Israel). After map-
ning the surface potentials in response to acoustic click
stimulation (40 dB above the auditory brainstem response
threshold) recorded using either an Ag/AgCl ball electrode or
a 16-channel surface grid electrode (Blackrock Microsystems,
Germany) [26], either a single-shank, 16 channel multi-elec-
trode array (A1 $\times$ 16-5mm-150-177) or a double-shank, 32
channel multi-electrode array (A2 $\times$ 16-10mm-150-500-177,
NeuroNexus, USA) was inserted perpendicularly to the cor-
tical surface at the position of highest response amplitude.
Electrodes were inserted manually with stereotactic microma-
ipulators (precision < 10 $\mu$m) to a depth of 2.5 mm, ensuring
a complete coverage of all six cortical layers. Electrode posi-
tion was additionally controlled by functional parameters
(current source density (CSD) profile) and verified by post-
experimental histology like presented in previous publications
[6, 25]. In both types of penetrating arrays, the same contact
size (177 $\mu$m$^2$), inter-electrode distance (150 $\mu$m) and number
of contacts per shank (16) were used. Cortical potentials were
recorded, bandpass filtered (1 Hz to 9 kHz), digitized (22 kHz
sampling rate), and stored for offline analysis.

Acoustic and electric microstimulation protocol

Acoustic click stimulation (50 $\mu$s condensation click) was pre-
stanted on the contralateral (left) ear using a loudspeaker (DT-
48; Beyerdynamic GmbH & Co. KG, Germany) connected to
the outer ear canal with a plastic cone. The amplitude of the click stimuli was calibrated offline using a 1/4″ condenser microphone (Type 4939 with a 2670 pre-amp and a Nexus conditioning amplifier, Brüel & Kjaer, Denmark) to dB SPL_{peak} equivalent (dB SPL_{pe}) values, i.e. having the same peak amplitude as a 1 kHz tone burst of a given intensity measured in dB SPL.

ICMS was performed in a monopolar fashion against the electrode in the neck. Stimulation consisted of single biphasic, charge-balanced, square-wave current pulses of either leading-phase polarities (200 µs/phase, no inter-phase gap, figure 1(b)). Stimulation intensity varied between 0.1 and 37 µA, as measured over a 100 Ω resistor placed in series in the current return path (see supplementary material, figure S1). Electrical stimuli were repeated 32 times with a repetition rate of ~1 Hz and the first and last trial were dropped. Acoustic stimuli were repeated 30 times.

**Data processing and statistical analysis**

The data was analyzed offline using custom MATLAB (The MathWorks, Inc., USA) scripts, which are accessible online (https://github.com/mbvoigt/AnodicICMS, DOI:10.5281/zenodo.2223067).

Electrical stimulation artefacts were blanked by linear interpolation (0–3 ms post-stimulation onset). In approx. 0.1% of all single recordings the stimulation artefact saturated the recording input range (486 of 468 480 single recordings). For the analysis of local-field potential (LFP) activity the data was lowpass-filtered using 2nd order, zero-phase Butterworth filtering (<150 Hz), and highpass-filtered (>300 Hz) for the analysis of multi-unit spiking activity (MUA). As in a previous publication [6], the CSD profile was calculated as the second spatial derivative of the LFP component as follows:

\[
\text{CSD}^2_z = \frac{\phi'_z - \phi'_{z+\Delta z} - 2\phi'_z + \phi'_{z-\Delta z}}{(\Delta z)^2}
\]  

where \(\phi'_z\) designates the LFP at time \(t\) and depth \(z\). \(\Delta z\) represents the distance between two adjacent recording contacts (here 150 µm). The CSD was inverted, to analyze current sinks, i.e. putative neuronal activation, with positive values. A single CSD signal is called a ‘CSD trace’. While the combination of all single traces from a concurrent recording is called...
the ‘CSD profile’. For the calculation of the CSD, edge electrodes of the LFP profile were doubled following Vaknin et al [27]. CSD traces were baseline corrected relative to the 50ms pre-stimulus. For automatic amplitude quantification of current sinks, the current sources were removed from the data (set to 0). Single spike events were classified from the MUA data using a thresholding procedure [28].

