Evidence for the origin of the binaural interaction component of the auditory brainstem response

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Abstract
The binaural interaction component (BIC) represents the mismatch between auditory brainstem responses (ABR) obtained with binaural stimulation and the sum of ABRs obtained with monaural left and right stimulation. It is generally assumed that the BIC reflects binaural integration. Its potential use as a diagnostic tool, however, is hampered by the lack of direct evidence about its origin. While an origin at the initial site of binaural integration seems likely, there is no general agreement on the contribution of the two primary candidate nuclei, the lateral and medial superior olives (LSO and MSO, respectively). Here, we recorded local field potentials (LFP) and responses of units in the LSO and MSO of Mongolian gerbils (Meriones unguiculatus), presenting clicks with an interaural time or level difference (ITD and ILD, respectively), while simultaneously recording ABR. We determined the BIC from the ABR and, importantly, from LFP and responses of units in the LSO and MSO. If stimulus-induced changes in the ABR-derived BIC have their source in the LSO and/or MSO, we expect coherent changes in the unit-derived and the ABR-derived BIC. We find that BIC obtained from LSO units exhibits the same ITD and ILD dependence as the ABR-derived BIC. Neither BIC obtained from MSO units nor LFP-derived BIC recorded in either LSO or MSO did. The data thus strongly suggest that it is the activity of LSO units in the gerbil that is decisive for the generation of the ABR-derived BIC, determining its properties.

KEYWORDS
in vivo extracellular recordings, interaural level difference, interaural time difference, Mongolian gerbil

1 | INTRODUCTION

Auditory brainstem responses (ABRs) are a widely used measure of summed neural activity that allows for an objective assessment of a listener's hearing ability. They are measured non-invasively through electrodes placed on the scalp and can thus be obtained from both human and animal subjects. ABRs are characterized by distinct positive and negative deflections that represent the activity of different stages along the auditory pathway (reviewed in Möller, 2009).
1994). The so-called binaural interaction component (BIC) is associated with deflections in the ABR that represent the activity of stages of the auditory pathway that process binaural information (reviewed in Laumen, Ferber, et al., 2016). The BIC is revealed if the sum of responses to monaural left stimulation and monaural right stimulation is subtracted from the response to binaural stimulation (Dobie & Berlin, 1979). The difference in the summed activation under monaural versus binaural stimulation is thought to be due to neuron populations that represent the sensory input to each of the two ears, interacting in a non-additive way (e.g., Dobie & Berlin, 1979; Levine, 1981).

The actual generators of the BIC are still being discussed (Laumen, Ferber et al., 2016; Palanca-Castan, Laumen, Reed, & Köppl, 2016). Various findings point towards a source in the superior olivary complex (SOC), particularly the lateral and medial superior olives (LSO and MSO, respectively). The input patterns of LSO and MSO neurons provide the basis for non-additive summation of information originating in the left and the right ear. Neurons in the LSO receive excitatory inputs from the ipsilateral ear via the cochlear nucleus and inhibitory inputs from the contralateral ear via the medial nucleus of the trapezoid body (MNTB; Boudreau & Tsuchitani, 1968; Guinan, Norris, & Guinan, 1972). Neurons in the MSO receive bilateral excitatory inputs from both cochlear nuclei and bilateral inhibitory inputs via the MNTB and the lateral nucleus of the trapezoid body (Cant & Hyson, 1992; Kuwabara & Zook, 1992). Modelling studies, especially those of the LSO, were able to predict properties of the BIC, amongst others the amplitude and latency during the presentation of clicks with interaural time differences (ITD; Gaumond & Psaltikidou, 1991; cat: Ungan, Yagcioğlu, & Ozmen, 1997; guinea pig: Goksoy, Demirtas, Yagcioğlu, & Ungan, 2005; human: Riedel & Kollmeier, 2006; rodent species: Benichoux et al., 2018). The latest attempt at uncovering the source of the BIC compared BIC properties across rodent species that originates in the SOC, they cannot distinguish between the contributions of LSO and MSO neurons (e.g., carnivore and rodent species: Huang, 1980; guinea pig: Gardi & Berlin, 1981; Wada & Starr, 1983, 1989; cat: Melcher, 1996; Zaaroor & Starr, 1991). The latest attempt at uncovering the source of the BIC compared BIC properties across rodent species that have different relative sizes of LSO and MSO (Benichoux et al., 2018). The study argued in favour of the LSO but again failed to provide direct evidence that can only be achieved by comparing BIC properties with both LSO and MSO neuron response properties.

Here, we made use of the fact that properties of the BIC change depending on ITD and interaural level differences (ILD), as demonstrated in various species (guinea pig: Dobie & Berlin, 1979; human: Furst, Levine, & McGaffigan, 1985; cat: Ungan et al., 1997; guinea pig: Goksoy et al., 2005; human: Riedel & Kollmeier, 2006; gerbil: Laumen, Tollin, et al., 2016; barn owl: Palanca-Castan et al., 2016; rodent species: Benichoux et al., 2018). We recorded responses of units in the LSO and MSO of Mongolian gerbils and local field potentials (LFPs) from the same sites to clicks with an ITD or an ILD while simultaneously recording ABRs. We derived BICs not only from the ABRs but also from the responses of LSO and MSO units and the LFPs. If ITD/ILD-induced changes in the ABR-derived BIC have their origin in the LSO and/or MSO, we expect coherent changes in the BIC derived from responses of LSO/MSO units and the ABR-derived BIC. Coherent changes in the ABR-derived BIC and the BIC derived from units in the LSO would suggest that the output of LSO units is essential for the generation of the ABR-derived BIC while coherent changes in the ABR-derived BIC and the BIC derived from units in the MSO would hint at the MSO output as the origin of the ABR-derived BIC.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects

Seventeen adult, agouti-coloured Mongolian gerbils (10 females, seven males) aged between 12 and 61 (median: 15, 25th–75th quartile range: 14–25) weeks served as subjects. All animals were bred in the animal facilities of the University of Oldenburg, Germany, and originated from animals purchased at Charles River laboratories. The care and treatment of the animals were in accordance with the procedures of animal experimentation approved by the Government of Lower Saxony, Germany (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit).

