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Exposing pathological sensory predictions in tinnitus using auditory intensity deviant evoked responses

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Competing interests

The authors declare no competing interests.

Abstract

We tested the popular, unproven theory that tinnitus is caused by resetting of auditory predictions towards a persistent low-intensity sound. Electroencephalographic mismatch negativity responses, which quantify the violation of sensory predictions, to unattended tinnitus-like sounds were greater in response to upward than downward intensity deviants in 26 unselected chronic tinnitus subjects with normal to severely-impaired hearing, and in 15 acute tinnitus subjects, but not in 26 hearing and age-matched controls ($p < 0.001$, ROC AUC 0.77), or in 20 healthy and hearing-impaired controls presented with simulated tinnitus. The findings support a prediction resetting model of tinnitus generation, and may form the basis of a convenient tinnitus biomarker, which we name Intensity Mismatch Asymmetry (IMA), that is usable across species, that is quick, tolerable and requires no training.

Significance statement

In current models, perception is based around the generation of internal predictions of the environment, which are tested and updated using evidence from the senses. Here, we test the theory that auditory phantom perception (tinnitus) occurs when a default auditory prediction is formed in order to explain spontaneous activity in the subcortical pathway, rather than ignoring it as noise. We find that chronic tinnitus patients show an abnormal pattern of evoked responses to unexpectedly loud and quiet sounds that both supports this hypothesis and provides fairly accurate classification of tinnitus status at the individual subject level. This approach to objectively demonstrating the predictions underlying pathological perceptual states may also have a much wider utility, for instance in chronic pain.

Introduction

Tinnitus, the persistent perception of an illusory sound, affects 13%, and significantly impairs the quality of life of 2%, of the population. The search for effective treatments is greatly hampered by limited understanding of its mechanisms. Its major risk factor is hearing loss, which leads to increased central gain (i.e. increased firing rate and/or synchrony in for a given input), though a review of current evidence suggests that these changes may be contributory to tinnitus, but not sufficient to cause it, while other evidence suggests that gain increases may be irrelevant, or even protective, with respect to tinnitus, and the presence or absence of hyperacusis can confound results. We have recently proposed a theory of tinnitus causation, which shares some features with an earlier theory, in which a crucial process is the learning of a default 'tinnitus prediction' by higher perceptual centres. Specifically, we suggested that the origin of the tinnitus signal is spontaneous firing in the ascending auditory pathway, but that this is usually successfully ignored as irrelevant noise. Furthermore, we proposed that once the brain has recognised the tinnitus signal as a sound source, it forms a default prediction of that sound continuing which prevents the spontaneous activity being ignored as noise, and that prediction ensures the persistence of tinnitus once present for a sufficient length of time. Similar *predictive coding* models have been proposed for many perceptual disorders, but actually demonstrating the aberrant predictions themselves is a much greater challenge, hence these models rely on circumstantial evidence. The present work aimed to search for evidence of the existence of a default auditory prediction that might underpin chronic tinnitus. We focused on the mismatch negativity (MMN) evoked response, which occurs

across many sensory modalities in response to stimuli that differ (for instance in frequency or intensity) from a series of preceding stimuli. Moreover, MMN magnitude quantitatively indicates the extent to which a particular stimulus violates a prior prediction of what that stimulus will be, making it a useful tool for inferring the content of sensory predictions. In this study, we compared MMN responses, obtained from a roving oddball paradigm featuring pure tones close to the tinnitus frequency, to upward and downward intensity deviants in order to expose alterations of auditory predictions that might be associated with tinnitus (Figure 1). Specifically, because tinnitus is a quieter sound than those used in the experiment, we hypothesised that the tinnitus prediction should skew predictions of intensity downwards, meaning that downward intensity deviants should produce smaller MMN responses, and upward intensity deviants produce larger responses, compared to matched controls. Although there have been numerous MMN studies in tinnitus, the present study differs importantly in that it both targets the tinnitus frequency and features deviants in intensity, and thus is uniquely able to address this hypothesis. Our results showed a striking asymmetry of MMN responses of exactly the type predicted, compared to age and hearing matched controls. This supports the prediction hypothesis of tinnitus (though other interpretations are possible), and can classify individual subjects' tinnitus status with a fair degree of accuracy. The work thus introduces a new potential tinnitus biomarker for further human and animal work.

