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1 **Human insula neurons respond to simple sounds during passive listening**

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4

5 **Contributions**

6 **Berger**: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data 7 Curation, Writing - Original Draft, Writing - Review & Editing, Visualization; **Kawasaki**: 8 Investigation, Writing - Review & Editing; **Banks**: Writing - Review & Editing; **Kumar**: 9 Writing - Review & Editing; **Howard**: Funding acquisition, Writing - Review & Editing;

10 **Nourski**: Conceptualization, Investigation, Visualization, Writing - Original Draft, Writing 11 - Review & Editing

12

13 **Abstract**

14 The insula is critical for integrating sensory information from the body with that arising 15 from the environment. Although previous studies suggest that posterior insula is 16 sensitive to sounds, auditory response properties of insula neurons have not previously 17 been reported. Here, we provide the first report of a population of human single neuron 18 data from the insula and provide comparative data from the primary auditory cortex, 19 recorded intracranially from human participants during passive listening. In each 20 condition, more than 330 single neurons were recorded in 11 participants. Almost a third 21 of neurons in posterior insula and a smaller subset in anterior insula responded to 22 simple tones and clicks. Responsive neurons were distributed throughout posterior and 23 anterior insula and showed preferred frequency tuning. Onset latencies in the insula 24 were similar to those in the primary auditory cortex but response durations were 25 significantly shorter. Overall, these data highlight that insula neurons respond to 26 auditory stimuli even in non-behaviorally relevant contexts and suggest an important 27 contribution of audition to the postulated integrative functions of insular cortex.

28

29 **Introduction**

30 The insula, buried within the lateral fissure, is a key brain region involved in processing 31 of interoceptive signals [1-5] which includes sensations not only from within the interior 32 or viscera of body (e.g. cardiac, respiration, hunger) but also those arising from the skin 33 (e.g. pain, temperature and affective touch; [1, 6] for a review, see [7]). Theoretical 34 models of insula function suggest that these interceptive sensations form the basis for 35 subjective feelings [8] and sense of bodily self [9]. By contrast, empirical evidence for 36 the role of insula in processing of exteroceptive signals, such as sounds that are

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37 external to the body and not associated with interoceptive signals, remains scant, 38 although there is some limited evidence implicating the insula in auditory processing. 39 Case reports have shown that damage to the insula region can cause alterations in 40 auditory perception [10-13], even without apparent damage to auditory cortex. 41 Additionally, studies in animals [14-18], human neuroimaging using functional magnetic 42 resonance imaging (fMRI) [19-22] and electrophysiology [23-29], suggest that activity 43 within the insula may be modulated by sound, particularly when it is emotionally 44 arousing (e.g. music) or behaviorally relevant (e.g. detection of an odd tone), while 45 recent intracranial local field potential data highlight that posterior insula represents 46 phonological features during both speech production and perception [30]. In 47 misophonia, a condition characterized as an intense emotional reaction to sounds of a 48 particular nature (e.g. chewing), the anterior insula is hyperactivated and 49 hyperconnected when listening to trigger sounds [31]. This finding has been interpreted 50 within the context of social cognition and interoceptive awareness [32, 33]. The insula 51 has also been implicated in auditory hallucinations [34], and intracranial stimulation of 52 this region can produce auditory sensations [35-37], although not always [38]. However, 53 electrophysiological and imaging data have almost always been obtained in behaviorally 54 salient contexts, such as music or speech, while lesion studies may not always be 55 specifically localized to insula exclusively, and there are currently no reports of single 56 neuron responses recorded in the human insula.

57 Prior studies also do not address whether there is any tuning to basic acoustic sound 58 attributes in the insula [39]. For example, neurons in primary auditory cortex are tuned 59 to specific spectral and temporal features of acoustic stimuli [40-42]. Can activity of 60 individual neurons in the insula also be modulated according to these fundamental 61 acoustic properties even when stimuli are not behaviorally relevant? If so, what are the 62 latencies and tuning properties of these neurons, and where are these located?