### 2.2 | Anaesthesia

Initially, animals were anesthetized with a mixture of xylazine hydrochloride and ketamine hydrochloride (Rompun 2%, Bayer, xylazine 6 mg/kg body weight and Ketamin 10%, CP-Pharma, ketamine 135 mg/kg body weight i.p. initial dose). To ensure a constant level of anaesthesia and hydration of the animals during the experiments, xylazine hydrochloride and ketamine hydrochloride in saline (0.33 and 7.5 mg/ml, respectively) were provided and administered with a syringe pump (AL-1000; World Precision Instruments), run at 300–450 µl/hr, through a needle placed subcutaneously. The animals’ body temperature was kept between 36 and 38°C throughout the experiment using a homeothermic heating blanket (Harvard Apparatus).
2.3 | Surgery

The skull of the animal was stereotaxically positioned by earbars, exposed along the midsagittal line, and levelled using the epidural marker bregma and lambda as references. A small metal bolt was positioned on bregma, glued to the skull’s surface using superglue and additionally steadied with dental cement (Paladur, Kulzer). The animal was then transferred to the sound-attenuated booth (IAC 401-A; Industrial Acoustics Company) and fixed in the recording setup using the metal bolt and a holder. Centred on the midline of the skull approximately 2,500 μm caudal to the lambda suture, a craniotomy (diameter 2,000 μm) was made to target units in the superior olivary complex (SOC).

2.4 | Recordings in the SOC and auditory brainstem recordings

The brain was entered dorsally with tungsten electrodes (FHC) with tip diameters from ≈1 to 3 μm and impedances ranging from 1.3 to 3.9 MΩ to record LFP and extracellular responses simultaneously from sites in the left SOC. A silver wire (EP1, World Precision Instruments) positioned within skin tissue close to the recording site served as reference electrode. Measured from the skull’s surface, the SOC, containing LSO and MSO, can be found in a depth of 7,000–8,200 μm. The location of the LSO and MSO was guided by the response properties of other nuclei of the auditory brainstem, mainly the MNTB. The MNTB responds to contralateral stimulation only and runs medially along the entire rostrocaudal axis of the SOC. The LSO is located lateral to the caudal third of the MNTB. The MSO shows its largest extent lateral to the rostral half of the MNTB. Simultaneously to recordings in the SOC, during the presentation of clicks with an ITD or ILD (see below), ABRs were made between a needle electrode placed in the skin just rostral of the bregma and a needle electrode placed under the skin in the neck of the animal. Wetting the electrodes with saline solution ensured low impedances.

Extracellularly recorded responses were amplified depending on the amplitude of the action potentials (×10³ or ×10⁴) to avoid overloading the amplifier, and ABRs were always amplified by ×10⁴ (two ISO 80; World Precision Instruments). Both signals were band-pass filtered (10 Hz to 7 kHz) (two ISO 80; World Precision Instruments) and digitized at 48 kHz sampling rate using a Hammerfall DSP (Multiface II, RME). In the offline analysis, the voltage signal from the ABRs was band-pass filtered between 100 and 1,500 Hz (800th-order finite impulse response [FIR] filter). The voltage signal from the SOC was band-pass filtered between 50 and 400 Hz for the extraction of the LFP (800th-order FIR filter) and between 500 Hz and 3 kHz for the extraction of the action potentials (spikes; 800th-order FIR filter). Spike times were extracted after triggering at a visually determined level. Based on auditory nerve fibre refractoriness (Heil, Neubauer, Irvine, & Brown, 2007), a unit was considered well-isolated, that is a single unit, if less than 1% of the first-order interspike intervals occurred during the time between 0 and 0.5 ms after a spike in a time window starting with click onset and lasting 30 ms. Only five of 32 LSO units were single units because they fulfilled this criterion. They were of the EE (n = 2) and E0 (n = 3) types (see Section 2.8 below). The remaining 27 LSO units were multi-unit recordings. For the same reasons, 4/11 units recorded in the MSO were likely single units, the remaining seven units were multi-unit recordings. Two of the four single units were of the EE type and two of the E0 type.

2.5 | Acoustic stimulation

Stimuli were generated using Matlab (R2013b; The Mathworks). They were produced at a sampling rate of 48 kHz by a Hammerfall DSP (Multiface II, RME) and presented through a closed-field delivery system via earphones (IE800; Sennheiser). In situ measurements of the earphones’ frequency characteristics (ER-7C; Etymotic) were made by presenting a sine sweep (0.02–22 kHz, logarithmic scaling at 1 octave/s) using the Hammerfall DSP (Multiface II, RME). FIR filters (300th order) derived from the impulse responses were then used during the experiment to correct the earphones’ outputs. The output levels of the earphones were adjusted to be flat for frequencies between 0.3 and 16 kHz. Control measurements showed that second and third harmonic components in the signal had levels more than 30 dB lower than the level of the fundamental at the highest output level. Acoustical cross-talk was measured between a speaker and the opposite probe microphone (ER-7C; Etymotic). The average interaural attenuation was ≥20 dB for frequencies between 250 Hz and 16.3 kHz and ≥30 dB for frequencies between 800 Hz and 11.4 kHz.