Materials and methods

Subjects

Unselected chronic tinnitus subjects (n = 26) were recruited from local research volunteer mailing lists, with the only inclusion criteria being age 18 or over, persistent tinnitus for longer than six months, ability to perform experiments, and absence of structural brain pathology or profound hearing loss in the tinnitus ear(s). Non-tinnitus controls (matched n = 26, and simulated tinnitus n = 20) were recruited from the same lists and subjected to pure-tone audiometry, with the best matches being invited to take part in the full study on a separate occasion. Acute tinnitus subjects (n = 15) were recruited via paid advertising on an Internet search engine, with the same inclusion criteria as for chronic subjects, but with tinnitus duration of less than six weeks. Group sizes were chosen as the minimum necessary to give a high chance of demonstrating the predicted effects. Subject characteristics can be found in Table 1 of the main text. No significant differences in hearing thresholds (see Figure 5) at any frequency were present between chronic tinnitus subjects and matched controls, except at 0.5 and 1 kHz in the right ear, which were remote from the stimulus and tinnitus frequencies ($p < 0.05$).

Recruitment and data collection occurred between November 2017 and October 2018, with additional simulated tinnitus data being collected in August 2019. The study was given a favourable opinion by the Newcastle University Research Ethics Committee, and all participants provided written informed consent according to the Declaration of Helsinki.

Clinical and psychophysical assessment

All research activity occurred within the Institute of Neuroscience, Newcastle University. Subjects completed a short questionnaire covering demographic details, health conditions and medications. Tinnitus subjects also indicated the duration, character and laterality of

their tinnitus, along with visual analogue scale (VAS) ratings of their average and current tinnitus loudness, average annoyance, and completed the Tinnitus Handicap Inventory (THI). All subjects underwent pure tone audiometry at octave intervals from 0.25 to 8 KHz, with the addition of 6 kHz.

Tinnitus subjects performed five rounds of tinnitus matching, using custom-made tools in Matlab (Mathworks, Natick, MA, USA), based on interactively tuning narrowband noise with a Hanning-shaped spectrum in terms of its centre frequency (CF), bandwidth (BW), intensity, and laterality balance. Each round used random starting parameters for CF and BW. Subjects were allowed to discard matches they regarded as sub-optimal, and the mean CF and BW across remaining matches were used as a starting point for stimulus generation. Control subjects used their matched tinnitus-subject's data for stimulus generation.

For each subject, two pure tone stimuli of different frequencies were created: one at the tinnitus match centre, and one at the lower edge of the Hanning passband. Tinnitus subjects had one opportunity to tune the frequency of these until perceived as in the centre of the tinnitus frequency band ('centre'), and the other as close as possible to the tinnitus frequency while being distinctly lower in frequency ('edge'). The rationale for using these frequencies was to test whether any tinnitus-related effects were tightly locked to the tinnitus frequency, and whether precise tinnitus frequency matching would be required to observe these effects. All subjects then iteratively tuned the following parameters in sequence, until a full round passed with no further changes: laterality balance (centre then edge), balancing the subjective loudness of both frequencies, tuning overall intensity of both frequencies. Control subjects were allocated the stimulus frequencies of the tinnitus subject with the closest audiometric thresholds in stimulus ear/frequency, and were asked

157 to make stimuli as loud as possible without resulting in even minor discomfort, or producing
158 distortions from the headphones. The additional constraint for tinnitus subjects was that
159 tinnitus must remain audible in the gaps between stimuli (i.e. tinnitus not be totally
160 attenuated by residual inhibition).

161

162 **Experimental design**

163 In a soundproof room, 64 channel electroencephalography (EEG) was recorded from
164 participants, using a Biosemi Activetwo system (Biosemi, Amsterdam, The Netherlands),
165 while they were passively presented with the experimental stimuli through Sennheiser HD
166 380 pro headphones, and watched a silent subtitled movie. Electrode offset (equivalent to
167 impedance) was kept within manufacturer-recommended limits of +/- 40 mV.