Normalized CSD peak amplitudes in the time window up to 50ms post-stimulation at varying stimulation current or varying acoustic click intensity were fit according to the following logistic function:

\[
\text{Amplitude}_{\text{CSD}}(c) = R_{\text{Low}} + \frac{R_{\text{High}}}{1 + e^{-w(c-c_{\text{sep}})}}
\]

(2) where \( c \) denotes the stimulation intensity in dB SPL re or dB re. 0.1 \( \mu \)A, \( R_{\text{Low}} \) is the baseline response amplitude, \( R_{\text{High}} \) is the upper asymptote, \( w \) denotes the steepness of the function and \( R_{50\%} \) is the point of inversion. We calculated the dynamic range as the difference between the stimulus intensities that satisfy the conditions \( \text{Amplitude}_{\text{CSD}}(c) = 0.75 \) and \( \text{Amplitude}_{\text{CSD}}(c) = 0.25 \), in order to facilitate comparisons to earlier studies [29–31].

For detailed analysis of the position of the evoked activity, the sink component of the CSD traces were normalized by the maximum sink amplitude of the CSD profile. When the normalized peak amplitude in the 50ms post-stimulation window exceeded a value of 0.8, the response was considered supra-threshold and the respective CSD trace was coded with a 1, otherwise with 0. This resulted in logical activity matrices. This difference is 0 if two matrices are the same and increasingly larger with increasing distance between both matrices.

All statistical analyses were performed in MATLAB. For statistical evaluation, the CSD amplitude was transformed to dB re. 1 mV mm\(^{-2}\) and the stimulation current to dB re. 1 \( \mu \)A. This allowed to investigate the relative effects of the experimentally varied factors across animals, even though the absolute amplitude values are expected to differ across animals. All statistical tests (e.g. Wilcoxon signed-rank tests, Kruskal–Wallis tests, repeated measures ANOVAs) were performed two-sided. Post-hoc testing was performed using Tukey’s honest significance difference (HSD). \( P \)-values < 0.05 were considered statistically significant.

**Results**

ICMS of medium strength (~6 \( \mu \)A, three times mean threshold value, see supplementary table S2 and figure S2) inside the auditory cortex evoked activity regardless of the leading phase polarity. Neuronal activity was visible as positive and negative deflections in the LFP profile in response to acoustic (figure 2(a)) as well as electric stimulation (figure 2(b)). Consequently, after localizing the generators of the LFPs within the electrode shanks there was a characteristic pattern of sinks in the respective current-source density (CSD) profiles. The CSD is calculated from the LFP signals as the second spatial derivative and is a representation of the underlying (subthreshold) current sinks and sources generating the LFP signal. Current sinks (depicted upwards, with black fill in figure 2) are assumed to correspond to cell membrane depolarizations and consequently neuronal activation. Quantifications of the CSD are therefore restricted to the sink component of the CSD in the following.

CSD profiles in response to anodic-leading ICMS were specific to the depth of stimulation as previously reported for cathodic-leading stimulation [6]. Both leading-phase polarities resulted in CSD profiles which were visually very similar to each other. For example, a stimulation on electrode 1 (figure 2(a)) evoked a strong but short-duration source followed by a longer-duration sink in the topmost CSD trace in both the cathodic-leading as well as the anodic-leading ICMS condition.