2.6 | Click stimuli

To test binaural response properties, clicks were presented to the left and the right ear at 80 dB SPL (in some cases at 70 or 90 dB SPL). ILDs of 0, ±10, ±20 and ±30 dB or ITDs of 0, ±125, ±500, ±2,000, and in most cases, ±1,000 μs were applied. ILDs were produced by increasing the SPL in one channel by 1/2*ILD and by decreasing the SPL in the other channel by 1/2*ILD. ITDs were produced by shifting the signal by 1/2*ITD in opposing temporal directions in the two channels. Click stimuli presented to either ear served as monaural control stimulations. They had the same level as the stimuli used during binaural stimulation. Details of the technical aspects of the stimulation with clicks can be found elsewhere (Beutelmann, Laumen, Tollin, & Klump, 2015; Laumen, Tollin et al., 2016).
2.7 | Tonal stimuli

Responses recorded extracellularly in the SOC were also characterized by their frequency-level response areas. They were measured by presenting pure tones (duration 100 ms including 10 ms raised cosine ramps at the onset and offset) at multiple frequency-level combinations (17 frequencies in a three-octave range around the characteristic frequency [frequency at which the unit is most sensitive], seven levels in steps of 5–10 dB) in randomised order 10 times each. Additionally interleaved, 17 × 10 times no tone was presented to estimate spontaneous activity. The inter-stimulus intervals were 300 ms. Three frequency-level response areas were measured; one with the tones presented to the ipsilateral ear, one with the tones presented to the contralateral ear and one with the tones presented binaurally.

2.8 | Type of binaurality

The type of binaurality of an LSO or MSO unit was determined based on its responses to clicks and on its responses to tones. In the case of click-based responses, the number of action potentials elicited during each of the 500 presentations of clicks to the ipsilateral ear was compared with the number of action potentials elicited during each of the 500 presentations of clicks to both ears (ITD=0 μs, ILD=0 dB). The number of spikes elicited during a single presentation of a monaural click or binaural click pair ranged from 0 to 19 (median: 5.5) spikes in a 30 ms time window after stimulus onset. To test whether the number of action potentials elicited during 500 repeats of ipsilateral stimulation was greater than the number of action potentials elicited during 500 repeats of binaural stimulation, a left-tailed Mann-Whitney U test was used. If the test yielded \( p \leq .05 \) a unit was classified as an EE unit (Figure S1, second panel in bottom row). If neither of these conditions applied, a unit was classified as EE unit (Figure S1, third and fourth panels in bottom row). If the more than 60% of frequency-level combinations showing significant rate changes at all were positive, a unit was classified as EI unit (Figure S1, bottom left). If the test yielded \( p \leq .05 \). Changes in spontaneous rate were estimated using the same procedure applied to recordings during which no tone was presented. Spontaneous rate differences indicated changes unrelated to the acoustic stimulation (e.g., the acquisition of the binaural and the ipsilateral FLRAs at different points in time) and served to normalize rate changes during acoustic stimulation. Differences in response rates between binaural and ipsilateral stimulation were normalized by subtracting the mean spontaneous rate change from every single frequency-level combination and dividing by the standard deviation of the spontaneous rate changes. Positive resulting values indicated an increase in response rate under binaural stimulation with respect to response rates observed under ipsilateral stimulation; negative values demonstrated a decrease in response rate. Values of greater than 2 or less than −2 indicated that the increase and decrease in response rates, respectively, exceeded rate changes occurring without acoustic stimulation by two standard deviations, thus corresponding to a rate increase or decrease at a significance level of \( p \leq .05 \).

If more than 60% of frequency-level combinations showing significant rate changes at all had negative normalized rate differences, a unit was classified as E0 unit (Figure S1, bottom left). If the test yielded \( p \leq .05 \). Changes in spontaneous rate were estimated using the same procedure applied to recordings during which no tone was presented. Spontaneous rate differences indicated changes unrelated to the acoustic stimulation (e.g., the acquisition of the binaural and the ipsilateral FLRAs at different points in time) and served to normalize rate changes during acoustic stimulation. Differences in response rates between binaural and ipsilateral stimulation were normalized by subtracting the mean spontaneous rate change from every single frequency-level combination and dividing by the standard deviation of the spontaneous rate changes. Positive resulting values indicated an increase in response rate under binaural stimulation with respect to response rates observed under ipsilateral stimulation; negative values demonstrated a decrease in response rate. Values of greater than 2 or less than −2 indicated that the increase and decrease in response rates, respectively, exceeded rate changes occurring without acoustic stimulation by two standard deviations, thus corresponding to a rate increase or decrease at a significance level of \( p \leq .05 \).

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If more than 60% of frequency-level combinations showing significant rate changes at all had negative normalized rate differences, a unit was classified as E0 unit (Figure S1, second panel in bottom row). Thus, all units were classified.

2.9 | Binaural interaction components

The BIC was derived from ABRs by subtracting the sum of responses to monaural left and monaural right stimulation from the response to binaural stimulation. To this end, the responses, that is the voltage traces, to each of 500 repetitions of monaural left, monaural right and binaural click presentations per ITD and ILD condition were averaged after collection (for details see Laumen, Tollin et al., 2016; Figure 1, top). Likewise, in the case of LFPs, voltage traces were collected and averaged (Figure 1, middle). In the case of spiking activity, peri-stimulus time histograms (PSTH) with a bin-width of 25 μs were constructed by collecting spike times from responses to the 500 presentations per stimulus condition. PSTH were subsequently smoothed with a Hann window of 0.5 ms duration (Figure 1, bottom). PSTH and averaged voltage traces from LFP recordings were then used for calculating an initial BIC for all ITD and ILD conditions, that is by subtracting the sum of the responses to monaural left...
and monaural right stimulation from responses to binaural stimulation. While the ABR-derived BIC occurs during the activation of both the left and the right brainstem, LFPs and spiking activity were only recorded from the left brainstem. Assuming mirror-image like activity in the right brainstem with respect to ITD and ILD effects, the final LFP-derived and PSTH-derived BIC were calculated by adding the initial BIC measured in the left brainstem for a certain ILD or ITD with the initial BIC for the corresponding sign-inverted ILD or ITD. Because this leads to the loss of information about the side a click stimulus originates from (i.e., if the click has a positive or negative ILD or ITD), the BIC derived from...
the ABR was averaged across positive and negative ITD and ILD values. The terms BIC\(_{\text{ABR}}\), BIC\(_{\text{LFP}}\) and BIC\(_{\text{PSTH}}\) will be used in the following for BIC that were derived from ABRs, the final BIC derived from LFPs, and the final PSTH-derived BIC, respectively.