168 The paradigm was a roving mismatch negativity (MMN) paradigm, in which 300 ms pure
169 tones (with 10 ms onset/offset ramps) were presented isochronously with a stimulus onset
170 asynchrony of 600 ms. Stimuli were presented to the tinnitus ear if entirely or mainly
171 unilateral (including to the matched control), and bilaterally in other cases. The roved
172 parameter was stimulus intensity, which randomly alternated between 0 and -6 dB (relative
173 to the subject-calibrated intensity) every four to eight stimuli. One block of the experiment
174 comprised 21 such intensity changes, and a total of 50 blocks were presented, alternating
175 between the centre and edge frequency. A control deviant condition was superimposed on
176 these sequences, whereby one in ten stimuli (1/6 probability, after minimum separation of
177 four stimuli) were duration deviants of 150 ms.

Simulated tinnitus subjects were simultaneously presented with ongoing narrowband noise on alternate blocks ('tinnitus on' condition). This noise had the spectrum of the tinnitus match of the acute or chronic tinnitus subject with the closest hearing thresholds at frequencies adjacent to their tinnitus frequency. To prevent the non-representative scenario of the stimuli and 'tinnitus' being perceptually identical, the bandwidth for simulated pure tone tinnitus was set to 1/40 octave. Intensity of the noise stimulus was set at the tinnitus match intensity initially, and the subject was asked to adjust the intensity, if necessary, to ensure it was loud enough to be audible over quiet speech, and quiet enough to not prevent a normal volume conversation. Such adjustments were not required in most cases.

EEG data processing

Data analysis was performed in Matlab, using the FieldTrip toolbox. EEG data were recorded at 1024 Hz, downsampled to 256 Hz and high-pass filtered from 0.3 Hz. Data were re-referenced to combined P9/P10, roughly corresponding to M1 and M2 locations. Bad channels were identified visually and reconstructed by interpolation. Data were epoched between -0.5 and 1 s peristimulus time, with demeaning and detrending. Epochs with grossly outlying maximum amplitudes, based on visual inspection, were excluded, followed by removal of ocular and muscle artefacts using independent component analysis (ICA). A mean of 23 components was removed per subject, with no significant difference in the number of components removed between tinnitus and control groups. Epochs were baseline corrected to -100-0 ms peri-stimulus time, and each epoch was summarised by four values derived from its (normalised within channel) time series: largest absolute amplitude in any channel at any time point; largest mean absolute deviation across channels at any

time point; largest mean absolute amplitude across time at any channel; largest mean absolute amplitude across time and channels. Histograms were plotted of the four values, and thresholds for trial rejection specified manually based on the point where the upper tail deviates from a normal distribution. Epochs were rejected if any of their four values exceeded its threshold, and approximately 10% of trials were rejected for each subject. Visual inspection of a subset of epoch waveforms confirmed that this method removed bad epochs successfully. Surviving epochs were averaged within their respective stimulus conditions, followed by low-pass filtering at 35 Hz.

Based on evoked peak topographies observed in pilot experiments, FCz was chosen as the sole channel from which to present time-domain data, and three time windows were determined that maximally captured the three deflections characterising the evoked response. The mean evoked potential within each time window was taken as the basis for statistical analysis.

Because we had no hypothesis about MMN latency, the duration of MMN responses were relatively long, and there were no clear differences in MMN latency, we did not subject MMN latency to any formal analysis. The primary outcome measure was MMN amplitude, with MMN-timeframe response magnitudes to standards, and N1 and P50 magnitudes as secondary outcome measures.

Statistical analysis

Statistical analysis was performed in Matlab. On account of Lillefort's test not indicating more datasets deviating from a normal distribution than expected by chance, ANOVA was

used as the basis for statistical analysis. For comparison of chronic tinnitus subjects and controls, a three-way ANOVA with full interaction terms was applied, with group (tinnitus or control), frequency (edge or centre) and intensity (low or high) as the factors of interest. As the acute tinnitus group was not matched to a control group, it was subject to a two-way ANOVA, with interaction term, with frequency and intensity as the factors of interest. The simulated tinnitus group was subject to a two-way ANOVA, with interaction term, with state ('tinnitus' on or off) and intensity as the factors of interest. Each ANOVA was performed separately on standards and deviants (deviants minus standards). The receiver-operator characteristic (ROC) curve was generated using standard Matlab functions.