63 We addressed this gap in knowledge by recording extracellular single neuron activity 64 from the insula in eleven humans and comparing these recordings to those obtained 65 from the adjacent posteromedial portion of Heschl’s gyrus (HGPM), corresponding to

66 primary auditory cortex [43]. Data were collected while participants passively listened to 67 auditory stimuli consisting of short click trains and pure tones of various frequencies. 68 This allowed us to determine (1) the extent of insula neurons that are responsive to 69 auditory stimulation; (2) whether these neurons showed frequency tuning; (3) the spatial 70 distribution of responsive neurons across posterior and anterior insula (InsP and InsA, 71 respectively); and (4) the latencies, magnitudes, and durations of these responses 72 compared to HGPM. Knowledge about the neuronal properties of this brain area in 73 response in audition is important for understanding how the human brain processes 74 sounds. This knowledge has additional relevance to auditory hallucinations and 75 disorders such as tinnitus [44].

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78 **Results**

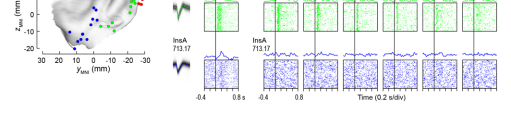
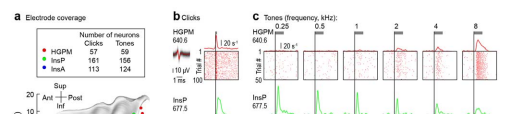
79 Electrodes were implanted for clinical purposes of monitoring seizures and were 80 localized based on pre- and post-operative neuroimaging (see *Methods*). Single neuron 81 responses to 40 ms 100 Hz click trains were recorded in a single 100-trial block. 82 Responses to 300 ms tones were recorded in a separate block while participants were 83 presented with pseudo-randomly ordered tones of varying frequency, from 0.25 kHz to 8 84 kHz, separated by octave steps. Each tone was presented 50 times, resulting in 300 85 trials total. Interstimulus intervals were 2 s in both experiments (see *Methods* for full 86 details of auditory stimulus presentation).

87 **Figure 1a** displays the locations from which single neurons were successfully isolated, 88 shown on a template reconstruction of the insula. The size of each marker indicates the 89 number of neurons isolated at a particular location. For click stimuli, a total of 331 90 neurons were isolated (57 HGPM neurons, 161 InsP neurons and 113 neurons in InsA). 91 For pure tone stimuli, a total of 339 neurons were isolated (59 HGPM neurons, 156 InsP 92 neurons and 124 InsA neurons). **Figure 1b** shows spike waveforms, trial rasters, and 93 kernel-smoothed spike density functions for exemplar neurons in HGPM, InsP and InsA 94 (top to bottom panels, respectively) in response to click train stimuli. In these examples, 95 HGPM shows a sharp onset response to the click train, while the InsP neuron responds 96 consistently across trials with a slightly longer latency than HGPM, and the InsA neuron 97 responds with an even longer latency, with visibly less consistent timing across trials. 98 **Figure 1c** shows examples for three single neurons in these same brain locations in 99 response to pure tone stimuli of different frequencies. The neurons in HGPM and InsP

100 showed clear evidence of frequency tuning (to 8 kHz and 0.25 kHz, respectively). 101 Tuning is less obvious in the InsA neuron, though significant suppression was observed 102 in response to tonal stimuli of 2 kHz.

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**Figure 1: Insula neurons respond to clicks and tones.**

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**a**, Locations and numbers of isolated putative single neurons in response to clicks and tones, plotted on a template reconstruction of insula. **b**, Example responsive neurons in HGPM (top panel), InsP (middle panel) and InsA (bottom panel). For each example, corresponding spike

