Auditory evoked fields measured noninvasively with small-animal MEG reveal rapid repetition suppression in the guinea pig

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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 after the sound onset. The mechanisms that produce MMN are the subject of some debate (May and Tiitinen 2010; Näätänen et al. 1978), but it is generally interpreted as reflecting the violation of predictions of a model tuned to an ongoing stimulus regularity (Lieder et al. 2013; Näätänen et al. 2007). Therefore, MMN 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 (Garrido et al. 2013; Lieder 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 paradigm method of eliciting 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). 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., Costa-Faidella et al. 2011; Haenschel et al. 2005). 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 (for a review, see Nelken and Ulanovsky 2007). Several investigations have failed to find an MN-like response (Fishman and Steinschneider 2012; Lazar and Metherate 2003; Umbricht et al. 2005), resulting in a persistent mystery with respect to the neural generators of 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 MMN or its precursors, has been demonstrated in various stages of the auditory system across a variety of species (Ayala and Malmierca 2012; Gutfreund 2012; Nelken and Ulanovsky 2007). 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 used the roving standard paradigm, adapted from recent work in humans (Costa-Faidella et al. 2011), to investigate auditory brain responses in the anesthetised guinea pig, a rodent commonly used as an auditory model system because its low-frequency hearing range is similar to that of humans. We exploited a series of advances in instrumentation (Miyamoto et al. 2008) and signal processing (de Cheveigné and Parra 2014; de Cheveigné and Simon 2007) to provide the first characterization of auditory evoked fields (AEFs) obtained noninvasively using a multi-channel magnetoencephalography (MEG) system designed for recording from small animals, allowing comparison with similarly noninvasive MEG measurements of auditory brain responses in humans.
Our results validated the use of small-animal MEG to measure auditory brain responses in rodents and revealed an extremely rapid reduction in AEF amplitude with tone repetition. Moreover, we demonstrated 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 anesthetised 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 nine 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 before 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 anatomic coregistration was attempted, as the limited signal-to-noise ratio and small number of sensors prevent accurate source localization. Our aim was to characterize the time course of responses rather than their spatial characteristics.

Subjects. All experiments were performed under licenses granted by the United Kingdom Home Office 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 characterization and 9 animals were used for experiments on response adaptation. All animals were anesthetised using 20% urethane (1.5g/kg body wt, single intraperitoneal injection) and 0.3 mg/ml buprenorphine (0.05 mg/kg body wt, 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 sign monitor (STARR Life Sciences) to track breathing rate, O₂ saturation, and heart rate. Once the animal was anesthetised, its head was shaved and then 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 was positioned dorsally over the vertex of the skull (Fig. 1A) in an entirely noninvasive 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 hessencephalic; that is, the cortical surface is smooth and 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 1991). 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 AEFs using this noninvasive approach (cf. Barth 1991; Barth et al. 1986) without resorting to more invasive methods, as 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 ~25 cm of 1.6-mm-inner diameter polyethylene 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, which was estimated before 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 ~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., Costa-Faidella et al. 2011; Cowan et al. 1993; Haenschel et al. 2005). 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 2 sound frequencies (hence, 183 frequency transitions). Each experiment involved the presentation of stimulus blocks with parameters chosen to optimize data collection either for analysis of basic characteristics of AEFs or for analysis of the reduction in amplitude of the evoked fields with tone repetition.

For basic response characterization, tone frequencies were separated by 0.25, 0.5, 1, or 2 octaves around a center 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 [interonset interval (IOI)] was always 400 ms. Responses to these stimuli were used to analyze AEF characteristics, including waveform shape, extrema and...
latency, and the frequency dependence of extrema latency and amplitude.

For experiments on the reduction in AEF amplitudes with tone repetition, we fixed tone frequencies at 800 and 3.2 kHz (2-octave separation) to maximize sound frequency change within the constraints imposed by the frequency response of the MEG-compatible sound delivery system and hearing range of the guinea pig. We then systematically varied the time between transitions [interdeviant interval (IDI)] and N in a $2 \times 2$ (IDI = 1.6 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/transition (e.g., Costa-Faidella et al. 2011). Our decision to use either 4 or 8 tones/transition was dictated by the need to have enough transition events in each experiment to overcome the low signal-to-noise ratio of noninvasive MEG measurements in small animals.

