Title:
Auditory evoked fields measured non-invasively with small-animal MEG reveal rapid repetition suppression in the guinea pig.

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Running title:
Repetition suppression in small-animal MEG.

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Abstract

In animal models, single-neuron response properties such as stimulus-specific adaptation (SSA) have been described as possible precursors to the mismatch negativity (MMN), a human brain response to stimulus change. Here, we attempt to bridge the gap between human and animal studies by characterising responses to changes in the frequency of repeated tone series in the anaesthetised guinea pig using small-animal magnetoencephalography (MEG). We show that: (1) auditory evoked fields (AEFs) qualitatively similar to those observed in human MEG studies can be detected non-invasively in rodents using small-animal MEG; (2) guinea-pig AEF amplitudes reduce rapidly with tone repetition, and this AEF reduction is largely complete by the second tone in a repeated series; and (3) differences between responses to the first (deviant) and later (standard) tones after a frequency transition resemble those previously observed in awake humans using a similar stimulus paradigm.

Keywords: adaptation; magnetoencephalography; roving standard

Introduction

The mismatch negativity (MMN) is one of the most investigated human brain responses. Classically, it is measured in the context of an “oddball paradigm” in which the brain response to rare deviant sounds is contrasted with that to a series of more common standards, revealing a negative shift in the evoked potential in the range of 100–250 ms post sound onset. The mechanisms that produce the MMN are the subject of some debate (Näätänen et al 1978; May and Tiitinen 2010), but it is
generally interpreted as reflecting the violation of the predictions of a model tuned to an ongoing stimulus regularity (Näätänen et al 2007; see also Lieder et al 2013). The MMN therefore has theoretical and practical appeal as a measure of brain activity that is hypothesized to tap sensory learning and adaptation to the statistics of the acoustic input. It has been put forward as a means of uncovering the mechanisms that enable listeners to adapt to complex listening environments (Lieder et al 2013; Garrido et al 2013), as a tool for probing perceptual representations, and as an assay for brain function in patients with neurological disorders (Boly et al 2011; Näätänen et al 2012).

Recently, the “roving-standard” paradigm (Fig. 1B) has gained popularity as an alternative to the “oddball” method of eliciting the MMN. The roving-standard paradigm involves the use of sequences of repeated tone series with occasional step-changes in frequency; tones immediately after frequency transitions are deviant, and repeated tones are standards. Both tone frequencies are equally represented in the stimulus, thus controlling for physical differences between standard and deviant tones, and the experimental yield is higher than with the oddball paradigm (Cowan et al 1993; Haenschel et al 2005; Garrido et al 2008). The MMN increases in size with increasing number of preceding standards, and recent research has shown that this is mostly due to a reduction in the response to the repeated standards (“repetition suppression”), rather than an increase in the response to the deviants (e.g. Haenschel et al 2005; Costa-Faidella et al 2011). This finding carries important implications for understanding the mechanisms that give rise to the MMN response (Haenschel et al 2005; Lieder et al 2013).

In animal models, large brain-volume scale (synaptic potential-based) MMN
studies using the classic oddball paradigm have yielded contradictory results (see review in Nelken and Ulanovsky 2007). Several investigations have failed to find an MMN-like response (Fishman and Steinschneider 2012; Umbricht et al 2005; Lazar and Metherate 2003), resulting in a persistent mystery with respect to the neural generators of the MMN. Such inconsistencies might stem from differing use of controls and the low yield associated with the oddball paradigm. At the single-neuron level, stimulus-specific adaptation (SSA; Ulanovsky et al 2003), a phenomenon thought to be related to the MMN or its precursors, has been demonstrated in various stages of the auditory system across a variety of species (Nelken and Ulanovsky 2007; Gutfreund 2012; Ayala and Malmierca 2012). However, the relationship between SSA and MMN remains unclear (Farley et al 2010; Taaseh et al 2011).

The present study constitutes part of an ongoing research effort to bridge the gap between human and animal investigations of MMN-like responses and SSA. We use the roving-standard paradigm, adapted from recent work in humans (Costa-Faidella et al 2011), to investigate auditory brain responses in the anaesthetised guinea pig, a rodent commonly used as an auditory model system because its low-frequency hearing range is similar to that of humans. We exploit a series of advances in instrumentation (Miyamoto et al 2008) and signal processing (de Cheveigné and Simon 2007; de Cheveigné and Parra 2014) to provide the first characterisation of auditory evoked fields obtained non-invasively using a multi-channel MEG system designed for recording from small animals, allowing comparison with similarly non-invasive MEG measurements of auditory brain responses in humans.

Our results validate the use of small-animal MEG to measure auditory brain responses in rodents, and reveal extremely rapid reduction in auditory evoked field
amplitude with tone repetition. Moreover, we demonstrate that differences between
deviant and standard responses in the roving standard paradigm analogous to reported
MMN-like responses in awake human listeners can arise even in the anaesthetised
guinea pig.

Methods

Small-animal MEG machine
Measurements were made using a purpose-built small-animal MEG machine
designed and built by the Applied Electronics Laboratory of the Kanazawa Institute of
Technology (Miyamoto et al 2008) (Fig. 1A). The machine features a single sensor
array with 9 SQUID magnetometers, each 2.5 mm in diameter, arranged in a 3 × 3
square array with 2.75 mm between each coil center. Three reference sensors,
arranged at right angles to each other, are placed along the sensor array shaft, and the
subject and sensor array sit inside a shielded box. In addition, an accelerometer is
mounted on the machine to provide a vibration reference. A trigger pulse delivered by
the audio system prior to each stimulus and recorded by the MEG system serves to
align stimulus and response. The sensor array was placed in the same position relative
to head features for all subjects (Fig. 1A), but no other anatomical co-registration was
attempted, as the limited signal-to-noise ratio and small number of sensors prevent
accurate source localisation. Our aim was to characterise the time course of responses
rather than their spatial characteristics.

Subjects
All experiments were performed in accordance with the United Kingdom
Animal (Scientific Procedures) Act of 1986. Subjects were 17 adult male Duncan-Hartley guinea pigs; 8 animals were used for basic response characterisation, and 9 animals for studies of response adaptation. All animals were anaesthetised using 20% urethane (1.5g/kg body weight, single intraperitoneal injection) and 0.3 mg/ml buprenorphine (0.05 mg/kg body weight, single subcutaneous injection); 0.6 mg/ml atropine sulphate (0.2 ml, single subcutaneous injection) was also administered to reduce respiratory secretions. The state of the animal was monitored through assessment of the pedal withdrawal reflex, and the use of a MouseOx vital signs monitor (STARR Life Sciences) to track breathing rate, oxygen saturation and heart rate. Once the animal was anaesthetised, its head was shaved and then it was placed into a custom head rest (Fig. 1A). The tube of a custom earpiece was inserted into the right ear just at the entrance to the auditory canal and then sealed into the outer ear using soft silicone ear plugs. The animal was then placed into the MEG machine and the sensor array positioned dorsally over the vertex of the skull (Fig. 1A), in an entirely non-invasive procedure. The plane of the sensor array was thus oriented parallel to cortical columns within the auditory cortex, which lies along the lateral surface of the brain in guinea pigs.