Results

Subject characteristics

Subject groups comprised 26 unselected chronic tinnitus subjects, 26 age and hearing-matched controls, 15 acute tinnitus subjects, with repeat assessment of seven of these in the chronic phase, and 20 healthy controls studied with and without the simultaneous presentation of simulated tinnitus based on tinnitus subjects' psychophysical tinnitus matches. Their characteristics, along with their individual tinnitus matches and derived stimulus parameters, are summarised in Table 1. Due partly to prioritising audiometric matches at the stimulus frequencies, there were significant differences, between chronic tinnitus and matched control groups, in sex and stimulus intensity. The latter may have reflected the matching procedure, or (appropriate) compensation for hyperacusis in the tinnitus group. Stimulus loudness at the tinnitus edge frequency was, on average, higher in

the control than chronic tinnitus group (Table 1), but overlap between groups was high. To ensure that this did not lead to spurious results, we repeated the primary analysis after excluding the six tinnitus subjects with the lowest stimulus intensities (in dB SL) at the edge frequency; this balanced the group means for the edge frequency stimulus intensity (41.0 vs. 42.7 dB SL, $p = 0.75$), and increased the statistical significance of the main finding (discussed in its respective section) from $p = 0.0009$ to $p = 0.0001$. The inclusion of unselected tinnitus subjects, including those with severe high-frequency hearing loss, older volunteers, and both tonal and narrowband noise types of tinnitus, potentially may have added noise and variance to the data, but we considered this inclusivity important to prove the applicability of any findings to the broader tinnitus population as opposed to a particular subset.

Spatiotemporal organisation of stimulus response

In a roving MMN paradigm (Figure 1), with isochronous 300ms pure tones matched to either the centre frequency or lower edge (subjectively defined as 'just outside' the tinnitus sound) of the tinnitus frequency band as the stimuli, and stimulus intensity as the roved parameter, the event-related potential was characterised (Figure 2) by approximately equally sized P50 and N100 responses, and a prolonged late negative potential, peaking at 200-450ms in keeping with the timeframe of mismatch negativity (MMN). This is long for MMN in general, but we note that the one study to examine intensity deviants in tinnitus showed later responses to these than other deviants, in keeping with what we observed here. Furthermore, it is recognised that smaller perceptual changes are associated with later MMN responses (17).

268

269 **Early auditory evoked potentials (P50 and N100) are unaffected by tinnitus**

270 There were no differences in standard or deviant P50 responses related to subject group,
271 stimulus frequency or stimulus intensity. In a three-way ANOVA (subject group, stimulus
272 frequency, and stimulus intensity), N100 response magnitudes to standard stimuli showed a
273 main effect of larger responses to high intensity stimuli ($p < 0.05$), which was an expected
274 and trivial finding. An equivalent analysis of deviant-minus-standard responses showed a
275 main effect of larger responses to the tinnitus edge (lower) than tinnitus centre (higher)
276 frequency ($p < 0.005$). The lack of differences between tinnitus and control groups in these
277 early responses makes simple acoustic differences in stimuli, such as loudness, an unlikely
278 explanation for the tinnitus-related changes described below.

279

280 **Tinnitus-irrelevant duration deviants**

281 To exclude broad differences in predictive processes not specifically related to tinnitus or
282 the underlying hypothesis, we incorporated occasional shorter 150 ms stimuli to serve as
283 duration deviants. Duration deviants, in the absence of intensity changes, elicited clear
284 MMN responses, which showed no significant differences on account of stimulus frequency
285 or intensity, or subject group.

286

287 **Late (MMN timeframe) responses to standard stimuli show small differences due**
288 **to tinnitus**

289 Standard responses in the MMN time window showed a main effect of being larger for high
290 as opposed to low intensity stimuli ($p < 0.005$), which was expected. As shown in Figure 3A,
291 there was a group x frequency interaction ($p < 0.05$), whereby the control group, but not the
292 tinnitus group, had larger responses to the lower (edge) frequency standards.