For further analysis, the amplitude and the latency of the largest negative deflection of the BIC in an analysis window starting 2 ms after stimulus onset and ending 10 ms after stimulus onset were extracted. In the BIC\(_{\text{ABR}}\), this negative deflection is often called DN1 and used to characterize the BIC\(_{\text{ABR}}\) (Laumen, Tollin et al., 2016). BIC amplitudes were standardized, that is, the signed number of standard deviations was calculated by which a BIC amplitude deviated from the mean absolute amplitude that was observed for a single unit, a single site in the case of the LFP or a single recording instant in the case of the ABR across the different ITD or ILD values tested. The standardization was chosen because the absolute amplitudes of the BIC\(_{\text{ABR}}\), the BIC\(_{\text{LFP}}\) and the BIC\(_{\text{PSTH}}\) were very different, multiple orders of magnitude, due to the acquisition of the raw data they were based on. BIC\(_{\text{ABR}}\) and BIC\(_{\text{LFP}}\) latencies differed from BIC\(_{\text{PSTH}}\) latencies (Figures 2 and 3). This can be attributed to the differences in band-pass filtering of the underlying waveforms during the offline processing of the signals. BIC latencies were therefore also standardized.

### 2.10 Statistical analysis

Standardized BIC amplitudes and standardized BIC latencies obtained while either recording from units in the LSO or MSO were submitted to general linear mixed-effects model analyses of variance (GLMM ANOVA, SPSS version 24.0, procedure mixed) with fixed factors absolute ITD (0, 125, 500, 1,000, 2,000 \(\mu\)s) or absolute ILD (0, 10, 20, 30 dB) and type of recording (ABR, LFP, PSTH). Because both single- and multi-units were recorded in the LSO and MSO, the additional fixed factor type of unit (single unit, multi-unit) was initially tested for effects on BIC\(_{\text{PSTH}}\) amplitudes and BIC\(_{\text{PSTH}}\) latencies. All eight GLMM ANOVA run on standardized BIC\(_{\text{PSTH}}\) amplitudes or standardized BIC\(_{\text{PSTH}}\) latencies yielded a \(p\)-value for the fixed factor type of unit exceeding the Bonferroni-corrected alpha level of 0.00625 (range of \(p\)-values: \(0.027 \leq p \leq 0.995\)). The fixed factor type of unit was therefore disregarded in any of the following analyses. Due to using the \(z\)-scores of BIC amplitudes and BIC latencies, the influence of the type of recording cannot have an effect but can only be inferred from the interaction of the fixed factors ITD or ILD and type of recording on BIC amplitudes or BIC latencies. Statistical differences between types of recording were tested with subsequent GLMM ANOVAs that included BIC amplitudes or BIC latencies from only two of the three types of recording, that is, ABR-derived BIC and LFP-derived BIC and ABR-derived BIC and PSTH-derived BIC.

Three of 32 LSO units were excluded from the statistical analysis of BIC amplitudes and BIC latencies during ITD stimulation because ITD of \(\pm 1,000\ \mu\)s was not tested. Two of 32 LSO units were not tested during ILD stimulation.

### 2.11 Verification of recording sites

Electrolytic lesions (\(\pm 7\) to \(\pm 13\) \(\mu\)A, \(5 \times 5\) s) were made in order to verify recording sites. They were placed at least 30 min before sacrificing the animal. In the majority of animals, the electrolytic lesions were placed right after finishing recording in a track. The time between making the lesion and sacrificing the animal was therefore often a few hours.
Animals were sacrificed with a lethal dose of sodium pentobarbital (Narcoren, Merial, 60–85 mg/100 g body weight, i.p.) and perfused transcardially with 0.1 M phosphate buffer followed by paraformaldehyde (4% in 0.1 M phosphate buffer, pH 7.4). Brains were post-fixed in paraformaldehyde, later immersed in 30% sucrose solution and then embedded in gelatin. The tissue was cut in two serial transverse sections (25 μm) using a freezing microtome (Leica CM 1950). Sections of both series were stained with cresyl violet. Sections of one series were additionally stained with luxol fast blue by Kluver and Barrera to reveal nerve fibres in order to facilitate locating the electrolytic lesions (Mulisch & Welsch, 2010). Sections were then examined under the light microscope.

3 | RESULTS

While simultaneously registering ABRs, we recorded responses of 78 units in the SOC and LFPs from the same sites in 17 Mongolian gerbils. Based on histological examinations, of those units, 32 units were identified as LSO units and 11 as MSO units. The remaining units were recorded either in regions of the SOC belonging to neither LSO nor MSO or were units whose recording site could not be recovered. LSO units were mainly recorded from the lateral limb of the LSO with characteristic frequencies between 0.5 and 16 kHz (median: 1.6, quartile range: 0.9–4.7 kHz). MSO units had characteristic frequencies between 0.7 and 5.0 kHz (median: 1.6, quartile range: 0.9–1.9 kHz).