### CSD amplitudes in the experimental group ‘Varying depth’

A repeated measures ANOVA with post-hoc Tukey’s HSD comparisons (performed on data transformed to dB re. 1 mV mm\(^{-2}\)) revealed significant amplitude differences due to the within-subject factor leading phase polarity (\( F(1,112) = 169.39, p < 0.0001 \)) and the between-subject factor stimulated electrode (\( F(15,112) = 4.19, p < 0.0001 \)), without interaction between these two factors (\( F(15,112) = 1.15, p = 0.3236 \)). The post-hoc pairwise comparison of CSD amplitudes (in dB) of both leading-phase polarities for each stimulated electrode revealed statistically significant differences in 14 out of 16 stimulated electrodes (supplementary table S3). Highest mean sink amplitudes were reached when stimulating in supra-granular layers, i.e. roughly electrodes 3–7. Averaged over all 16 stimulated electrodes the mean CSD sink peak amplitude was 0.56 ± 2.50 dB re. 1 mV mm\(^{-2}\) for cathodic-leading stimulation and −3.34 ± 2.62 dB re. 1 mV mm\(^{-2}\) for anodic-leading stimulation (Wilcoxon signed-rank test, \( z = -3.52, p = 0.0004 \)). This amounts to a mean difference between cathodic-leading ICMS and anodic-leading ICMS of 3.9 dB. In total, cathodic-leading stimulation led to higher LFP peak-to-peak (figure 3(a)), as well as CSD peak amplitudes (figures 3(b) and (c)) than anodic-leading stimulation in the experimental group ‘Varying depth’.
For CSD sink peak latency (figure 3(d)) a repeated measures ANOVA with post-hoc Tukey’s HSD comparisons showed no significant influence of the factor leading phase polarity ($F(1,112) = 0.05, p = 0.8260$), but a significant influence of the factor stimulated electrode ($F(15,112) = 2.14, p = 0.0125$). The grand mean sink latency was $15.07 \pm 1.15$ ms and $15.15 \pm 1.98$ ms for cathodic- and anodic-leading ICMS, respectively. Peak latencies were lowest when stimulating electrodes 7–9 and highest when stimulating the most superficial and most deep electrodes (figure 3(d)). Correlation coefficients between sink peak amplitudes (in dB) and latencies were negative, i.e. latencies were lowest where the amplitude was highest and vice versa (cathodic: Spearman’s rho: $-0.54, p = 0.0338$, anodic: Spearman’s rho: $-0.51, p = 0.0450$).

CSD amplitudes in the experimental group ‘Varying current’

Both LFP amplitude [6] and CSD sink peak amplitude increased with stimulation current (figure 4(a)) in the animals of the experimental group ‘Varying current’. Statistical evaluation of CSD amplitudes (in dB re. 1 mV mm$^{-2}$) using a repeated measures ANOVA with Tukey’s HSD post-hoc testing revealed significant influences of the between-subject factors stimulation current (current measured in dB re. 1 µA, $F(14,315) = 198.10, p < 0.0001$) and stimulated electrode ($F(2,315) = 184.71, p < 0.0001$), with a significant interaction between these factors ($F(28,315) = 6.54, p < 0.0001$). However, the within-subject factor leading phase polarity ($F(1,315) = 0.65, p = 0.4198$) was not significant, neither was...
the three-way interaction ($F(28,315) = 0.79, p = 0.7684$). The interaction between factors current and leading phase polarity also did not reach significance with a threshold of $p = 0.05$, but showed a trend ($F(14,315) = 1.63, p = 0.0698$). The most influential factor for the resulting CSD amplitude, judging by the fraction of each factors sum of squares over the total sum of squares, was the stimulation current with an $R^2 = 0.7077$, followed by the stimulated electrode, $R^2 = 0.0943$ and stimulus polarity with $R^2 < 0.0001$.

Comparison of dynamic range for ICMS and acoustic stimulation

While the CSD amplitude growth in response to different ICMS stimulation intensities was very uniform between the different animals (supplementary figure S3), the CSD amplitude growth in response to acoustic click stimulation of varying intensity showed more variation between the animals (figure 4(b)). In some animals, the CSD amplitude increased very strongly over a few decibels of stimulus level increase. The mean of all animals, on the other hand, showed a smooth incline over the tested click level range (figure 4(c)). The steepness of these amplitude growth functions was quantified between 25% and 75% of the normalized CSD amplitude (figure 4(d)). The grand mean of the dynamic range was $5.718 \pm 1.436$ dB (mean ± standard deviation) for cathodic-leading ICMS and $4.607 \pm 0.818$ dB for anodic-leading ICMS. The acoustic stimulation had a dynamic range of $19.972 \pm 15.441$ dB. A Kruskal-Wallis test over all seven groups (3 stimulated electrodes × 2 leading-phase polarities + acoustic stimulation) was statistically significant (d.f. = 6, $\chi^2 = 23.61, p = 0.0006$). A post-hoc Tukey’s HSD comparison between the groups revealed statistically significant differences between the median of the acoustic dynamic range and the anodic-leading ICMS on electrode 9 and anodic- and cathodic-leading ICMS on electrode 16.