Tone sequences used for experiments on the 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 h of data per subject, with more data collected 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 h of data per subject, with more data collected if conditions permitted.

Data acquisition and analysis. MEG and reference data were collected using custom software provided by the Applied Electronics Laboratory (SQUIDLab). Sensor signals were band-pass filtered in hardware between 0.5 and 500 Hz and sampled at 1 kHz. Sampled signals were digitally high-pass filtered at 1 Hz, smoothed with a four-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 denoising techniques: outlier rejection, time-shift principal components analysis (TSPCA) (de Cheveigné and Simon 2007), and denoising source separation (DSS) (de Cheveigné and Parra 2014; Särelä and Valpola 2005) (Fig. 1C).

Outlier rejection is a standard procedure that is recommended before applying least-squares methods such as averaging, regression, or principal components analysis because these procedures are sensitive to large deviations from the mean. We removed outliers both before and after denoising steps, such as TSPCA and DSS, since each denoising 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 individually. This denoising 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 denoising technique that derives linear combinations of channels that optimize the signal with respect to a defined criterion, such as repeatability over trials or differentiation of stimulus conditions (de Cheveigné and Parra 2014; de Cheveigné and Simon 2008). 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 denoised sensor waveforms (Fig. 1E), which we then averaged to obtain our best estimate of the cortical response (see APPENDIX: THE DE NOISING PROCESS for full details). Thus, AEF used for all further analyses was the average of the denoised sensor waveforms obtained by backprojecting 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 labeled these three extrema by their approximate latencies as $M_{20}$, $M_{50}$, and $M_{140}$, where the subscript c is a species designator taken from the guinea pig genus name: Cavia. The value of 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 $M_{20}$, 25–75 ms for $M_{50}$, and 100–175 ms for $M_{140}$). The relative polarities of these extrema are well defined, but the absolute polarities are not. (To infer the polarities of the source currents within the auditory cortex from the signal-to-noise ratio for MEG measurements was consistently highest for this extrema transition events in each experiment to overcome the low signal-to-noise ratio of noninvasive MEG measurements in small animals.

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 to address the specific question: how quickly does $M_{50}$ magnitude reduce with repetition of 3.2-kHz tones? We focused on $M_{50}$ magnitude because the signal-to-noise ratio for MEG measurements was consistently highest for this extrema; 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 $M_{50}$ magnitude to the first stimulus in a series ($m_1$) occurring at time $t_1$ has a value equal to the maximum $M_{50}$ magnitude ($M$). After any activity, the responsiveness of the system is immediately suppressed to a fraction $\sigma e^{-\tau (t - t_1)}$ of its previous responsiveness, which then recovers back to its baseline state with a time constant ($\tau$). So, for the $n$th stimulus, $M_{50}$ magnitude $m_n$ at time $t_n$ can be defined as follows:

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

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 $\sigma$ 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 nine-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 nine sensors were denoised (Fig. 1C) and linearly combined to obtain a representation of AEF that optimized the reliability of stimulus-evoked responses and differentiation of stimulus conditions (Fig. 1E; optimal linear combination of sensor signals shown backprojected onto different channels to indicate signal strength at each sensor location). We defined AEF to be this optimized estimate of the auditory evoked response (the first DSS component, backprojected into sensor space and averaged across sensors; see METHODS) and used this representation for all further analyses (Fig. 1, D and F, and subsequent figures).

Basic response characteristics. While 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 denoted the three reliable extrema by their approximate latencies as _M20_, _M50_, and _M140_.

Studies in humans have shown 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 electroencephalography (EEG)] also have extrema with comparable latencies (Ehlers et al. 1994; Sam-beth et al. 2003; Siegel et al. 2003; Umbricht et al. 2004, 2005), although 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 latencies of AEF extrema are known to be dependent on the frequency of the tone stimulus (Roberts et al. 2000). In guinea pigs, AEF extrema amplitudes increased in absolute magnitude with increasing tone frequency (Fig. 2, regressions for _M50_ and _M140_ significant at _P_ < 0.01, regression for _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 ~50 Hz to 50 kHz. It is possible that 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 analyze 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 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).