3.1 | Relating single-unit responses with neural measures of summed activity

The aim of the study was to directly compare ABR with LFP and responses obtained from units in the same two potential source regions of the BIC<sub>ABR</sub>, the LSO and the MSO. Responses of units can be considered the output of a nucleus, while the LFP are assumed to be derived from the synaptic currents of a small population of neurons in the vicinity of the recording electrode. To allow for a comparison between the three measures of neural activity, the responses were analysed in the same way. In the case of responses of units, PSTHs formed the basis for the analysis (Figure 1, bottom). In the case of LFPs and ABRs, the averaged voltage traces were used (Figure 1, middle and top). The BIC resulted from the difference in voltage trace/PSTH obtained with binaural stimulation and the sum of voltage trace/PSTH obtained with summed monaural left and monaural right stimulation (Figure 1, left column, dash-dotted [orange] lines). We presented clicks with varying ITD or ILD in order to compare changes in BIC<sub>ABR</sub> with changes of BIC<sub>PSTH</sub> and BIC<sub>LFP</sub> obtained from LSO and MSO. Coherent changes in different BIC due to the manipulation of ITD or ILD should provide an indication about the origin of those changes and thus about the origin of the BIC<sub>ABR</sub>

A common and prominent feature of all BIC, that is, the BIC<sub>ABR</sub>, the BIC<sub>LFP</sub> and the BIC<sub>PSTH</sub>, was the occurrence of distinct peaks and troughs (Figure 1, right column). For further analysis, all BICs were characterized by the amplitude and latency of the largest negative deflection in each stimulus condition (Figures 1 (triangles), 2 and 3). BIC amplitudes were standardized for further analysis (see Section 2). Thus, the analysis focused on the manner the BIC amplitude changed across ITD or ILD. Note that positive z-scores mark low and negative z-scores mark high BIC amplitude values due to mean BIC amplitudes, used for the standardization, being negative. For this reason and to get a better intuition for standardized BIC amplitudes, we will speak of small standardized BIC amplitudes in the case of positive z-scores and of large standardized BIC amplitudes in the case of negative z-scores in the following. For the same reasons, the ordinates were reversed in the figure panels showing standardized BIC amplitudes (Figures 2–5). In the following analyses and descriptions, we will use the term BIC amplitudes to refer to the standardized values.

3.2 | BIC properties varying with ITD

Numerous studies showed that BIC<sub>ABR</sub> properties change characteristically with ITD (e.g., Furst et al., 1985; Laumen, Tollin et al., 2016; Riedel & Kollmeier, 2006) rendering ITD the ideal acoustic cue to manipulate in order to correlate potential changes in BIC<sub>LFP</sub> and BIC<sub>PSTH</sub> with changes in the BIC<sub>ABR</sub>. In the present study, BIC amplitudes depended on the absolute ITD (Figure 2, light to dark colour), the type of recording (ABR, LFP, PSTH) and the site of recording (LSO, MSO) (Figure 4, top row). Smallest BIC amplitudes for all recording types and recording sites were observed at an absolute ITD of 2,000 μs. BIC<sub>ABR</sub> amplitudes decreased as the absolute ITD increased from 0 to 1,000 μs (Figure 4, top row, ABR, LFP, dashed lines and diamonds). Consequently, largest BIC<sub>ABR</sub> amplitudes were observed at an ITD of zero. The BIC<sub>ABR</sub> obtained from scalp electrodes while recording from LSO or MSO units showed no differences in amplitude. This was expected because the BIC<sub>ABR</sub> simply stem from ABR recordings obtained at different points in time from the same or a similar set of animal subjects. The ITD dependence of BIC<sub>LFP</sub> amplitudes obtained while recording from LSO and MSO units was different from the ITD dependence of BIC<sub>ABR</sub> amplitudes. They remained rather constant at ITDs from 0 to 1,000 μs with mean z-scores roughly between 0 and 0.5 (Figure 4, top row, LFP, dash-dotted lines and triangles). BIC<sub>PSTH</sub> amplitudes depended strongly on ITD and, importantly, on the recording site (LSO, MSO). In LSO units, BIC<sub>PSTH</sub> amplitudes decreased with increasing
ITD (Figure 4, top row, PSTH, circles and solid lines) and therefore mirrored very much the behaviour of BIC<sub>ABR</sub> amplitudes. In MSO units, however, BIC<sub>PSTH</sub> amplitudes were rather constant at ITD between 0 and 1,000 μs with z-scores between 0 and 0.5 and thus did not mirror the behaviour of BIC<sub>ABR</sub> amplitudes.

To statistically verify those observations, standardized BIC amplitudes were submitted to two GLMM ANOVAs, one for BIC amplitudes obtained while recording from LSO units and one for BIC amplitudes obtained while recording from MSO units (see Section 2). The GLMM ANOVA including BIC amplitudes obtained while recording from LSO units detected a significant effect of ITD \( F_{4,456} = 122.958, p < .001 \) and an interaction between ITD and type of recording \( F_{8,456} = 7.915, p < .001 \). To clarify whether the BIC<sub>ABR</sub> resembles the BIC<sub>LFP</sub> or the BIC<sub>PSTH</sub>, two subsequent GLMM ANOVAs were done. The first GLMM ANOVA included BIC<sub>ABR</sub> and BIC<sub>LFP</sub> amplitudes and detected a significant effect of ITD \( F_{4,280} = 78.396, p < .001 \) and, notably, a significant interaction between the type of recording and ITD \( F_{4,280} = 12.274, p < .001 \) indicating that BIC<sub>ABR</sub> amplitudes and BIC<sub>LFP</sub> amplitudes changed differently with ITD.

In contrast, the second GLMM ANOVA, including BIC<sub>ABR</sub> and BIC<sub>PSTH</sub> amplitudes detected a significant effect of ITD \( F_{4,280} = 116.777, p < .001 \) but failed to detect a significant interaction between the type of recording and ITD \( p = .128 \) indicating a similar ITD dependence of BIC<sub>ABR</sub> and BIC<sub>PSTH</sub> amplitudes.