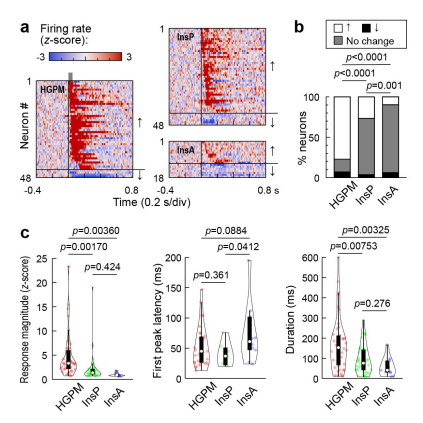
density functions are shown above raster plots. Codes under location names indicate neuron ID. Grey horizontal bars indicate duration of the stimulus. Time on x-axis is relative to stimulus onset (vertical line). **c**, Example responsive neurons for the same locations in response to tones of differing frequencies. For these examples, the corresponding best frequencies were 8 kHz, 0.25 kHz and 2 kHz, respectively, with the InsA neuron exhibiting significant suppression to the tonal stimulus. Throughout, red: HGPM, green: InsP, blue: InsA.

103 Information such as response latencies can provide crucial information about the 104 position of a region within the hierarchy of an information processing network [45-48]. 105 Thus, to characterize the properties of neuronal responses to clicks across the 106 population, we examined response magnitudes, latencies, and durations for modulated 107 neurons in each region. For each neuron, significant modulation in response to auditory 108 stimuli was determined by comparing the spike rates following the stimulus to the pre 109 stimulus baseline (see Methods). Trial-averaged raster plots for all significantly 110 modulated neurons in each of the three regions are shown in **Figure 2a**. These are 111 separated according to whether they increased or decreased their activity (indicated by 112 the arrow directions). The overall proportion of modulation response types is then 113 shown in **Figure 2b**. As is evident from both plots, the majority of modulated neurons 114 increased their firing rates in response to click-train stimuli. Across all recorded neurons, 115 30.4% of neurons in InsP were click-responsive (26.7% increasing, 3.73% decreasing)

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116 and 15.9% were modulated to clicks in InsA (9.73% increasing, 6.19% decreasing). By 117 comparison, 84.2% of neurons in HGPM modulated significantly to clicks (77.2% 118 increasing their activity, 7.02% decreasing). Fisher’s Exact probability tests with the 119 Freeman–Halton extension (to allow for preserving modulation direction with a 2 x 3 120 test) revealed that there were significant differences in the proportions of modulated 121 neurons between HGPM and both InsP and InsA (*p* < 0.0001 for both comparisons), 122 and between InsP and InsA (*p* = 0.001), highlighting that a significantly larger proportion 123 of neurons were modulated in InsP than in InsA, while both regions were proportionally 124 less modulated than HGPM.

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**Figure 2: Insula responses to clicks are prominent and have similar latencies to HGPM. **

**a**, Averaged raster plots for all modulated neurons, organized by region and sorted according to first peak latency. Arrows indicate neurons that either increased (up arrow) or decreased (down arrow) in response to clicks. **b**, Proportion of response types to click-trains for the three different

regions. *P*-values based on Fisher’s Exact probability tests. **c**, Violin plots showing response magnitudes (left panel), latencies (middle panel) and durations (right panel) for all three regions, across positively modulated neurons. White symbols denote medians, bars indicate Q1 and Q3, and whiskers show the range of lower and higher adjacent values (i.e., values within 1.5 interquartile ranges below Q1 or above Q3, respectively). *P*-values based on linear mixed effects models.