Spatial pattern of activity in the experimental group

Varying depth

To determine whether the change in ICMS leading phase polarity leads also to a change in the spatial pattern of the excited neuronal activity, we generated ‘activity matrices’ out of the CSD sink amplitude data of the experimental
The nominal difference between activity matrices was calculated into four quadrants (figure 5(c)). Calculating the nominal difference of the first and the fourth quadrant between the animals revealed higher variance in the fourth quadrant in anodic-leading stimulation as well as the difference matrices (figures 6(b) and (c)). This documents that anodic-leading stimulation fails to generate the characteristic spatial pattern of local activity if stimulating the lower half of the electrode array. Consequently, while cathodic-leading stimulation evoked the typical local activity regardless of stimulation depth, anodic-leading stimulation evoked such local activity only if stimulating superficial positions.

The nominal difference between activity matrices was quantified by calculating normalized Frobenius norms, i.e. the Euclidean distance of 2D matrices (see material and methods, supplementary figure S7). This difference revealed that the similarity between ICMS of each polarity between animals is the same as the difference between the polarities in a single animal (Kruskal–Wallis test, df = 2, $\chi^2 = 4.68, p = 0.0966$; figure 6(a)). In other words, for both leading phase polarities the variation from one animal to the next was the same as the difference between cathodic-leading and anodic-leading in a single animal. Subsequently, the activity matrices were separated into four quadrants (figure 5(c)). Calculating the nominal difference of the first and the fourth quadrant between the animals revealed higher variance in the fourth quadrant in anodic-leading stimulation as well as the difference matrices (figures 6(b) and (c)). This documents that anodic-leading stimulation fails to generate the characteristic spatial pattern of local activity if stimulating the lower half of the electrode array. Consequently, while cathodic-leading stimulation evoked the typical local activity regardless of stimulation depth, anodic-leading stimulation evoked such local activity only if stimulating superficial positions.

**Multi-unit activity in the experimental group ‘Varying depth’**

We additionally analyzed the multi-unit activity response to ICMS. This has the advantage of documenting the suprathreshold response as opposed to subthreshold activity revealed by the CSD. As with the CSD, the general shape of the spiking response to cathodic- and anodic-leading ICMS was generally similar (figures 7(a) and (b)). While the CSD response showed peak latencies of around 15 ms, most multi-unit activity was found in the first 10 ms post-stimulation (figure 7(c)).

The number of the spikes generated in the first 10 ms post-stimulus showed a similar dependence on stimulation depth as the CSD amplitude (figure 8(a)), with highest amplitudes reached when stimulating electrodes 4–6. A repeated measures ANOVA showed the significant influence of the factors stimulated electrode ($F(15,112) = 2.80, p = 0.0010$) and leading-phase polarity ($F(1,112) = 127.24, p < 0.0001$) on the MUA amplitude, and a significant interaction ($F(15,224) = 3.53, p < 0.0001$). Averaged over all 16 stimulated electrodes the mean

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**Figure 5.** (a) Preparation of activity matrices out of activity vectors by thresholding the normalized CSD sink activity profile in the ‘varying depth’ group. Source activity was removed before normalization against the peak amplitude of the CSD profile. (b) Example activity matrices for cathodic-leading (left), anodic-leading (middle) and the difference between the two (right). For activity matrices for each single animal see supplementary figure S6. (c) Mean of activity matrices over eight animals. For detailed analysis the activity matrices were split into four quadrants, numbered as depicted in the right most matrix.
amplitude for cathodic-leading stimulation was $219.24 \pm 100.21$ spikes (mean ± standard deviation, summed over 15 recording electrodes and 30 trials), and $132.28 \pm 56.30$ spikes for anodic-leading stimulation (figure 8(b)). Again this was statistically significant (Wilcoxon signed-rank test, $p = 0.0156$), and additionally confirmed the stronger cortical response to cathodic-leading ICMS than to anodic-leading ICMS.