The GLMM ANOVA including BIC amplitudes derived from ABR, LFP and PSTH obtained while recording from MSO units detected a significant effect of ITD \( F_{4,150} = 43.802, p < .001 \) and the interaction between ITD and type of recording \( F_{8,150} = 4.418, p < .001 \) on BIC amplitudes. Again to clarify whether the BIC<sub>ABR</sub> resembles the BIC<sub>LFP</sub> or the BIC<sub>PSTH</sub>, two subsequent GLMM ANOVAs were run. ITD significantly influenced BIC amplitudes in both GLMM ANOVAs [ABR and LFP: \( F_{4,100} = 75.002, p < .001 \); ABR and PSTH: \( F_{4,100} = 26.374, p < .001 \)] and so did the interaction of ITD and type of recording [ABR and LFP: \( F_{4,100} = 10.332, p < .001 \); ABR and PSTH: \( F_{4,100} = 5.521, p < .001 \)] confirming the results described above that the ITD dependence of BIC<sub>ABR</sub> amplitudes differs both from the ITD dependence of BIC<sub>PSTH</sub> and BIC<sub>LFP</sub> amplitudes obtained while recording in the MSO.

In summary, the coherent changes of BIC<sub>ABR</sub> and BIC<sub>PSTH</sub> amplitudes obtained while recording in the LSO with ITD and the lack of such coherent changes of BIC<sub>ABR</sub>, BIC<sub>LFP</sub> and BIC<sub>PSTH</sub> amplitudes obtained while recording in the MSO suggest that the output of LSO units, but not MSO units, forms the basis for the ITD dependence of the BIC<sub>ABR</sub>.
lack of different ITD dependences of BIC_{ABR}, BIC_{LFP} and BIC_{PSTH} latencies prevents a similar conclusion.

### 3.3 BIC properties varying with ILD

Compared with studies on the ITD dependence of the BIC_{ABR}, there are only few studies that measured the ILD dependence of the BIC_{ABR} (Furst et al., 1985; Laumen, Tollin et al., 2016). This seems surprising considering that neurons in the LSO, which are often favoured as the origin of the BIC (e.g., Benichoux et al., 2018; Ungan et al., 1997; Zaaroor & Starr, 1991), are particularly sensitive to ILD (e.g., Caird & Klinke, 1983; Irvine, Park, & McCormick, 2001; Sanes & Rubel, 1988). In the present study, BIC_{ABR} amplitudes tended to decrease with increasing ILD, as did BIC_{PSTH} amplitudes obtained while recording in the LSO. BIC_{LFP} amplitudes did not vary much with ILD and neither did BIC_{PSTH} amplitudes obtained while recording in the MSO (Figure 5, top row).

Statistical testing showed that BIC amplitudes obtained while recording from LSO units depended on ILD \([F_{3,368} = 31.135, p < .001, \text{GLMM ANOVA}]\) and the interaction of ILD and type of recording \([F_{6,368} = 8.535, p < .001, \text{GLMM ANOVA}]\). To clarify whether the BIC_{ABR} resembles the BIC_{LFP} or the BIC_{PSTH}, two more GLMM ANOVAs were conducted including BIC amplitudes of two of the three types of recordings. Both GLMM ANOVAs detected a significant effect of ILD \([\text{ABR and LFP: } F_{3,248} = 13.643, p < .001; \text{ABR and PSTH: } F_{3,244} = 56.486, p < .001]\) and the interaction of ILD and type of recording \([\text{ABR and LFP: } F_{3,248} = 11.123, p < .001; \text{ABR and PSTH: } F_{3,244} = 3.697, p = .012]\) on BIC amplitudes. Thus, the ILD dependence of BIC_{LFP} and BIC_{PSTH} amplitudes obtained while recording from LSO units was different from the ILD dependence of BIC_{ABR} amplitudes. The same picture emerged when submitting BIC amplitudes obtained while recording from MSO units to GLMM ANOVAs. BIC_{ABR}, BIC_{LFP} and BIC_{PSTH} amplitudes all differed in their ILD dependence, evident from the significant effects the interaction of ILD and type of recording had on BIC amplitudes \([\text{ABR, LFP, PSTH: } F_{6,120} = 3.351, p = .004; \text{ABR and LFP: } F_{3,80} = 6.962, p < .001; \text{ABR and PSTH: } F_{3,80} = 4.564, p = .005]\) and the effect of ILD \([\text{ABR, LFP, PSTH: } F_{3,80} = 2.989, p = .034; \text{ABR and LFP: } F_{3,80} = 3.685, p = .015; \text{ABR and PSTH: } F_{3,80} = 7.017, p < .001]\).

BIC_{ABR}, BIC_{LFP} and BIC_{PSTH} latencies did not change consistently with ILD in either nucleus (Figure 3, colour coding/greyscale). Because BIC_{PSTH} latencies tended to be smaller than BIC_{ABR} and BIC_{LFP} latencies (Figure 3) due to the different band-pass filtering of the underlying waveforms, BIC latencies were standardized. A GLMM ANOVA detected a significant influence of the interaction of ILD and type of recording on standardized BIC latencies obtained while recording in the LSO \([F_{3,368} = 3.973, p = .001]\). Subsequent GLMM ANOVAs, conducted to clarify whether the BIC_{ABR} resembles the BIC_{LFP} or the BIC_{PSTH}, detected a significant interaction of ILD and type of recording \([\text{ABR and LFP: } F_{3,248} = 8.461, p < .001; \text{ABR and PSTH: } F_{3,244} = 6.181, p = .001]\) but not when BIC_{ABR} and BIC_{PSTH} latencies were tested \((p = .207)\) indicating that the ILD dependence of BIC_{ABR} and BIC_{PSTH} latencies was not different. Standardized BIC latencies obtained while recording in the MSO were significantly influenced by the interaction of ILD and type of recording \([F_{6,120} = 3.552, p = .003, \text{GLMM ANOVA}]\). Both subsequent GLMM ANOVAs conducted to clarify whether the BIC_{ABR} resembles the BIC_{LFP} or the BIC_{PSTH} detected a significant interaction of ILD and type of recording on BIC latencies \([\text{ABR and LFP: } F_{3,80} = 6.181, p = .001; \text{ABR and PSTH: } F_{3,80} = 4.690, p = .005]\) indicating that the ILD dependence