293

294 **Asymmetry of MMN responses to intensity deviants differentiates tinnitus subjects**
295 **from controls**

296 There was a main effect of larger deviant minus standard responses to the lower (edge)
297 than higher (centre) frequency ($p < 0.0001$). This mirrored the equivalent difference seen in
298 the N1 responses to deviants, and is likely to be for the same reason. As shown in Figure 3B,
299 our principal finding was in line with our hypothesis, in that there was a group x direction
300 interaction ($p < 0.001$), whereby tinnitus subjects had larger responses to upward deviants
301 (minus standards), while matched controls had larger responses to downward deviants
302 (minus standards). The effect, which we term Intensity Mismatch Asymmetry (IMA), applied
303 to both tinnitus centre and tinnitus edge frequencies but, in keeping with the main effect of
304 frequency, the absolute effect appeared larger at the edge frequency, though the
305 contribution of frequency to this interaction was not statistically significant. Repeating the
306 analysis after excluding the six tinnitus subjects with the least intense edge frequency
307 stimuli (in order to balance mean intensity with the control group) showed the same finding,
308 but with the greater level of statistical significance of $p = 0.0001$.

309 To assess whether IMA can serve as a biomarker for tinnitus, we used the simple metric
310 (averaged across stimulus frequencies) of upward deviants (minus standards) minus
311 downward deviants (minus standards). Figure 4 shows the receiver-operator characteristic

(ROC) curve for this metric, which results in an area under the curve of 0.77, indicating the favourable end of 'fair' diagnostic accuracy. We aimed to avoid the use of a more complicated classifier metric, which might have produced greater accuracy, to constrain our findings to those that might be ported directly to animal studies. No significant linear correlations were observed between this metric and either THI or VAS loudness score.

Tinnitus-related MMN changes are present in the acute, as well as chronic, stages

We have previously hypothesised that there is a window of reversibility following initial tinnitus onset, before the tinnitus prediction becomes pervasive, though the length of this window would be unclear, potentially ranging from a scale of days to months. In a 2-way ANOVA (intensity and frequency), the group of 15 subjects with new-onset tinnitus (usually within the past 3-4 weeks) showed a main effect of upward intensity deviants yielding larger MMN responses than downward deviants ($p < 0.05$).

Absence of equivalent changes in simulated tinnitus

The above finding of IMA could theoretically be for the reason hypothesised, that the aberrant prediction responsible for tinnitus skews sensory predictions, or for the more trivial, but still diagnostically useful, reason that the presence of *any* quiet continuous sound alongside the stimuli skews predictions towards that quiet intensity. To distinguish these possibilities, we conducted the same experiment in 20 healthy controls, with and without hearing loss, with half the blocks containing the addition of simulated tinnitus based on tinnitus subjects' matching data. To maintain a sufficient number of trials, only stimuli at the

tinnitus centre frequency were used. No significant differences were found between the 'tinnitus on' and 'tinnitus off' state; in a two-way ANOVA featuring deviant direction (up or down) and state (on or off), the p values for main effects of state and state x direction interaction were 0.81 and 0.77 respectively.

Discussion

Intensity Mismatch Asymmetry (IMA) differentiates tinnitus subjects from controls

We tested the hypothesis that development of chronic tinnitus requires formation of a pervasive 'default' prediction of a (usually quiet) constant sound within a specific frequency band, and that this prediction favours perceptual recognition of tonotopically-specific spontaneous firing in the auditory pathway as a real sound (i.e. tinnitus) rather than ignoring as noise. Processing of auditory stimuli within or close to the relevant frequency band, might be altered by skewing of all predictions towards the characteristics of the default prediction (Figure 1). We hypothesised that these skewed predictions would be detectable in MMN responses to intensity deviants around the tinnitus frequency; because downward deviations in intensity involve stimuli becoming quieter, therefore more similar to the default prediction, tinnitus subjects would show reduced response magnitudes. Conversely, upward deviations in intensity involve stimuli becoming louder, hence more distant from the default prediction, tinnitus subjects would show increased response magnitudes. Thus we hypothesised that contrast between upward and downward intensity

355 deviants might serve as an objective marker of tinnitus, and our results support this
356 hypothesis in both the acute (around 4 weeks from onset) and chronic stages of tinnitus.