Guinea pigs, like other small rodents, are lissencephalic; that is, the cortical surface is smooth, lacking gyri, and cortical currents therefore tend to be radial to the skull surface. For a uniform spherical conductor, radial current dipoles produce no measurable magnetic field outside the sphere, as a result of the balance between primary and return currents (Baule and McFee 1965). However, the shape of the guinea pig brain differs significantly from a sphere, and primary currents may not be perfectly radial, so measurable magnetic fields may be present outside the head (Barth...
We positioned the MEG sensors on the dorsal surface of the skull, in a plane parallel to the expected direction of current flow in the auditory cortex, and were able to pick up magnetic fields produced by primary or return currents induced by auditory stimuli. Notably, we were able to obtain clear auditory evoked fields using this non-invasive approach (cf. Barth et al 1986; Barth 1991), without resorting to more invasive methods used in some previous studies (Barth and Sutherling 1988; Bowyer et al 1999).

Auditory stimuli

Stimuli consisted of sequences of tone pips that alternated between two sound frequencies (Fig. 1B). The choice of stimulus conditions was limited by constraints specific to MEG recording. Transducers had to be placed outside the magnetically shielded box and stimuli were therefore transmitted via tubing to the animal's ears, limiting their spectral range. Furthermore, a large number of repeats of each stimulus was needed to overcome magnetic noise, limiting the number of different stimulus conditions. Stimuli were synthesised in MATLAB (Mathworks) and played out using PureData (http://puredata.info) via ER-2 transducers (Etymotics), which were located in a magnetically shielded box outside the MEG machine and connected to the custom earpiece via approximately 25 cm of 1.6 mm ID polythene tubing. Sound levels were calibrated in situ with a microphone (40BF free-field microphone, G.R.A.S Sound & Vibration) inserted into the custom ear piece. Calibrations included a compensation for attenuation of the sound signal between the microphone and the eardrum, estimated prior to the experiments using a simulated ear canal. The frequency response characteristics of the transducers, combined with low-frequency filtering
effects of the tubing, limited the range of effectively transmitted sound frequencies to approximately 0.5–4 kHz, which is at the lower end of the guinea pig hearing range. A roving-standard stimulus paradigm (Fig. 1B; Haenschel et al 2005) was used to achieve efficient yield of data for analysis of deviant and standard responses and to facilitate comparison with the growing number of studies in awake humans using this paradigm (e.g., Cowan et al 1993; Haenschel et al 2005; Costa-Faidella et al 2011). Tone sequences were composed of alternating series of repeated tones. Tones were 30 ms in duration (with 5-ms cosine-squared rise/fall times), isochronously presented, and alternated between two different sound frequencies in each stimulus block (Fig. 1B). A block consisted of 92 repeats of the tone series at each of the two sound frequencies (hence 183 frequency transitions). Each experiment involved presentation of stimulus blocks with parameters chosen to optimise data collection either for analysis of basic characteristics of auditory evoked fields, or for analysis of reduction in amplitude of the evoked fields with tone repetition.

For basic response characterisation, the tone frequencies were separated by 0.25, 0.5, 1, or 2 octaves around a centre frequency of 1.6 kHz. The number of tones in a series before a frequency transition (N) was always 4, and the interval between tone onsets (inter-onset interval, IOI) was always 400 ms. Responses to these stimuli were used to analyse auditory evoked field characteristics including waveform shape, extrema latency, and frequency dependence of extrema latency and amplitude.

For studies of reduction in auditory evoked field amplitudes with tone repetition, we fixed the tone frequencies at 800 Hz and 3.2 kHz (2 octaves separation) in order to maximise sound frequency change within the constraints imposed by the frequency response of the MEG-compatible sound delivery system and the hearing
range of the guinea pig. We then systematically varied the time between transitions (the inter-deviant interval, or IDI) and the number of tones per transition (N), in a 2x2 (IDI=1.6 s or 3.2 s, N=4 or 8) design. Previous studies in humans using roving-standard stimuli have sometimes observed effects of increasing N even further, e.g. to 12 tones per transition (Costa-Faidella et al 2011). Our decision to use either 4 or 8 tones per transition was dictated by the need to have enough transition events in each experiment to overcome the low signal-to-noise ratio of non-invasive MEG measurements in small animals.

Tone sequences used for studies of reduction in evoked field amplitudes therefore included tone series with four different possible temporal patterns (at each of the two possible alternating tone frequencies): 200 ms IOI, N=4; 400 ms IOI, N=4; 400 ms IOI, N=8; or 800 ms IOI, N=4. (Nominal IOIs were augmented by a small amount (1.14 ms) to ensure that power line interference components (50 Hz and harmonics) were not reinforced by trial averaging.) At every frequency transition in the tone sequence, the temporal presentation pattern for the next tone series was chosen randomly from among the four possible conditions, so that an equal number of each type of series would be presented in each hour. We aimed to collect a minimum of 6 hours of data per subject, with more data collected if conditions permitted.

Data acquisition and analysis

The MEG and reference data were collected using custom software provided by the Applied Electronics Laboratory (SQUIDLab). Sensor signals were bandpass filtered in hardware between 0.5 Hz and 500 Hz, and sampled at 1 kHz. Sampled signals were digitally high-pass filtered at 1 Hz and smoothed with a 4-sample boxcar
filter, and then separated into trials of either 350 ms duration (basic response analysis) or 175 ms duration (analysis of response reduction with tone repetition).

The aim of the data analysis was to extract the weak stimulus-evoked magnetic response from a combination of high-amplitude environmental and physiological noise. To achieve the highest possible signal-to-noise ratio in the processed data, we averaged signals across repeated trials and also applied three de-noising techniques: outlier rejection, time-shift principal components analysis (TSPCA; de Cheveigné and Simon 2007) and de-noising source separation (DSS; de Cheveigné and Parra 2014; Särelä and Valpola 2005) (Fig. 1C).

Outlier rejection is a standard procedure, recommended before applying least-squares methods such as averaging, regression or PCA because these procedures are sensitive to large deviations from the mean. We removed outliers both before and after other de-noising steps such as TSPCA and DSS, since each de-noising step revealed new outliers that were previously masked by noise. These successive outlier removal steps had little impact on final estimates of the MEG signal in most cases, but were necessary to counteract the effects of occasional large signal glitches due to transient environmental noise.