Furthermore, we calculated a spike index as the ratio of spikes in the first ms post-artefact over the total number of spikes in the first 7 ms post-stimulation (i.e. 3–10 ms post-stimulation, figure 8(c)). The mean of the spike index averaged over all 16 stimulation channels for cathodic-leading stimulation ($0.41 \pm 0.14$) was statistically higher than the spike index for anodic-leading stimulation ($0.28 \pm 0.05$, Wilcoxon signed-rank test, $p = 0.0156$, figure 8(d)). This demonstrates that the distribution of spikes is biased to the first ms in cathodic-leading stimulation, whereas in anodic-leading stimulation the multi-unit activity is more evenly distributed in time.

**Discussion**

Both cathodic- and anodic-leading biphasic ICMS pulses evoked local cortical activity next to the stimulated electrode, albeit with a substantially reduced dynamic range compared to acoustic stimulation. The response to cathodic-leading stimuli was significantly larger than the response to anodic-leading stimuli, regardless of analyzed response modality (LFP, CSD, or MUA). This difference in effectivity of neuronal activation led to a differential effect when stimulating with reduced current in the deep cortical layers, where cathodic-leading stimuli still evoked a local neuronal response, while anodic-leading ICMS failed to do so. In consequence, the present study provides in vivo evidence for a higher effectiveness of cathodic-leading symmetric pulses in ICMS.

Simulations [32–34] and electrophysiological studies [35] consistently showed differential effects of the stimulus polarity on neuronal activation. The simplified prediction of the ‘activation function’ of extracellular stimulation, the second spatial derivative of the extracellular potential, is that cathodic stimuli have a higher probability to activate neurons (figure 1(a)). From this perspective, our finding of an increased response amplitude to cathodic-leading stimuli is not surprising. However, the effect of stimulus polarity on neuronal activation is actually more complex than this simple prediction. Simulations using the activation function found complex relationships between stimulus polarity and electrode-neuron distance, with some spatial arrangements (e.g. electrode close to the axon initial segment) leading to a preference for cathodic stimuli and other arrangements (e.g. electrode close to the cell body) to a preference for anodic stimuli [15, 32–34], or a differential effect according to the neuron orientation relative to the electrode [36].

Assuming that anodic stimuli preferentially activate local cell bodies and cathodic stimuli preferentially local axons, it might be concluded that cathodic stimulation leads to smaller
action potential initiation latencies. Our present data support such a notion by showing more short-latency multi-unit activity in cathodic-leading than in anodic-leading stimulation. However, there is a substantial limitation in the present approach. The artefact blanking limited our data analysis to responses 3 ms after stimulus onset and consequently impeded conclusions about the direct effect of stimulation. Furthermore, it is generally assumed that the bulk of the neuronal activation seen after ICMS originates from trans-synaptically activated neurons [37, 38]. Consistently with these suggestions, we consider the spiking data presented here to originate mainly from trans-synaptically activated neurons.

There are also several limitations to the concept of the activation function itself (see for example [39, 40]). By definition, it is only concerned with the geometry of the extracellular potential and ignores the characteristics of the physiology of the neurons inside the potential gradient (e.g. strength-duration relationships [35]), as well as ignoring the effects of time. Therefore, it cannot be used straightforwardly to predict the neuronal activation when adding a second phase to the electrical stimulus, i.e. applying biphasic stimuli. Indeed, simulations suggest that (symmetric) biphasic stimuli of either leading-phase polarity (as were used in this study) may not have the same selectivity as monophasic pulses [15, 36]. This highlights the fact that during biphasic stimulation, both phases of the stimulus might contribute to the resulting neuronal activation. And as a consequence, differential effects between stimuli of different polarity are actually not to be expected for biphasic stimuli, in contrast to monophasic stimulation.