357

358 **The IMA effect reflects tinnitus specifically**

359 In theory, differences in MMN responses might occur simply because of an ongoing sound
360 filling in the inter-stimulus gaps, in which case IMA would be an epiphenomenon of tinnitus,
361 rather than a causative factor. To differentiate these possibilities, we studied 20 non-
362 tinnitus controls with and without the simultaneous presentation of narrowband noise
363 derived from subjects' tinnitus matches. Short-term simulated tinnitus should not alter
364 default predictions of the kind hypothesised to underlie tinnitus, because: 1) we only
365 presented it for around 60 seconds at a time, which would not be a long enough timescale
366 to form pervasive default predictions; 2) not everybody would necessarily change their
367 default prediction even after a sufficiently long duration.

368 The addition of simulated tinnitus within this second control group did not produce any
369 appreciable change in MMN responses, suggesting a specific role in tinnitus.

370

371 **Mechanisms potentially underlying IMA**

372 Auditory MMN is generated by a bilateral network of primary and non-primary auditory
373 cortex and inferior frontal gyrus (IFG). Reciprocal interaction between these centres is
374 argued to comprise the bottom-up propagation of prediction errors, which signal
375 discordance between prior prediction and sensory input, and the top-down updating of
376 sensory predictions in light of this new evidence, though other explanations include sensory

memory, local adaptation to a stimulus and change detection. MMN amplitude is also sensitive to higher level statistical structure in stimulus sequences, and therefore also provides a quantitative indication of the improbability of a stimulus based on prior predictions. Ventrolateral prefrontal cortex (including IFG) has been argued to form part of a 'tinnitus core' network, which also includes auditory, inferior parietal and parahippocampal cortex (PHC). PHC has shown altered resting-state activity contralateral to the tinnitus ear and resting-state fMRI correlation with auditory cortex, and based on its prominent role in auditory memory is a potential source of persistent auditory predictions. While these networks are likely contributors to the IMA effect we observed here, as the present study does not provide source-resolved activity, it does not in itself specify the brain basis of the effect. Future work might address this issue with imaging modalities with higher spatial resolution.

MMN magnitudes might be affected by changes in central gain, including related to hyperacusis, or deficient noise cancellation via frontostriatal gating which amounts to a gain control mechanism. P50 suppression is often used as a marker cortical input gating, and might have been expected to be a sensitive marker of any gating changes if present. However, there were no differences in any evoked response magnitudes to standard stimuli between tinnitus and control groups, suggesting against a straightforward gain or hyperacusis-related explanation. There are, however, more nuanced aspects of gain, such as dynamic range adaptation, and so we cannot altogether rule out changes in gain in the broader sense as a contributory factor.

We attempted to standardise attention by having all subjects watch a subtitled movie they found engaging, and all subjects claimed they were able to largely ignore the auditory

stimuli and attend to the movie. However, this was not formally quantified, hence some differences between groups cannot be ruled out. Similarly, subjects with substantial tinnitus-related distress might attend more to auditory stimuli, or perceive intensity increases in a more threatening way. However, we observed no correlation between magnitude of IMA effect and THI score

Previous MMN studies in tinnitus

Previous MMN and equivalent studies, have varied according to the type of deviant, the paradigm used, control matching for hearing loss and, importantly, whether stimulus frequencies were standardised or targeted to subjects' tinnitus. Studies with non-targeted stimulus frequencies have reported slightly smaller MMN responses to deviants of all types tested, and minor differences in P300 oddball responses to auditory and visual stimuli. At the audiometric (not tinnitus) edge frequency, tinnitus patients showed larger MMN responses (in the N1 timeframe) to downward frequency deviants than hearing unmatched controls, and unchanged responses one octave lower. Frequency deviants, with the deviant at the tinnitus match frequency, and the control frequency 10% different, have been found to be increased compared to controls, with partial resolution of the difference following successful tinnitus retraining therapy. Using standardised stimulus frequencies around 8 kHz (irrespective of tinnitus frequency), smaller MMN responses were observed in tinnitus patients with high levels of distress only. These studies set a precedent for there being small differences in sensory, mnemonic and/or predictive processing relevant to the MMN in tinnitus. Our present study is the first to feature intensity deviants targeted to the tinnitus

422 frequency, and as such our results show a much stronger effect, and may provide a way
423 forward for this specific field in tinnitus research.