TSPCA was applied to the recordings from each MEG channel separately. This de-noising method effectively suppresses environmental noise such as 50 Hz signals from electrical equipment (see de Cheveigné and Simon 2007 for details).

Recordings from different channels were then combined by applying DSS, a de-noising technique which derives linear combinations of channels that optimise the signal with respect to a defined criterion, such as repeatability over trials or differentiation of stimulus conditions (de Cheveigné and Simon 2008; de Cheveigné
DSS produces a set of mutually uncorrelated component signals, ordered by decreasing criterion score. For our analysis we used the first DSS component, representing the linear combination of channel signals with the highest possible signal-to-noise ratio. This first DSS component was projected back into sensor space to produce a set of de-noised sensor waveforms (Fig. 1E), which we then averaged to obtain our best estimate of the cortical response (see Appendix: De-noising Process for full details). Thus, the auditory evoked field (hereafter, AEF) used for all further analyses was the average of the de-noised sensor waveforms obtained by back-projecting the first DSS component into sensor space.

The average over trials of this AEF waveform typically consisted of a series of three deflections. Adopting nomenclature similar to that used in human studies, we labelled these three extrema by their approximate latencies as the cM20, the cM50, and the cM140, where “c” is a species designator taken from the guinea pig genus name Cavia. The value of the AEF at each of these extrema was quantified as the most extreme value within a small temporal window around the reference latency (window bounds: 10–30 ms for cM20, 25–75 ms for cM50, and 100–175 ms for cM140). The relative polarities of these extrema are well defined, but not the absolute polarities. (To infer the polarities of the source currents within the auditory cortex from the weighted sum of brain activity in the MEG signal, we would need precise knowledge of the position of the sensors relative to auditory cortex; a forward model specific to the guinea pig head; and the simplifying assumption of a dipolar source.)

The magnitude of the AEF extrema usually decreased between the first and subsequent tones of a series (4 or 8 tones of same frequency). For each extremum, the degree of AEF reduction was quantified using the reduction index (RI), defined as the
distance in standard deviations between the observed AEF extremum amplitude for non-initial tones and the mean AEF extremum amplitude that would be obtained under the null hypothesis of no reduction with tone repetition. This z-scored measure was used to assess the significance of AEF reduction in individual subjects, since it took into account the noise level of the recording for each subject. To facilitate comparison with previous studies, we also analysed AEF reduction for each subject using the more conventional approach of normalising the AEF extremum magnitude for non-initial tones by the AEF extremum magnitude for initial tones (see Fig. 3C).

Model

To quantify the time-course of the AEF reduction with same-frequency tone repetition, we adapted a model previously used to describe short-term synaptic depression (Dayan and Abbott 2001). Similar models have recently been used to model the dependence of SSA in single neurons on tone frequency and probability (Taaseh et al 2011). Here, we used a simpler version in order to address the specific question: how quickly does cM50 magnitude reduce with repetition of 3.2 kHz tones? We focused on cM50 magnitude because the signal-to-noise ratio for MEG measurements was consistently highest for this extremum; likewise, we considered only responses to 3.2 kHz tones in this analysis because the strongest MEG responses were evoked at this tone frequency.

Assuming an initial state of an extended period of silence, the cM50 magnitude to the first stimulus in a series, \( m_1 \), occurring at time \( t_1 \), has a value of \( M \), the maximum cM50 magnitude. Following any activity, the responsiveness of the system is immediately suppressed to a fraction \( \alpha \in [0, 1] \) of its previous responsiveness,
which then recovers back to its baseline state with a time constant $\tau$. So, for the $n$th stimulus, the cM50 magnitude $m_n$ at time $t_n$ can be defined as

$$m_n = \alpha m_{n-1} + \left( M - \alpha m_{n-1} \right) \left( 1 - e^{-\left( t_n - t_{n-1} \right)/\tau} \right)$$

The value of $M$ was estimated from the mean AEF amplitude for the initial tone in all 3.2 kHz tone series, aggregating across different IOI and N conditions. We divided the data into 10 parts, and fit model parameters $\alpha$ and $\tau$ to 9/10 of the data from all IOI and N conditions using the simplex search method (Lagarias et al 1998) with cross-validation on the remaining 1/10 of the data. This procedure was repeated 10 times, with disjoint subsets of the data used for cross-validation. Model parameters reported here are, for each subject, the average parameters obtained from the 10 cross-validated model fits.

**Results**

The 9-sensor array in the small-animal MEG machine was positioned on the dorsal surface of the animal's head to detect signals arising from radial current flow in the laterally positioned auditory cortex (Fig. 1A). Data from each of the 9 sensors were de-noised (Fig. 1C) and linearly combined to obtain a representation of the auditory evoked field that optimised the reliability of stimulus-evoked responses and differentiation of stimulus conditions (Fig. 1E; optimal linear combination of sensor signals shown back-projected onto different channels to indicate signal strength at each sensor location). We defined the AEF to be this optimised estimate of the auditory evoked response (the first DSS component, back-projected into sensor space and averaged across sensors; see Methods), and used this representation for all further analysis (Fig. 1D, 1F and subsequent figures).
Basic response characteristics

While the AEF in response to a 30-ms tone pip varied in magnitude and in shape between individual subjects (Fig. 1F), three clearly defined extrema were consistent across subjects: two early ones sharing the same polarity, and a later extremum of opposite polarity (Fig. 1D). (As shown in Fig. 1F, in some subjects there was another early extremum of the same polarity as the late extremum, but in other subjects this additional extremum was absent; since it was not present in the population average, we do not discuss it further here.) As explained in Methods, we denote the three reliable extrema by their approximate latencies as the $c$M20, the $c$M50, and the $c$M140, where “c” is a species designator taken from the guinea pig genus name Cavia.

Studies in humans show a similar overall AEF profile, with the latencies of the extrema approximately twice what we observe here. Auditory evoked potentials (AEPs) measured in other rodents (using EEG) also have extrema with comparable latencies (Ehlers et al 1994; Sambeth et al 2003; Siegel et al 2003; Umbricht et al 2004; Umbricht et al 2005), though rodent AEPs exhibit additional, longer-latency components than reported here, and the shortest latency components may be reversed in polarity relative to other extrema (see Discussion).