Whereas there were no significant changes in extremum latency for _M20_ or _M50_, the _M140_ latency was longer for deviant than standard tones, particularly at the shortest IOI of 200 ms (Fig. 3B). At 200-ms IOI, we also observed a strong reduction in AEF amplitude with tone repetition for all three extrema (Fig. 3A). To facilitate comparison with similar analyses in previous MEG studies, in Fig. 3C we show AEF extremum magnitude for later (standard) tones normalized by AEF extremum magnitude for initial (deviant) tones. Across subjects, this measure of the relative response to repeated versus initial tones was significantly <1 for 200-ms IOI (_P_ < 0.01 by Wilcoxon rank-sum test on medians). Similar results were obtained for 200-ms IOI using a _z_-scored measure (RI; see
exceptions were as follows: two subjects, deviant M50 magnitude smaller at IDI = 1.6 s than IDI = 3.2 s, P < 0.01; one subject, deviant M140 weakly dependent on N, P < 0.05; and one subject, deviant M140 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. 4C). 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 (α ∈ [0, 1]) and τ for the recovery of responsiveness between tone presentations. We fit the model only to M50 data for repeated 3.2-kHz tones, which produced the strongest and most reliable responses. For M20, the responses were weak for several subjects, making for unreliable fits; for M140, 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 τ and α were fit to the data and another in which only τ was fixed and α 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 α to 0 and fitting only the τ parameter. Model fits produced τ 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 τ values 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 M50 magnitude with tone repetition as a function of IOI. These data demonstrate that between the first tone and later tones in a series, the M50 magnitude drops by nearly 50% for 200-ms IOI but <20% for 400-ms IOI and <10% for 800-ms IOI. These data are consistent with the short τ estimates obtained from model fits. In fact, the predicted population mean M50 magnitude based on...
Effects of the number of tone repetitions. The fact that \( \tau \) values of the model fits were on the order of the smallest IOIs used in this study raises the possibility that the \( \tau \) 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 \( \tau \) values for AEF reduction with tone repetition were short even for subjects for which the 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 \( \tau \) values like those reported in comparable human studies, perhaps because only large changes in \( \tau \) 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 percent reduction in \( \tau \) amplitude that could have been resolved at \( P < 0.01 \) for recordings from each animal, given the \( \tau \) 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 \( \tau \) reduction (range: 15–43%). Importantly, however, estimated \( \tau \) values for AEF reduction with tone repetition were short even for subjects for which the minimum resolvable \( \tau \) reduction was smallest (Fig. 5B).

Effects of the length of preceding tone series. For comparison with MMN-like response in humans. For comparison with MMN-like responses detected in comparable studies of awake human subjects, Fig. 7 shows grand-average (cross-trial and population) AEFs for the IOI = 400 ms condition plotted as for human data in Fig. 2A of Costa-Faidella et al. (2011): responses to standard (second and last) tones (Fig. 7A), responses to deviant (first) tones (Fig. 7B), and the difference between responses to deviant and standard tones.
DISCUSSION

Here, we have presented evidence that 1) AEFs with deflections resembling those observed in human MEG studies can be detected noninvasively in rodents using small-animal MEG; 2) AEF amplitudes in the anesthetised 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 characterize 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, 1999) and to examine large-scale changes in the overall cortical state, such as spreading depression (e.g., Eiselt et al. 2004; Gardner-Medwin et al. 2004; Lazar and Metherate 2003; Umbricht et al. 2005). Nevertheless, the present pattern of results raises the possibility that, rather than representing an exogenous response, the late MMN-like deflection that we observed might arise from the same low-level adaptive processes that produce a shorter \(t_M140\) latency for standard (repeated) relative to deviant (initial) tones.

We have no evidence that \(t_M50\) is functionally homologous to 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 observed might arise from the same low-level adaptive processes that produce a shorter \(t_M140\) latency for standard (repeated) relative to deviant (initial) tones.

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 analog to human MEG, which is 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...
Fig. 6. No dependence of AEF reduction with tone repetition on the number of repeated tones. The magnitudes of the AEF at M20 (diamond), M50 (star), and M140 (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, \( p > 0.05 \)).