FIGURE 5 Binaural interaction component (BIC) amplitudes and BIC latencies during interaural level difference (ILD) stimulation. Average z-transformed amplitudes (top) and averaged z-transformed latencies (bottom) of the largest negative deflection of the BIC_{ABR} (diamonds), the BIC_{LFP} (triangles) and the BIC_{PSTH} (circles) during stimulation with clicks with an ILD for lateral superior olive units (left column, 31 units) and for medial superior olive units (right column, 11 units). Error bars denote standard error of the mean. [Colour figure can be viewed at wileyonlinelibrary.com]
of BIC$_{\text{LFP}}$ and BIC$_{\text{PSTH}}$ latencies differs from the one observed in BIC$_{\text{ABR}}$ latencies.

In summary, ILD stimulation does not seem to be suitable to conclusively judge the contribution of LSO and MSO units to the emergence of BIC$_{\text{ABR}}$ properties. This is justified by the facts that neither BIC$_{\text{PSTH}}$ amplitudes obtained in the LSO nor BIC$_{\text{PSTH}}$ amplitudes obtained in the MSO resembled BIC$_{\text{ABR}}$ amplitudes and the lack of ILD dependence in either nucleus due to the inconsistent ILD dependences of BIC$_{\text{ABR}}$, BIC$_{\text{LFP}}$ and BIC$_{\text{PSTH}}$ latencies.

### 3.4 Binaural response properties and their influence on BIC amplitudes and BIC latencies

In the LSO, units typically respond to binaural tonal stimulation with lower response rates than to ipsilateral stimulation alone because under binaural stimulation the excitatory inputs that LSO units receive from the ipsilateral cochlear nucleus interact with inhibitory inputs received from the contralateral cochlear nucleus via the MNTB. When assessing the influence of contralateral stimulation on responses of LSO units to ipsilateral stimulation by comparing responses to clicks presented ipsilaterally with responses to binaurally presented clicks (Figure S2, first three rows), we found that only 5 of 32 LSO units were classified as EI units. Nineteen units were classified as EE units. Eight units were classified as E0 units. Classification based on tonal stimulation identified 15 EI units, 12 EE units and five E0 units (examples for all three types in Figure S1). Those numbers indicate that the binaural response properties based on tonal stimulation did not necessarily match the binaural response properties based on click responses (Table S1). While for EE and E0 responses, the classification based on tones matched the classification based on clicks, many of the units classified EI with tones were classified E0 (4/15) or EE (7/15) when stimulated with clicks.

In the MSO, units respond to binaural stimulation with higher response rates than the response rates observed during ipsi- or contralateral stimulation alone. Nine out of 11 units recorded in the MSO with click stimulation were classified as EE units, the remaining two as E0. In the case of the classification according to tonal stimulation, all units (11/11) were classified as EE units.

The type of binaurality might be decisive for the way BIC$_{\text{PSTH}}$ properties change with ITD or ILD. We therefore tested its effect on BIC$_{\text{PSTH}}$ amplitudes and BIC$_{\text{PSTH}}$ latencies by conducting GLMM ANOVAs with the fixed factors ITD or ILD and type of binaurality (EI, EE, E0). The units were classified according to the type of binaurality observed under click stimulation because the data presented were also based on responses to click stimuli. LSO units of any type of binaurality (EE, EI, E0) showed a very similar ITD and ILD dependence of BIC$_{\text{PSTH}}$ amplitudes and BIC$_{\text{PSTH}}$ latencies (Figures S3 and S4, blue). Statistically testing indeed showed that the interaction of type of binaurality with either ITD or ILD did not significantly influence BIC$_{\text{PSTH}}$ amplitudes in LSO units (all $p \geq .719$). The same was true for BIC$_{\text{PSTH}}$ latencies in LSO units both during ITD and ILD stimulation (all $p \geq .785$; Figures S3 and S4). BIC$_{\text{PSTH}}$ amplitudes and BIC$_{\text{PSTH}}$ latencies obtained while recording in the MSO were not statistically tested due to the small sample size in the E0 group ($n = 2$). In summary, this indicates that BIC amplitudes and BIC latencies obtained while recording from units with different types of binaurality showed the same ITD or ILD dependence in LSO units. This suggests that the differences in the observed ITD dependence of BIC$_{\text{ABR}}$, BIC$_{\text{LFP}}$ and BIC$_{\text{PSTH}}$ amplitudes described above are determined by the site of recording, that is the LSO and MSO.