424

425 **Potential use as a biomarker**

426 Successful animal research into tinnitus mechanisms and treatments requires knowing
427 which animals experience tinnitus. Numerous methods have been developed to determine
428 this, and broadly fall into two categories. Conditioned behaviour models are often regarded
429 as the more accurate, and require lengthy prior training of animals to perform or refrain
430 from certain behaviours, such as licking, during the presence of an ongoing sound.
431 Automatic response methods have the advantage of requiring no training, and exploit
432 involuntary responses, such as the acoustic startle response, in conjunction with stimuli
433 related to the possible tinnitus (such as a short gap in an ongoing pure tone) to modify this
434 depending on tinnitus status, but are subject to caveats and controversies, and show
435 inconsistent replicability in humans. The two types of approach have shown limited
436 correlation with each other, and with the presence or absence of an auditory insult
437 potentially sufficient to induce tinnitus. However, because there is no gold standard in
438 animals against which to test the sensitivity and specificity of a diagnostic tinnitus test, the
439 performance of these measures remains unquantified. Potential biomarkers derived from
440 human tinnitus studies have mainly focused on whole-brain resting-state imaging of
441 electrical activity or large scale correlations in cerebral blood flow, but these measures are
442 inherently non-transferrable to animals. The IMA technique, as reported here, has the
443 potential to constitute a diagnostic test that is quantifiable in its diagnostic performance, on
444 account of being developed in humans, applicable across species, free from training

445 requirements and quick to perform. The presence of the IMA effect even just outside the
446 tinnitus frequency suggests that its success does not depend upon highly specific tinnitus
447 matching. Another recent study has had the same aim, based on quantifying the acoustic
448 change complex, an evoked response to a change during a stimulus. It yielded only slightly
449 lower ROC performance, but we note that the study was subject to numerous limitations,
450 including only yielding this result at uncomfortably loud stimulus levels, and excluding
451 subjects who were older or had significant hearing loss. Although there are a number of
452 factors to address in follow-up studies (e.g. tuning curves over stimulus frequency and
453 intensity, optimising stimulus timing and duration, standardised diagnostic cut-offs), we
454 believe the IMA technique might have the potential to serve as a convenient and robust
455 biomarker for future animal studies of tinnitus.

456

457 **Parallels with other perceptual disorders**

458 Predictive coding accounts of perception, are popular in neuroscience, and our predictive
459 coding tinnitus model joins other predictive coding models of tinnitus, and other
460 pathological perceptual states including chronic pain, musical hallucinosis, psychosis, and
461 functional neurological disorder. These theoretical models generally lack support by
462 measurement of the pathological predictions themselves. Here, we demonstrate proof of
463 concept that pathological predictions can be measured using cheap, widely-available tools.
464 As it shares many parallels with tinnitus, chronic pain would be a logical condition to extend
465 this approach to next.

466

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591 Figure legends

592 **Figure 1:** Experimental hypothesis and paradigm. The paradigm is a roving mismatch
593 negativity (MMN) paradigm, with 300ms pure tones (black bars) interspersed with 300ms
594 intervals. Stimulus frequency is matched to within or adjacent to the individual tinnitus
595 frequency band. Intensity is the roved parameter, between 0 and -6 dB relative to an
596 individualised reference intensity (R). We hypothesised that control subjects would make
597 optimal predictions of upcoming stimuli (blue line) based on recent stimulus history, the
598 presence of a tinnitus prediction (T and grey line) would result in tinnitus subjects forming
599 an intermediate prediction (red line) between the optimal stimulus-based prediction and
600 the tinnitus prediction. This would result in an asymmetry between MMN responses to
601 upward and downward intensity deviants in tinnitus subjects (red arrows) compared to
602 controls (blue arrows). Note that many aspects of stimulus sequences are predicted, but
603 illustrated predictions here refer only to the intensity of the next upcoming stimulus. Also
604 note that because control subjects' default prediction is of no sound at all, rather than an
605 auditory percept (such as tinnitus) but with zero intensity, this does not impact the
606 predicted intensity of upcoming stimuli.

607

608 **Figure 2:** Spatiotemporal distribution of evoked responses. (A): group mean scalp
609 topographies within colour-coded time windows capturing the three dominant waveforms.
610 Illustrative responses are shown for the high intensity standards, and upward deviant minus
611 high standard responses, only. Std. = standard. Dev. = deviant. (B): Evoked waveforms from
612 the FCz electrode, highlighted in red in (A), to both standard (dashed) and intensity deviant

(solid) stimuli at the tinnitus centre (black) and edge (red) frequencies. Grey horizontal bars indicate stimulus presentation, and coloured vertical bars indicate the colour-coded time windows corresponding to P50, N100 and MMN, as shown in (A), and forming the basis of statistical analyses.