Fig. 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). A–C: 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 8 (thick) conditions. Shaded regions indicate 1 SD of the bootstrap estimate of the SE (overlapping for \( N = 4 \) and 8 conditions). Grand-average AEFs for deviant tones differed significantly from grand-average AEFs for standard tones in both \( N = 4 \) and 8 conditions (with no significant differences between \( N = 4 \) and 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 8 conditions. For comparison with a very similar analysis performed on AEFs from awake humans, see Fig. 2A in Costa-Faidella et al. (2011).
stimulus-specific adaptation in human MEG can elicit effects with interstimulus intervals of 1 s or longer (e.g., Briley 2011; Salminen et al. 2009), whereas in our data, only the 200-ms IOI tone series reliably generated observable amounts of AEF reduction. A comparable EEG study in rats (Lazar and Metherate 2003) also reported a significant reduction to ~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; whereas all of the 200-ms IOI series had eight tones, for 400-ms IOI we had an equal number of series with four or eight tones, and we saw no effect of series length there (Fig. 6).

Possible explanations for this apparent discrepancy between 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 (Lazar and Metherate 2003)]. A fourth possibility is that the adaptation of the MEG signal we recorded in the guinea pig was driven primarily by subcortical rather than cortical processes. However, our analysis of AEF reduction was conducted on M50, and the most likely analog of this extremum in human AEF is M100, which is thought to originate in primary and association cortices (Papanicolaou et al. 1990; Rogers et al. 1990). Moreover, guinea pig M50 is clearly similar to rat P2 analyzed in Lazar and Metherate (2003), both in timing and polarity relative to other components of the rat AEP; Lazar and Metherate (2003) found a substantial reduction in 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; Costa-Faidella et al. 2011; Haenschel et al. 2005; Rosburg et al. 2010; Soros et al. 2009), where a dependence of the AEF reduction on tone series length was 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 ≥12, whereas studies that used shorter series reported no effect (Budd et al. 1998; Rosburg et al. 2010). Similarly, in our data, the differences between AEFs evoked by four- and eight-tone series were not significant (Figs. 4 and 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 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 standard AEF relative to deviant AEF. Human studies using the roving standard paradigm have reached similar conclusions (Costa-Faidella et al. 2011; Cowan et al. 1993; Haenschel et al. 2005). Apparently consistent with our work, evoked potential studies in the anesthetised rat (Lazar and Metherate 2003) and mouse (Umbrecht et al. 2005) reported no differences in the 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) argued that such controls are overly strict and suggested instead a “many-standards” control. However, results with such controls have proven ambiguous, with some reporting clear changes relative to the oddball-deviant model (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 (Taasen 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 AEF, as is the case for responses labeled as MMN in previous human studies [although the latency shift is larger in awake humans for some stimulus paradigms (Kretzschmar and Gutschalk 2010; Nätänen et al. 1989; Winkler et al. 1997) compared with others (Costa-Faidella et al. 2011; Haenschel et al. 2005)]. 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 (Fishman and SteinSchneider 2012; Javitt et al. 1994; von der Behrens et al. 2009). This temporal shift is considered one of the key pieces of evidence in support of the idea that MMN is generated by a novelty-driven process distinct from the generators of N100 and other components of AEP (Nätänen 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 N100; in our data, the most obvious explanation arises from the reduction in the latency of M140 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 later 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 anesthetised (with urethane), whereas 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 MMN 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 magnetic counterpart of
APPENDIX: THE DENOISING PROCESS

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 denoising 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 denoising 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 denoising process, since each denoising 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 adaptation-related responses. We therefore confined our analysis to that component alone.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: G.B.C., M.C., A.d.C., and J.F.L. conception and design of research; G.B.C. and J.F.L. performed experiments; G.B.C. and A.d.C. analyzed data; G.B.C., M.C., A.d.C., and J.F.L. interpreted results of experiments; G.B.C. and A.d.C. prepared figures; G.B.C. and M.C. drafted manuscript; G.B.C., M.C., A.d.C., and J.F.L. edited and revised manuscript; G.B.C., M.C., A.d.C., and J.F.L. approved final version of manuscript.

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