Asymmetric biphasic pulses, with one phase reduced in amplitude but proportionally extended in time to retain charge-balancing, showed the same selectivity as monophasic stimulation [15]. In the present study we did not test other pulse shapes than the symmetric, biphasic, square wave pulses, and therefore cannot conclude on possible polarity differences in eliciting local neuronal activation with asymmetric or mono-/tri-phasic pulses. However, a study testing behavioral detection thresholds of these asymmetric pulse shapes in the auditory cortex found no difference between different amplitude/duration ratios of the charge-balancing phase [41]. Instead the authors reported that detection thresholds depended solely on the magnitude of the cathodic phase. Again, as this does not change with changing the leading-phase polarity in the symmetric pulses used here, we did not necessarily expect to find the differential neuronal activation shown here in figure 3. It has to be noted though that in the previous study symmetric biphasic stimuli were reported to have higher detection thresholds than the other stimuli [41].

In behavioral experiments, anodic-leading stimulation is generally harder to detect (i.e. had higher current thresholds) than cathodic-leading stimulation, as seen in the auditory cortex of rats [41], monkey V1 [4] and human V1 [42]. Given our results presented here, this is a direct consequence of the lower amount of cortical activity evoked by anodic-leading stimulation. There are, however, contrasting reports about the interaction between leading-phase polarity and the depth of stimulation below the cortical surface. While saccade

Figure 7. (a) Overlays of n = 8 raster plots of multi-unit activity for cathodic-leading ICMS. Electrode 9 was stimulated. Each dot marks a single spiking event. For each electrode 30 trials are shown stacked. For the data of single animals see supplementary figure S8. (b) Same as panel (a), but for anodic-leading ICMS. (c) Sum of spikes over all 15 electrodes (and 30 trials) for cathodic-leading and anodic-leading ICMS on electrode 9, binned in 500 ms bins (mean over animals ± SEM).
layers) [44]. This might indicate that such observations are specific to the target structure.

Furthermore, the reduced response amplitude generated by anodic-leading stimulation could be considered another evidence for the preferential activation of fibers of passage over cell bodies. It is generally assumed that at any given depth below the cortical surface there are relatively more fibers of passage than cell bodies in the vicinity of the electrode. This should result in a bias towards the effectiveness of cathodic-leading stimulation, regardless of the stimulation depth. This is what we have found in the present data and it corresponds well to the non-uniform, sparse cell activation seen with ICMS [10, 22].

In deep layers the anodic-leading ICMS failed to raise the cortical response amplitude above our detection threshold, whereas in cathodic-leading stimuli such layer-dependent effect was not observed (figure 9). It is unlikely that the mechanism of stimulation changes with stimulation depth. If we assume that anodic-leading stimulation preferentially activates local cell bodies, then the cell bodies stimulated in the deep layers are most likely not significantly driving other neurons in the vicinity, i.e. failing to generate a local response. Cathodic-leading stimulation, on the other hand, activates axons from the local neurons, but also from other neurons projecting onto the cells in the vicinity of the electrode contact. These latter axons might explain why cathodic-leading stimulation activates deep layers, but anodic fails to do so.

The absence of a statistically significant influence of the leading-phase polarity in the experimental group ‘Varying current’ is likely to be related to the low stimulation current data (close to stimulation thresholds). It is reasonable to assume the influence of the leading phase polarity to increase with increasing stimulation current (figure 4(a)). Statistically, this should have led to a significant interaction between the stimulation current and the leading phase polarity. The here presented data showed a trend towards this interaction ($p \approx 0.07$). However, due to stimulation safety concerns, we limited the maximum amount of current applied per pulse.