Figure 3: Mismatch negativity (MMN) amplitudes in chronic tinnitus and control subjects. Bars indicate group mean and standard error. Colour coding indicates subject group, with red denoting chronic tinnitus and blue matched controls. The upper plot indicates MMN timeframe responses to standard stimuli, while the lower plot indicates MMN responses to deviant minus standard responses. Significant differences relevant to tinnitus status are indicated by p values and nested brackets, with black brackets indicating variables, or interactions, associated with significant effects based on ANOVA analyses, and grey brackets indicating variables not significantly contributing to effects. The core finding was a significant ($p < 0.001$) group (chronic tinnitus vs. control) by direction (upward vs. downward) interaction in deviant MMN responses.

Figure 4: Receiver-operator characteristic (ROC) curve for classification of subjects as chronic tinnitus or matched control.

Figure 5: Pure tone audiometry of subject groups. Plots indicate group mean and standard error at each frequency/ear. Asterisks indicate differences between chronic tinnitus (T)

634 subjects and matched controls significant at $p < 0.05$ uncorrected, which were only present
635 at frequencies remote from any experimental stimuli used.

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639 Tables

Mean (SD)	Chronic T	Control	p = (T vs C)	Acute T	Simulated T
Demographics					
Age	55.4 (13.6)	59.7 (15.3)	0.31	53.8 (12.5)	45.0 (19.1)
Sex	13F 13M	19F 7M	0.014 *	6F 9M	10F 10M
Tinnitus characteristics					
Duration	15.5 (16.7) years			4.2 (1.7) weeks	
THI	31 (28.1)			27 (23.2)	
T ear	11/15/0 L/C/R			4/8/3 L/C/R	
T character	13/13 T/N			9/6 T/N	
VAS loudness	5.0 (2.1)			4.8 (1.9)	
VAS distress	4.8 (2.9)			4.8 (2.8)	
% awareness	55.2 (34.3)			44.6 (23.7)	
T match CF (Hz)	6777 (2009)			7047 (2536)	
T match BW (oct)	0.25 (0.25)			0.17 (0.20)	
Stimulus and hearing characteristics					
Centre F (Hz)	7709 (2706)			7582 (2517)	7164 (2861)
Edge F (Hz)	5901 (1948)			6028 (2418)	
Edge F to centre F (oct)	0.37 (0.27)			0.39 (0.27)	
Edge F to match lower bound (oct)	0.078 (0.27)			0.18 (0.18)	
Thresh centre (dB)	44.8 (26.1)	37.9 (21.1)	0.19	34.3 (20.6)	20.3 (19.6)
Thresh edge (dB)	40.3 (21.1)	35.4 (17.0)	0.25	31.6 (22.7)	
SPL centre (dB)	78.1 (18.4)	78.2 (11.2)	0.99	79.5 (13.0)	79.0 (14.1)
SPL edge (dB)	72.8 (16.4)	78.9 (9.68)	0.043 *	76.3 (12.6)	
SL centre (dB)	33.3 (23.3)	40.2 (20.6)	0.15	45.2 (23.6)	58.7 (20.6)
SL edge (dB)	32.5 (20.6)	43.4 (18.3)	0.012 *	44.7 (26.8)	

640 **Table 1:** Subject, tinnitus and stimulus characteristics. Numbers inside and outside of

641 parentheses indicate mean and standard deviation, respectively, unless otherwise indicated.

T = tinnitus. C = control. THI = Tinnitus Handicap Inventory. VAS = visual analogue scale. CF = centre frequency. BW = bandwidth. Oct = octaves. Thresh = hearing threshold (at specified frequency). SPL = sound pressure level (of stimulus). SL = sensation level (of stimulus). L/C/R = left (predominant)/centre/right (predominant). T/N = tonal/narrowband noise. F = female. M = male. * indicates $p < 0.05$. Shaded cells indicate measures not applicable to particular groups.