In humans, both the magnitudes and the latencies of AEF extrema are known to be dependent on the frequency of the tone stimulus (Roberts et al 2000). In guinea pigs, the AEF extrema amplitudes increased in absolute magnitude with increasing tone frequency (Fig. 2, regressions for $c$M50 and $c$M140 significant at $p < 0.01$, regression for $c$M20 significant at $p < 0.05$), but there was no dependence of AEF
extrema latencies on tone frequency (all regressions $p > 0.1$; data not shown). However, experimental time constraints and equipment considerations limited us to collecting data for only a small portion of the guinea pig hearing range, which extends from approximately 50 Hz to 50 kHz. It is possible that the AEF extrema latencies might show frequency dependence over a larger frequency range, similar to the weak frequency dependence of AEF extrema latencies in humans (Roberts and Pöppel 1996).

### AEF changes with tone repetition

To analyse changes in AEF waveforms with tone repetition, we focused on responses to tones at 3.2 kHz sound frequency, for which the AEF magnitude, and our statistical power to resolve changes, was highest. Within each series of repeated tones, the waveform shape of the AEF remained relatively stable, but the latency of the latest of the extrema, and the overall amplitude of all three extrema, differed between the first tone after a frequency transition (deviant) and subsequent (standard) tones, especially at fast repetition rates (Fig. 3A).

While there were no significant changes in extrema latency for $\text{cM20}$ or $\text{cM50}$, the $\text{cM140}$ latency was longer for deviant than standard tones, particularly at the shortest IOI of 200ms (Fig. 3B). At 200ms IOI, we also observed strong reduction in AEF amplitude with tone repetition for all three extrema (Fig. 3). To facilitate comparison with similar analyses in previous MEG studies, in Fig. 3C we show AEF extremum magnitude for later (standard) tones normalised by AEF extremum magnitude for initial (deviant) tones. Across subjects, this measure of the relative response to repeated versus initial tones was significantly less than 1 for 200 ms IOI.
(Wilcoxon rank-sum test on medians, \( p < 0.01 \)). Similar results were obtained for 200 ms IOI using a z-scored measure (reduction index RI; see Methods) to take into account differences between subjects in the signal-to-noise level of recordings (5/9 subjects with RI > 2.5 for \( cM20 \), 9/9 for \( cM50 \), and 8/9 for \( cM140 \)). However, for longer IOIs, reductions in extrema amplitude with tone repetition were neither significant across subjects (Fig. 3C) nor consistently observed in individual subjects. More precisely, at the intermediate IOI of 400 ms, only a subset of the subjects showed significant AEF reduction with tone repetition at 400 ms IOI (RI > 2.5: 1/9 for \( cM20 \), 5/9 for \( cM50 \), and 5/9 for \( cM140 \)), and at the largest IOI of 800 ms, few subjects showed AEF reduction with tone repetition (0/9 for \( cM20 \), 1/9 for \( cM50 \), and 2/9 for \( cM140 \)).

In principle, the AEF amplitude reduction with tone repetition evident in Figure 3A could arise either from a reduction of the standard AEF response (e.g. adaptation), or from an augmentation of the deviant AEF response (e.g. a novelty effect), or both. If it were a novelty effect, we would expect the size of the deviant AEF response to increase with the time elapsed between transitions (inter-deviant interval, or IDI; Fig. 4A), or the number of tones between transitions (N; Fig. 4B). We therefore compared the deviant AEF magnitude between the IDI=1.6 s and IDI=3.2 s conditions, and also between the N=4 and N=8 conditions for each subject. For all three extrema and for most subjects, there were no significant differences in deviant AEF magnitude between IDI conditions or N conditions (Fig. 4C). (The only exceptions were: two subjects, deviant cM50 magnitude smaller at IDI=1.6 s than IDI=3.2 s, \( p < 0.01 \); one subject, deviant cM140 weakly dependent on N, \( p < 0.05 \); one subject, deviant cM140 weakly dependent on IDI, \( p < 0.05 \).) Moreover, there was
no significant change in deviant extremum latency for any of the extrema or conditions (Fig. 4D). Therefore, overall, we did not observe a large or consistent dependency of deviant AEF response on either N or IDI, suggesting that novelty effects were minimal for the stimulus parameters tested.

Dependency of AEF reduction on IOI

To quantify the dependency of AEF reduction on IOI, we fit a model based on a widely used mathematical description of synaptic depression (see Methods) with two parameters: the degree of responsiveness immediately after a tone presentation ($\alpha \in [0,1]$), and the time constant for recovery of responsiveness between tone presentations ($\tau$). We fit the model only to cM50 data for repeated 3.2 kHz tones, which produced the strongest and most reliable responses. For cM20 the responses were weak for several subjects, making for unreliable fits, and for cM140 the latency shift was not consistent with the model’s assumption of a simple scaling of the response. We tested two versions of the model, one in which both $\tau$ and $\alpha$ were fit to the data, and another in which only $\tau$ was fit and $\alpha$ was fixed to 0. There were no significant differences in cross-validation performance between the two models (data not shown), suggesting minimal cumulative effect of tone repetitions after the second tone in a series. We were therefore able to simplify the model by fixing $\alpha$ to 0 and fitting only the $\tau$ parameter. Model fits produced $\tau$ estimates for recovery from depression with a median across animals of 251 ms, and 25% and 75% quartiles of 167 and 833 ms respectively. These time constants are comparable to the duration of the IOIs themselves, again implying little cumulative effect of tone repetition, and rapid recovery between tone presentations.
Figure 5A shows the reduction in cM50 magnitude with tone repetition as a function of IOI. These data demonstrate that between the first tone and later tones in a series, the cM50 magnitude drops by nearly 50% for 200 ms IOI, but less than 20% for 400 ms IOI and less than 10% for 800 ms IOI. These data are consistent with the short τ estimates obtained from model fits. In fact, the predicted population mean cM50 magnitude based on the best-fit depression models for each subject (solid line in Fig. 5A) slightly overestimates the degree of AEF reduction at larger IOIs (note offset between solid line and data), suggesting that recovery from successive tone presentations may actually be a faster process than the simple exponential recovery in the model.

We wondered if the relatively high noise levels in our guinea pig MEG data could have obscured detection of longer time constants like those reported in comparable human studies, perhaps because only large changes in cM50 amplitude with tone repetition would have been resolvable. To find out, we estimated the resolvability of AEF reductions for each of our subjects, by calculating the minimum percentage reduction in cM50 amplitude that could have been resolved at p < 0.01 for recordings from each animal, given the cM50 amplitude for the first tones in each tone series and the variance of the signal. There was a large amount of variation between animals in the minimum resolvable cM50 reduction (range 15–43%). Importantly, however, estimated time constants for AEF reduction with tone repetition were short even for subjects for which the minimum resolvable cM50 reduction was smallest (Fig. 5B). Of particular note is the fact that the minimum resolvable cM50 reduction was less than 25% in 6 out of 9 subjects. In human studies, AEF reductions of 25% are observed at IOIs of 1 s (Briley 2011); therefore, we conclude that we should have
been able to detect comparably long timescales for AEF reduction with tone repetition in the guinea pig if present.