**Implications for the development of neuroprosthetic devices**

The observed commonalities and differences between cathodic- and anodic-leading ICMS pulses have several
implications when considering to use ICMS in neuroprosthetic devices, i.e. stimulating cortical implants. The most robust observation of the present study was that cathodic-leading stimulation led to higher response amplitudes than anodic-leading ICMS. This suggests the use of cathodic-leading stimulation in potential clinical applications of ICMS. Achieving the same amount of cortical response with lower stimulation intensities is beneficial not only in terms of power consumption of the stimulation, but also in regard to the safety of the stimulation, both for the cortical tissue and the stimulation electrodes [13]. However, in a clinical setting there might be other factors to consider besides absolute thresholds, for example available therapeutic windows (difference between effect thresholds and side-effect thresholds), which might also be influenced by stimulus polarity.

The dynamic range of the electrical stimulation in the auditory cortex was found to be significantly smaller than the dynamic range of physiological, acoustic stimulation. But while the inter-experimental variation in ICMS was relatively low, acoustic stimulation showed a high variability of the dynamic range from one penetration to the next. This most likely reflects a difference in the amount of neuronal recruitment, which in the case of artificial electrical stimulation depends mostly on the extent of the supra-threshold extracellular potential due to a specific stimulation current. In the case of acoustic stimulation, on the other hand, the neuronal recruitment is dependent on several factors besides the stimulation intensity, like the specific electrochemical properties of the neurons or the cortical state at the time of stimulation [45, 46]. The dynamic range seen here for electrical stimulation of the auditory cortex is well within the range of values found for electrical stimulation in several other stations of the auditory pathway (table 1). It has to be kept in mind, however, that electrical stimulation does not always lead to cortical response saturation before a safety limit is reached with the stimulation current. Exact dynamic ranges are therefore hard to compare and the definitions might vary between studies. It is important to note that deaf patients can achieve useful speech recognition scores with cochlear implants also with a relatively narrow dynamic range [47].

Conclusion

Supra-threshold ICMS inside the auditory cortex using symmetrical, charge-balanced pulses evoked neuronal activity regardless of leading-phase polarity. However, cathodic-leading pulses were more effective than anodic-leading stimulation, particularly in deep cortical layers. Therefore, with the exception of special research conditions, cathodic-leading stimuli are preferable for ICMS when using symmetric biphasic pulses.

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Declarations of interest

None.

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References

[1] Histed M H, Ni A M and Maunsell J H R 2013 Insights into cortical mechanisms of behavior from microstimulation experiments Prog. Neurobiol. 103 115–30

[2] Lebedev M A and Nicolelis M A L 2017 Brain–machine interfaces: from basic science to neuroprostheses and neurorehabilitation Physiol. Rev. 97 767–837r

[3] Cicmil N and Krug K 2015 Playing the electric light orchestra—how electrical stimulation of visual cortex elucidates the neural basis of perception Phil. Trans. R. Soc. B 370 20140206
[4] Bartlett J R et al 2005 Psychophysics of electrical stimulation of striate cortex in macaques. *J. Neurophysiol.*, 94 3430–42

[5] Tehovnik E J 1996 Electrical stimulation of neural tissue to evoke behavioral responses. *J. Neurosci. Methods* 65 1–17

[6] Voigt M B, Hubka P and Kral A 2017 Intracortical microstimulation differentially activates cortical layers based on stimulation depth. *Brain Stimul.* 10 684–94

[7] Butovas S and Schwarz C 2003 Spatiotemporal effects of microstimulation in rat neocortex: a parametric study using multielectrode recordings. *J. Neurophysiol.*, 90 3024–39

[8] Tehovnik E J and Slocum W M 2009 Depth-dependent detection of microampere currents delivered to monkey V1. *Eur. J. Neurosci.* 29 1477–89

[9] Brock A A, Friedman R M, Fan R H and Roe A W 2013 Optical imaging of cortical networks via intracortical microstimulation. *J. Neurophysiol.* 110 2670–8