### 4 DISCUSSION

The search for the origin of the BIC$_{\text{ABR}}$ reaches almost as far back as its discovery (reviewed in Laumen, Ferber et al., 2016). More and more evidence has accumulated that the LSO in the auditory brainstem might be its source (computational model: Gaumont & Psaltikidou, 1991; cat: Ungan et al., 1997; guinea pig: Goksoy et al., 2005; human: Riedel & Kollmeier, 2006; rodent species: Benichoux et al., 2018). Here, we provide direct evidence that the BIC$_{\text{ABR}}$ originates in the output of the LSO. We used the Mongolian gerbil as a model system because of its well-developed LSO and MSO. We recorded responses of units and LFPs, as indicators of neural outputs and local neural activity, respectively, of the LSO and the MSO during the presentation of clicks with an ITD or an ILD while simultaneously recording ABRs. We not only derived the BIC from the ABR but also determined the BIC from the PSTHs of unit responses and from LFPs. The BIC$_{\text{PSTH}}$ obtained from LSO units showed the same ITD dependence as the BIC$_{\text{ABR}}$ (Figures 3 and 4). Importantly, this was neither the case for the BIC$_{\text{PSTH}}$ obtained from units in the MSO nor for the BIC$_{\text{LFP}}$ recorded in either LSO or MSO excluding LFPs and the output of the MSO as the origin of the BIC$_{\text{ABR}}$. The data thus strongly support the view that it is the activity of the projection neurons in the LSO that is decisive for the generation of the BIC$_{\text{ABR}}$, determining its properties.

Earlier approaches investigating the source of the BIC$_{\text{ABR}}$ derived their conclusions (I) from observations made after lesioning potential source regions (e.g., carnivore and rodent species: Huang, 1980; guinea pig: Gardi & Berlin, 1981; guinea pig: Wada & Starr, 1989; cat: Melcher, 1996), (II) from correlating the BIC$_{\text{ABR}}$ with ABR waves (e.g., guinea pig: Gardi & Berlin, 1981; humans: Jones & Van der Poel, 1990; Levine, 1981), whose sites of generation are thought to be known (reviewed in Moller, 1994), (III) from comparing...
BIC$_{ABR}$ properties with properties of LFP recorded from candidate source regions (cat: Sontheimer, Caird, & Klinke, 1985; Ungan & Yagcioglu, 2002), and (IV) from modelling studies that included type of synaptic interaction of candidate source regions (Benichoux et al., 2018; Gaumond & Psaltikidou, 1991; Goksoy et al., 2005; Riedel & Kollmeier, 2006; Ungan et al., 1997). These studies accumulated evidence that the integrity of the SOC is essential for the occurrence of the BIC$_{ABR}$ and strengthened the original idea that binaural integration, likely under inhibitory influence, gives rise to the BIC$_{ABR}$. Because both the LSO and the MSO receive inputs from the left and the right ear allowing for the integration of binaural information (e.g., Boudreau & Tsuchitani, 1968; Caird & Klinke, 1983; Guinan et al., 1972; Irvine et al., 2001; Joris & Yin, 1995), they are likely candidate source regions.

Our approach differed from previous studies in that we compared the properties of the BIC$_{ABR}$ under ITD or ILD stimulation with the properties of BIC derived from responses of LSO and MSO units during the same stimulation. It is based on two considerations: firstly, the amplitude and latency of the BIC$_{ABR}$ change characteristically with the ITD and, to a lesser extend, with the ILD of the stimulus. Observed consistently across species, irrespective of the relative sizes of their LSO and MSO (Irving & Harrison, 1967) or their head size (Benichoux et al., 2018; Laumen, Ferber et al., 2016), the BIC$_{ABR}$ amplitude decreases with increasing ITD or ILD and the latency of the BIC$_{ABR}$ increases with increasing ITD (guinea pig: Dobie & Berlin, 1979; human: Furst et al., 1985; cat: Ungan et al., 1997; guinea pig: Goksoy et al., 2005; human: Riedel & Kollmeier, 2006; gerbil: Laumen, Tollin et al., 2016; barn owl: Palanca-Castan et al., 2016; rodent species: Benichoux et al., 2018). And secondly, any such dependence observed in a measure derived from a far-field potential such as the BIC$_{PSTH}$ might not be representative of the overall activity of a limited number of neurons that is registered for the integration of binaural information (e.g., Boudreau & Tsuchitani, 1968; Caird & Klinke, 1983; Guinan et al., 1972; Irving et al., 2001; Joris & Yin, 1995). Therefore, we compared the properties of the BIC$_{ABR}$, interactions of bilateral excitatory inputs, that is EE processes, cannot be excluded as contributing to or even generating the BIC$_{ABR}$. For example, owls have negative BIC$_{ABR}$ though EE processes are thought to be dominant in the lower stages of their auditory brainstems, that is, the likely source of the BIC$_{ABR}$ (Palanca-Castan et al., 2016). Furthermore, models assuming an EE process as the basis for the generation of the BIC$_{ABR}$ can predict its negative amplitude (Gaumond & Psaltikidou, 1991). While the present data provide clear evidence that the BIC$_{ABR}$ originates from LSO neurons, irrespective of their unit type (EI, EE, E0), resembled BIC$_{ABR}$ response characteristics while MSO units did not (data not shown).

Although EI processes are thought to play a major role in the generation of the BIC$_{ABR}$, interactions of bilateral excitatory inputs, that is EE processes, cannot be excluded as contributing to or even generating the BIC$_{ABR}$. For example, owls have negative BIC$_{ABR}$ though EE processes are thought to be dominant in the lower stages of their auditory brainstems, that is, the likely source of the BIC$_{ABR}$ (Palanca-Castan et al., 2016). Furthermore, models assuming an EE process as the basis for the generation of the BIC$_{ABR}$ can predict its negative amplitude (Gaumond & Psaltikidou, 1991). While the present data provide clear evidence that the BIC$_{ABR}$ originates from LSO neurons, irrespective of their unit type (EI, EE, E0), resembled BIC$_{ABR}$ response characteristics while MSO units did not (data not shown).
in the LSO but not the MSO, it is not unlikely that both EE processes and EI processes complementing one another contribute to its generation.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

DATA ACCESSIBILITY

All data reported in this study that have been used for statistical analysis will be available on the journal’s Figshare page.

AUTHOR CONTRIBUTIONS

S.T. and G.M.K. designed research; S.T. performed research and analysed data; S.T. drafted the manuscript; S.T. and G.M.K. revised the paper.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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