Effects of the number of tone repetitions

The fact that the time constants $\tau$ of the model fits were on the order of the smallest IOIs used in this study raises the possibility that cM50 reduction with tone repetition might have been largely complete after a single repetition (i.e., two tone presentations), and that effects of the length of the series of repeated tones might be minimal. This conclusion was confirmed by direct comparison of cM50 amplitudes between AEFs for the second tones and the last (i.e., either fourth or eighth) tones in the tone series (Fig. 6). In no subjects was there any condition in which we observed a significant difference in AEF magnitude across the length of the tone series (t-test with Holm-Bonferroni correction, $p > 0.05$). Thus, at least for the acoustic stimuli used in this study, there was no significant effect of the number of repeated tones on AEF reduction with tone repetition in the guinea pig.

In theory, optimizing the signal-to-noise ratio of the difference between responses to first and subsequent tones, as we did during the de-noising process (see Methods), might conceivably attenuate differences between responses to later tones. To address this concern, we modified the de-noising process to optimise the difference between responses to second and subsequent tones (ignoring all responses to first tones; see Methods), and repeated the previous analysis shown in Figure 6. Again, we found no difference in cM50 amplitudes between AEFs for the second and last tones ($t$-test with Holm-Bonferroni correction, $p > 0.05$). This additional analysis supports the conclusion that AEF reduction with tone repetition was essentially
complete by the second tone in the repeated series.

Comparison with MMN-like response in humans

For comparison with MMN-like responses detected in comparable studies of awake human subjects, Figure 7 shows grand-average (cross-trial and population) AEFs for the IOI=400 ms condition plotted as for human data in Figure 2A of Costa-Faidella et al (2011): responses to standard (second and later) tones (Fig. 7A), responses to deviant (first) tones (Fig. 7B), and the difference between responses to deviant and standard tones (Fig. 7C). Our results in anaesthetised guinea pigs show many similarities to those measured in awake humans using a similar paradigm.

Costa-Faidella et al (2011) examined responses to standards and deviants in a roving standard paradigm (with 25 frequencies between 880 and 2921 Hz) using tone series of length 3, 6 or 12. In that study, subtracting the standard response from that of the deviant revealed a negative MMN-like response that peaked after the N100 onset response (at the time of its downward slope); however there was no effect of tone series length on responses to either standard or deviant tones except for very long series (12 tones; see also Haenschel et al 2005). Consistent with those findings in humans, the guinea pig MEG data reveals a grand-average difference waveform that peaks after, rather than at the same time as, the cM50; and there is no effect of tone series length (N=4 versus N=8) on responses to either standard or deviant tones. The latency shift in the deviant-standard difference waveform, although comparatively small (7.9 ms), was significant at the population level for both N=4 and N=8 tone series lengths (bootstrap, $p < 0.05$ for grand averages; not significant in individual subjects).
We have no evidence that the cM50 is functionally homologous to the N100; moreover, the degree to which the MMN-like response observed in roving-standard paradigms constitutes a bona fide novelty response is not clear (Nelken and Ulanovsky 2007). Nevertheless, the present pattern of results raises the possibility that, rather than representing an exogenous response, the late MMN-like deflection that we observe might arise from the same low-level adaptive processes that produce a shorter cM140 latency for standard (repeated) relative to deviant (initial) tones.

Discussion

Here, we have presented evidence that: (1) auditory evoked fields with deflections resembling those observed in human MEG studies can be detected non-invasively in rodents using small-animal MEG; (2) AEF amplitudes in the anaesthetised guinea pig reduce rapidly with tone repetition, and this AEF reduction is largely complete by the second tone in a repeated series; and (3) differences between responses to the first (deviant) and later (standard) tones after a frequency transition resemble those previously observed in awake humans using a similar roving-standard stimulus paradigm.

Small-animal MEG

Standard whole-head MEG machines designed for human use have previously been used to characterise auditory and somatosensory evoked fields in the macaque monkey (Zhu et al 2009; Zurner et al 2010). However, there have been no previous studies of sensory or cognitive processing using MEG in rodents, because small-animal MEG is a relatively novel technology. Small-animal MEG machines have
been used to study the generative mechanisms of the MEG signal (e.g. Okada et al 1997; Okada et al 1999) and to examine large-scale changes in overall cortical state such as spreading depression (e.g. Gardner-Medwin et al 1991; Eiselt et al 2004) or epileptic seizures (e.g. Barth et al 1984). All previous incarnations of the technology have included some aspect of the preparation beyond anaesthesia that is not comparable to the use of MEG in human sensory studies: for example, a surgically invasive (e.g. Nowak et al 1999) or in vitro (e.g. Okada et al 1997) approach, a focus on very low-frequency activity (e.g. Gardner-Medwin et al 1991; Eiselt et al 2004), or the use of a single sensor (e.g. Barth et al 1984). With the advent of a compact multi-sensor MEG array (Miyamoto et al 2008) and with the aid of advances in de-noising methods (Särelä and Valpola 2005; de Cheveigné and Simon 2007; de Cheveigné and Parra 2014), we have been able to perform a sensorineural MEG study on par with previous human MEG studies and existing small animal EEG studies (e.g. Kraus et al 1994; Lazar and Metherate 2003; Umbricht et al 2005).

Comparison with EEG

As an experimental technique for studies of auditory function, small-animal MEG has several advantages over EEG. MEG is appealing for comparative physiology: magnetic fields are less distorted by the skull and scalp than electrical fields (Okada et al 1999), so MEG signals are less susceptible to species-specific distortions due to gross morphological differences in head shape. Moreover, small-animal MEG is a direct analogue to human MEG, commonly used for auditory cortex studies because the positioning of the auditory cortex in the human brain makes evoked magnetic fields relatively easy to record at the skull surface. Small-animal
MEG measurements are also less invasive than most small-animal EEG measurements, which typically involve the use of subdural or at least subdermal electrodes (e.g., Lazar and Metherate 2003). This advantage is offset by a greater susceptibility of MEG to environmental noise, for which we compensate here with a relatively sophisticated de-noising process. One specific disadvantage of MEG relative to EEG is that the superconducting MEG sensors (SQUIDs) require cooling with liquid helium, which is a limited natural resource with a volatile global market (Nuttall et al 2012).