[10] Overstreet C K, Klein J D and Helms Tillery S I 2013 Computational modeling of direct neuronal recruitment during intracortical microstimulation in somatosensory cortex. *J. Neural. Eng.* 10 66016

[11] Yamamura D, Sano A and Tateno T 2017 An analysis of current source density profiles activated by local stimulation in the mouse auditory cortex in *vivo* Brain Res. 1659 96–112

[12] Koiwani M, Wilks S J, Woolley A J and Otto K J 2011 Multimodal, longitudinal assessment of intracortical microstimulation. *Prog. Brain Res.* 194 131–44

[13] Merrill D R, Bikson M and Jefferys J G R 2005 Electrical stimulation of excitable tissue: design of efficacious and safe protocols. *J. Neurosci. Methods* 141 171–98

[14] Lilly J C, Hughes J R, Galkin T W and Alvord E C 1955 Brief, single, 33–100 microsecond impulse to the thalamus causes depolarization of central nervous system neurons by nonuniform electric fields. *J. Physiol.* 76 878–88

[15] Rattay F 1975 The basic mechanism for the electrical stimulation of the nervous system. *Neuroscience* 83 335–46

[16] Rattay F and Weng C 2010 Which elements of the mammalian central nervous system are excited by low current stimulation with microelectrodes? *Neuroscience* 170 399–407

[17] Ranck J B 1975 Which elements are excited in electrical stimulation of mammalian central nervous system: a review. *Brain Res.* 98 417–40

[18] Anderson D N, Duffley G, Vorwerk J, Dorval A D and Butson C R 2019 Anodic stimulation misunderstood: preferential activation of fiber orientations with anodic waveforms in deep brain stimulation. *J. Neural Eng.* 16 016026

[19] Hussin A T, Boychuk J A, Brown A R, Pittman Q J and Teskey G C 2015 Intracortical microstimulation (ICMS) activates motor cortex layer 5 pyramidal neurons mainly transsynaptically. *Brain Stimul.* 8 742–50

[20] Jankowska E, Pedal Y and Tanaka R 1975 The mode of activation of pyramidal tract cells by intracortical stimuli. *J. Physiol.* 249 617–36

[21] Altman K W and Ponsrey R 1990 Analysis of excitable cell activation: relative effects of external electrical stimuli. *Med. Biol. Eng. Comput.* 28 574–80

[22] Warman E N, Grill W M and Durand D 1992 Modeling the effects of electric fields on nerve fibers: determination of excitability thresholds. *IEEE Trans. Biomed. Eng.* 39 1244–54

[23] Koivuniemi A S and Otto K J 2011 Asymmetric versus symmetrical pulses for cortical microstimulation. *IEEE Trans. Neural Syst. Rehabil. Eng.* 19 468–76

[24] Schmidt E M, Bak M J, Hambrecht F T, Kuhta C V, O’Rourke D K and Vallabhanath P 1996 Feasibility of a visual prosthesis for the blind based on intracortical microstimulation of the visual cortex. *Brain* 119 507–22

[25] Tehovnik E J, Slocum W M and Schiller P H 2003 Saccadic eye movements evoked by microstimulation of striate cortex. *Eur. J. Neurosci.* 17 870–8

[26] Yazdan-Shahmorad A et al 2011 Estimation of electrode location in a rat motor cortex by laminar analysis of
electrophysiology and intracortical electrical stimulation

[45] Buonomano D and Maass W 2009 State-dependent computations: spatiotemporal processing in cortical networks Nat. Rev. Neurosci. 10 113–25

[46] Arieli A, Sterkin A, Grinvald A and Aertsen A 1996 Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses Science 273 1868–71

[47] Loizou P C, Dorman M, Poroy O and Spahr T 2000 Speech recognition by normal-hearing and cochlear implant listeners as a function of intensity resolution J. Acoust. Soc. Am. 108 2377–87

[48] Takahashi H, Nakao M and Kaga K 2005 Accessing ampli-tonotopic organization of rat auditory cortex by microstimulation of cochlear nucleus IEEE Trans. Biomed. Eng. 52 1333–44