In general, the AEF waveforms that we measured non-invasively in guinea pigs were similar in shape to AEPs measured in other rodents using invasive EEG recording techniques (Ehlers et al 1994; Sambeth et al 2003; Siegel et al 2003; Umbricht et al 2004; Umbricht et al 2005), with polarity reversed from that typically used to display AEPs (as is conventional also for many human MEG studies). The strong cM50 AEF extremum we observe in guinea pigs most likely corresponds to the large negative extremum that occurs at a latency of 40-80 ms in the rat AEP (Sambeth et al 2003) and 30-50 ms in the mouse AEP (Umbricht et al 2004), and which is considered a possible analogue of the human N100. Similarly, the cM140 extremum of opposite polarity following the cM50 in guinea pig may correspond to the positive extremum in the rat and mouse AEP with latency 80-150 ms in rats (Sambeth et al. 2003) and 70-120 ms in mice (Umbricht et al. 2004); this wave is considered a possible analogue of the human P200. An early component like the cM20 in the guinea pig AEF is also sometimes observed in the rat and mouse AEP at similar latencies. This early extremum, when it appears, usually has polarity opposite to that of the large N100-like extremum in the rat and mouse AEP (Sambeth et al 2003;
Umbricht et al 2004; Siegel et al 2003), while in most cases the cM20 had the same polarity as the cM50 in the guinea pig AEF. In addition, longer latency components that are sometimes evident in rodent AEPs were not detected in the guinea pig AEFs.

These differences in the characteristics and visibility of the weaker extrema in the guinea pig AEF versus rat and mouse AEP might reflect species differences or differences in the measurement techniques. However, the features of rodent AEPs that are most consistent across EEG studies were reliably observed here in guinea pig AEFs, providing confidence that the recorded MEG signals arise from similar auditory cortical sources as EEG signals.

Effects of tone repetition on the AEF

Our finding that reduction of the cM50 magnitude occurs only for short IOIs (Fig. 3) appears to be at odds with results of several previous experiments in humans and animals. Paradigms designed to probe stimulus-specific adaptation in human MEG can elicit effects with inter-stimulus intervals of a second or longer (e.g. Salminen et al 2009; Briley 2011), while in our data only the 200 ms IOI tone series reliably generated observable amounts of AEF reduction. Comparable EEG studies in rats (Lazar and Metherate 2003) have also reported a significant reduction to approximately 55% of the unadapted response at 500 ms IOI (and single-neuron studies of SSA in cats and rats have reported effects even at 1 or 2 s IOI; e.g. Ulanovsky et al 2004; Antunes et al 2010). It does not seem likely that the dependence on IOI was confounded by train length; while all of the 200 ms IOI series had 8 tones, for 400 ms IOI we had an equal number of series with 4 or 8 tones, and we saw no effect of series length there (Fig. 6).
Possible explanations for this apparent discrepancy between the present and previous results include differences in species, stimulation paradigm (roving-standard versus oddball paradigm; see Bäuerle et al 2011), and stimulus characteristics (e.g., use of tone frequencies at the low-frequency end of the animal’s hearing range here due to technical limitations of the small-animal MEG setup, versus tone frequencies in the middle of the hearing range in Lazar and Metherate 2003). A fourth possibility is that the adaptation of the MEG signal we recorded in guinea pig was driven primarily by subcortical rather than cortical processes. However, our analysis of AEF reduction was conducted on the cM50, and the most likely analogue of this extremum in the human AEF is the M100, which is thought to originate in primary and association cortices (Papanicolaou et al 1990; Rogers et al 1990). Moreover, the guinea pig cM50 is clearly similar to the rat P2 analysed in Lazar and Metherate (2003), both in timing and in polarity relative to other components of the rat AEP; and Lazar and Metherate (2003) found a substantial reduction in the P2 with tone repetition at 500 ms IOI.

Effects of the number of tone repetitions

In line with the observation of strong AEF reduction only at the shortest IOIs, we also found that AEF reduction was essentially complete after a single tone repetition. This result is consistent with findings from human MEG studies (Budd et al 1998; Soros et al 2009; Rosburg et al 2010; Haenschel et al 2005; Costa-Faidella et al 2011), where a dependence of the AEF reduction on tone series length is usually observed for long, but not short, tone series. Both Haenschel et al (2005) and Costa-Faidella et al (2011) reported strong effects of tone series length only when the comparisons included tone series of length greater than or equal to 12, while studies
that used shorter series reported no effect (Rosburg et al 2010; Budd et al 1998).

Similarly, in our data, the differences between AEFs evoked by 4- and 8-tone series were not significant (Fig. 4 and Fig. 7), but it is possible that longer series might have revealed an effect.

**Differences between deviant and standard AEFs**

We found no reliable dependence of the deviant AEF on stimulus history. Thus, it would appear that the observed dependence of the deviant-standard difference response on the structure of the stimulus is dominated by changes in the standard AEF relative to the deviant AEF. Human studies using the roving-standard paradigm have reached similar conclusions (Cowan et al 1993; Haenschel et al 2005; Costa-Faidella et al 2011). Apparently consistent with our work, evoked-potential studies in the anaesthetised rat (Lazar and Metherate 2003) and mouse (Umbricht et al 2005) reported no difference in response between deviant tones in a classic oddball paradigm and a “deviant-alone” control in which the standard tones are effectively replaced with silence, and those authors thus concluded that there was no evidence for change detection (as reflected by an increase in deviant AEF amplitude). Nelken and Ulanovsky (2007) argue that such controls are overly strict, and suggest instead a “many-standards” control. However, results with such controls have proven ambiguous, with some reporting clear changes relative to the oddball-deviant (Nakamura et al 2011) and some reporting no effect (Fishman and Steinschneider 2012); recent evidence suggests that the many-standards control is extremely sensitive to the range over which the standards are distributed (Taaseh et al 2011). It is also possible that the isochronous tone series and roving-standard stimulus design used
here and in previous human studies might fail to produce changes in the deviant response that would be evoked by more substantial alterations in stimulus statistics (Yaron et al 2012).

The latency of the peak of the deviant-minus-standard difference waveform (Fig. 7) was clearly distinct from the latency of the extrema of the AEF, as is the case for responses labeled an MMN in previous human studies (although the latency shift is larger in awake humans for some stimulus paradigms; Näätänen et al 1989; Winkler et al 1997; Kretzschmar and Gutschalk 2010; but compare with Haenschel et al 2005; Costa-Faidella et al 2011). MMN-like responses measured in local field potentials and current source densities in the awake rat and macaque also appear to display similar or larger latency shifts (Javitt et al 1994; von der Behrens et al 2009; Fishman and Steinschneider 2012). This temporal shift is considered one of the key pieces of evidence in support of the idea that the MMN is generated by a novelty-driven process distinct from the generators of the N100 and the other components of the auditory evoked potential (Näätänen et al 2005). However, the fact that we observed a delay in the latency of the difference waveform in our data, even though we found no other evidence for changes in the deviant response, suggests that such latency shifts may not arise from brain processes related to the novelty of the deviant response. May and Tiitinen (2010) have argued that the temporal separation between the peak of the difference response and the extrema of deviant or standard responses might in fact be due to variability in the latency of the N100; in our data, the most obvious explanation arises from the reduction in the latency of the cM140 for second and later tones (Fig. 3). These data raise the possibility that MMN-like latency shifts can arise through differential effects of tone repetition on the latency of early versus late deflections in
the tone-evoked brain response. In any case, the remarkable similarity between the
results obtained in anesthetized guinea pigs and awake humans using a similar
stimulus paradigm (Costa-Faidella et al 2011) suggests that the effect is generated by
low-level, automatic mechanisms, independent of conscious awareness.

Possible effects of anaesthesia

One major difference between this work in guinea pigs and MEG studies in
humans is that the guinea pigs were anaesthetised (with urethane), while human MEG
experiments are more often performed in awake subjects. The brain states evoked by
urethane anaesthesia are thought to be very similar to those evoked by sleep (Clement
et al 2008). Previous studies in humans have indicated that the mismatch negativity
response is reduced under anaesthesia (Simpson et al 2002; but see Koelsch et al
2006) and during slow-wave sleep (Csépe et al 1987), and the MMNm is also known
to be attenuated by anticholinergic drugs (Pekkonen et al 2001) such as the atropine
administered here to reduce bronchial secretions in the anaesthetised animals.

Therefore it is possible that the deviant-standard difference responses we recorded in
anaesthetised, atropine-treated guinea pigs were weaker than those that would be
obtained in awake animals. On the other hand, it should be noted that SSA has been
repeatedly demonstrated and most often characterised in urethane-anaesthetised
animals (Antunes et al 2010; Duque et al 2012), and therefore anaesthesia does not
undermine the validity of our results regarding SSA. Indeed, the fact that we observed
differences between responses to deviant and standard tones in the anaesthetised
guinea pig that resemble differences previously described as an MMN in awake
humans strengthens our conclusion that MMN-like responses can arise from low-level
adaptive processes independent of conscious awareness.

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Competing Financial Interests

The authors declare no competing financial interests.
To achieve the highest possible signal-to-noise ratio for the stimulus-evoked magnetic response, we averaged signals across repeated trials, as in most studies, and applied three additional de-noising techniques: outlier rejection to ensure that transient environmental noise did not bias our estimates of mean evoked fields; time-shift principal components analysis (TSPCA; de Cheveigné and Simon 2007) to remove environmental noise; and de-noising source separation (DSS; Särelä and Valpola 2005; de Cheveigné and Parra 2014) to derive the linear combination of channels that provided the most reliable estimate of the evoked signal that differed between stimulus conditions.

Outlier rejection was performed at multiple stages in the de-noising process, since each de-noising step revealed new outliers that were previously masked by noise. In the first stage, performed on the raw data from each channel, trials with clipping on more than 2% of samples on any channel (including reference sensors) were discarded, as were trials that differed from the average over trials by more than 2 standard deviations. In the second stage, performed on the processed data following TSPCA (see below), trials deviating more than 1.5 standard deviations from the average TSPCA-transformed signal across trials were discarded. The third stage of outlier rejection was performed on the DSS component representing the most reliable stimulus-dependent signal that could be obtained from a linear combination of data from the nine MEG sensors (see below). Outliers in this stage were defined as trials in which this DSS component deviated by more than 2 standard deviations from its average across trials. Overall, typically 20% of trials were discarded, leaving at least 1000 trials per stimulus condition.
TSPCA was applied to the recordings from each channel to suppress environmental noise recorded on the reference sensors. TSPCA projects out magnetic fields recorded from the reference sensors, with time shifts to compensate for any convolutional mismatch between reference and main sensors.

The TSPCA-transformed data from all nine MEG sensors was then combined using the DSS procedure, to derive the linear combination of channels that optimised the reliability of stimulus-evoked responses and their differentiation across stimulus conditions. We first applied DSS with a reliability constraint, to obtain the linear combination of channels that optimised the reliability of stimulus-dependent activity.

The first DSS component from this optimisation process was used for the final stage of outlier rejection, to identify and exclude trials in which the estimate of stimulus-dependent signal deviated significantly from the mean across repeated trials.

After the final outlier removal step, the DSS algorithm was applied once more, this time to optimise the difference between the response to the first tone and subsequent tones in a series. Specifically, for each condition (first or subsequent) we subtracted from each trial the average over all trials of the other condition. These data were then submitted to DSS to find the linear combination of channels for which this difference was most reliable. The weights obtained in this way were applied uniformly to all the data of both conditions. Normalising this component, and multiplying by the RMS over sensors of its associated sensor-space projection, provides our best estimate of the magnetic field associated with the adaptation-related cortical response. (As a control, we also used a variation of the above procedure in which we replaced the first tone by the second tone, comparing it to the average over all trials from third or later tones in the series; see Results.) The DSS algorithm
provides additional optimised components orthogonal to the first, but in these experiments, for all subjects, only a single component of the DSS displayed adaption-related responses. We therefore confined our analysis to that component alone.
Figures and Figure Legends

Figure 1: Brain responses to tone pips in the guinea pig, measured non-invasively with small-animal MEG. A: The MEG experimental setup. Left, a view of the small-animal MEG machine with the helium dewar visible on top of the recording chamber. Middle, close-up view of the recording chamber, with adjustable platform for the animal below the extremity of the dewar containing the MEG sensors. Right, an anaesthetised guinea pig with its head resting on the head-holder, and the dewar containing MEG sensors pressed lightly on the dorsal surface of the head. Plastic tubing held in place near the ears is attached to speakers outside the recording chamber, so that acoustic stimuli can be delivered directly into the auditory canal. B: In the roving-standard paradigm, a series of tones of the same frequency and at the same inter-onset interval (IOI) is followed by a series at a new frequency separated by $\Delta f$, with possibly a different IOI and number of tone repetitions in the series. The initial tone in each series (red) acts as a deviant stimulus compared to the standard stimuli of remaining tones in the series (blue). C: Individual channel waveforms evoked by a 3.2 kHz tone. Left, mean raw waveform averaged over ~14000 repeats; grey shading indicates bootstrapped standard deviation in the mean (equivalent to standard error here; bootstrap used for consistency with other analyses). Middle, waveform following TSPCA, which removes environmental noise such as 50 Hz or machine-induced magnetic fields. Right, waveform following TSPCA and DSS (see Methods); figure shows the first DSS component (the most reliable linear combination of channels) back-projected into sensor space to produce the de-noised auditory evoked field. D: Population average AEF for the 3.2 kHz tone, averaged across all tone presentations and all 9 animals used for studies of response adaptation. The
amplitude is calculated as the RMS amplitude across sensors. Shaded region indicates a bootstrap estimate of one standard deviation around the population mean (i.e., standard error). The guinea pig AEF is characterised by three extrema, with latencies of 10–30 ms (cM20), 25–75 ms (cM50), and 100–175 ms (cM140) relative to stimulus onset (see Methods). E: Spatial variation in the AEF for one animal, illustrated by back-projecting the first DSS component into sensory space for all 9 sensors. Color indicates waveform polarity; arrangement of plots reflects arrangement of the 9 magnetometers in the sensor array. F: AEFs for responses to a 30 ms, 3.2 kHz tone pip from four different animals used for studies of response adaptation. Responses are averaged across both standard and deviant conditions, with shading indicating bootstrapped standard deviation of the mean. These panels demonstrate the consistency of MEG responses both within and across animals.

**Figure 2**: Magnitudes of AEF extrema as a function of tone frequency. The magnitude (absolute value of response amplitude) of each of the AEF extrema increased with increasing frequency of the tone pip from 800 Hz to 3.2 kHz. Plots show data from all 8 animals used for basic response characterisation experiments, with linear regression lines (note that abscissa is logarithmic). A: cM20; linear regression 4.6 fT/kHz + 8.2 fT, p < 0.05. B: cM50; linear regression 13.5 fT/kHz + 15.0 fT, p < 0.01. C: cM140; linear regression 6.4 fT/kHz + 16.6 fT, p < 0.01.

**Figure 3**: Dependence of MEG responses to tones on inter-onset interval. A: Population average AEFs for 3.2 kHz tones reveal that compared to responses to the initial tones (red solid line), responses to later tones in 200 ms IOI series (dark blue
dashed line) were markedly reduced in amplitude. For tone series with 400 ms IOI (blue dotted line) and 800 ms IOI (light blue dashed-and-dotted line), the degree of AEF reduction was considerably less. Ellipses indicate approximate positions of the extrema (black, cM20; green, cM50; purple, cM140). B: Latency of the cM20 and cM50 did not vary significantly with IOI (RM ANOVA, $p > 0.1$); however, there was a significant effect of IOI on latency of the cM140 (RM ANOVA, $p < 0.01$). At faster IOIs, the latency of the cM140 was shorter for later tones than for initial (“Unadapted”) tones in the sequences. C: Box-and-whisker plots showing magnitudes of responses to repeated tones across subjects, for the three AEF extrema and for different IOI conditions. Response magnitude is quantified as the average AEF extremum magnitude for later tones in a series, normalised by AEF extremum magnitude for the initial (deviant) tone, to illustrate degree of AEF reduction. For all three extrema, the amount of AEF reduction with tone repetition decreased with increasing IOI (RM ANOVA, $p < 0.01$). This effect arose primarily from AEF reduction with tone repetition at the fastest IOI, since only the 200 ms IOI condition produced a consistent reduction in AEF magnitude for later relative to initial tones (Wilcoxon rank-sum test for difference in medians at 200 ms IOI, $p < 0.05$ for cM50, $p < 0.01$ for cM140, with similar trend at $p = 0.06$ for cM20). See RI analysis results in text for similar results obtained using a z-scored measure taking into account the signal-to-noise levels in recordings from different subjects.

Figure 4: Deviant AEF response shows no consistent dependence on either inter-deviant interval (IDI) or length of preceding tone series (N). Deviants were separated by one of two IDIs (1.6 or 3.2 s), and one of two possible tone series lengths
Population grand averages of the deviant responses for the two different IDI (A) or N (B) conditions showed little difference. C: Change in deviant response magnitude between IDI conditions or N conditions, for each of the three AEF extrema; more positive values signify a larger magnitude AEF for 3.2s IDI or N=8 conditions, respectively. There were no significant changes in deviant extrema magnitudes between either IDI conditions or N conditions across subjects (Wilcoxon rank-sum test), nor in most individual subjects (see text). D: Change in deviant response latency between IDI conditions or N conditions, for each of the three AEF extrema; more positive values signify a longer latency for 3.2s IDI or N=8 conditions, respectively. We observed no significant differences in extrema latency between any of the conditions for any extrema, either overall or in individual subjects.

**Figure 5:** Mathematical modelling of AEF reduction with tone repetition. A: Reduction in cM50 magnitude for repeated versus initial tones in tone series is plotted as a function of IOI, along with the mean across the population of the predictions from the depression model (solid line). B: Time constants $\tau$ from the model fits are plotted against the minimum resolvable reduction in the cM50 (a measure of noise level in the recordings) for each subject. Short time constants were obtained even in subjects for which the minimum resolvable cM50 reduction was less than 25% (6/9 subjects), which would have been sufficient to resolve ERP reductions at 1000 ms IOI in human studies (Briley et al. 2011).

**Figure 6:** No dependence of AEF reduction with tone repetition on the number of repeated tones. The magnitudes of the AEF at cM20 (diamond), cM50
(star), and cM140 (circle) for the last tones in all tone series (y-axis) are plotted against the AEF magnitudes for second tones in all tone series (x-axis) for each subject; dashed line indicates equal magnitude (slope=1). The last tone was the eighth tone for 200 ms IOI (A) and 400 ms IOI (B) series, and the fourth tone for 800 ms IOI series (C). There was no significant difference between the AEF extrema magnitudes for the second versus last tones in any of the stimulus conditions, for any of the subjects (t-test with Holm-Bonferroni correction, \( p > 0.05 \)).

Figure 7: Deviant-minus-standard differences in grand-average AEFs resemble those observed using similar stimulus paradigms in awake humans (cf. Costa-Faidella et al 2011). Grand-average AEFs for standard (A) and deviant (B) tones and the deviant-minus-standard difference waveform (C), for 400 ms IOI and either N=4 (thin) or N=8 (thick) conditions. Shaded regions indicate one standard deviation of the bootstrap estimate of the standard error (overlapping for the N=4 and N=8 conditions). Grand-average AEFs for deviant tones differed significantly from grand-average AEFs for standard tones in both the N=4 and N=8 conditions (with no significant differences between N=4 and N=8 responses). The horizontal bars indicate the region near 50 ms latency where the waves are significantly different at \( p < 0.05 \) for the N=4 and N=8 conditions. For comparison with a very similar analysis performed on AEFs from awake humans, see Figure 2A in Costa-Faidella et al. (2011).
A

AEF mag. (fT)

Time (ms)

Initial tone

200ms IOI

400ms IOI

800ms IOI

M20

M50

M140

B

Latency (ms)

IOI (ms)

Unadapted

C

AEF extremum mag. (fraction of deviant)

IOI (ms)

200 400 800

200 400 800

200 400 800

M20

M50

M140