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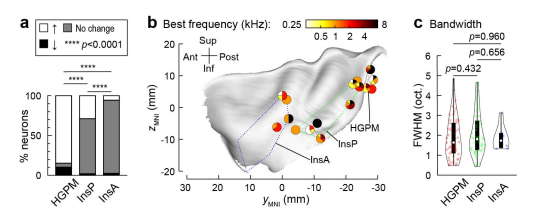
126 The magnitudes, latencies and durations of all positively modulated responses to click 127 train stimuli are shown in **Figure 2c**. The average magnitudes of responses following 128 stimulus presentation (0 to 350 ms post-stimulus onset) in InsP and InsA were 129 significantly smaller than HGPM (*p* = 0.00170 and *p* = 0.00360, respectively), with no 130 significant difference between InsP and InsA (*p* = 0.424). Positively modulated InsP 131 neurons exhibited similar latencies to HGPM neurons (*p* = 0.361), with median latencies 132 of 44.0 ms (interquartile range [IQR] = 48.8) and 48.5ms (IQR = 45.5), respectively 133 (middle panel of 2C), which is suggestive of parallel hierarchical auditory processing 134 between the two regions, while InsA latencies were notably slower (median = 67.0 ms, 135 IQR = 57.2). InsP and InsA responses were significantly more transient than HGPM 136 responses (*p* = 0.008 and *p* = 0.003, respectively; right panel in **Figure 2c**), with InsP 137 showing a median duration of 76 ms (IQR = 102.0) compared to a median of 153 ms 138 (IQR = 146.5) in HGPM, highlighting that there were differences in response patterns 139 between insula and HGPM neurons. InsA responses were even more transient (median 140 = 41 ms, IQR = 58.5), though there was no significant difference overall between the 141 response durations of InsP and InsA neurons (*p* = 0.276). Modulated neurons were 142 distributed throughout each subregion of insula, though there was an absence of 143 modulation in the most ventral aspects of InsA (see **Supplementary Figure 2**).

144 Figure 3 shows the response properties of the different regions for tones. Approximately 145 one third of InsP were modulated by tones (30.8% total; 28.8% increasing, 1.92% 146 decreasing). A higher proportion of neurons in HGPM were modulated by tones (94.9% 147 total; 84.8% increasing, 10.2% decreasing), with a lower proportion in InsA modulated in 148 response to tones compared to clicks (8.07% total; 5.65% increasing, 2.42% 149 decreasing). As with click trains, there were significantly higher proportions of 150 modulated neurons in HGPM compared to both InsP and InsA (*p* < 0.0001 for both 151 comparisons) and in InsP compared to InsA (*p* < 0.0001). See **Supplementary Figure** 152 **3** for the spatial distribution of the proportion of tone-modulated neurons.

153 We examined whether there were differences in frequency tuning for the different 154 regions by determining the proportion of neurons showing a preference to each of the 155 tone frequencies (**Figure 3b**). As the electrodes used in this study do not allow for 156 determining the precise spatial location of wires relative to one another (they only show 157 as a cluster of microwires on the magnetic resonance images [MRI] and do not clearly 158 map to particular channels), along with the relative sparsity of sampling of certain areas 159 of insula, we could not reliably obtain tonotopic maps for the individual electrode arrays. 160 However, as with clicks, there was an absence of response modulation in ventral 161 anterior insula. There was no clear preference for a particular tone frequency across 162 neurons, although fewer neurons in HGPM modulated maximally to 4 kHz than other 163 frequencies. To determine whether there were differences in how precisely tuned insula 164 neurons were, we assessed the bandwidth of responses to different tones (see

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**Figure 3: Tuning properties of insula and HGPM neurons.**

**a**, Proportion of response types for the three different regions. *P*-values based on Fisher’s Exact probability tests. **b**, Proportion of modulated neurons showing best responses to each tone frequency for each recording location. Each marker represents a recording location, with the proportion of modulated neurons responding to different frequencies represented according to the pie chart within that marker, i.e. if 100% of modulated neurons responded to 1 kHz, this would be represented by a fully orange circle. **c**, Violin plots showing bandwidth of all modulated neurons, measured in octaves according to full-width half maximum (FWHM; see Methods). White symbols denote medians, bars indicate Q1 and Q3, and whiskers show the range of lower and higher adjacent values (i.e., values within 1.5 interquartile ranges below Q1 or above Q3, respectively). *P*-values based on a linear mixed effects model.

165 Methods). This is shown in **Figure 3c** for the three different regions, with the spatial 166 distribution shown in **Supplementary Figure 3**. The median bandwidth for positively 167 modulated neurons was 1.64 octaves for HGPM (IQR = 1.54), 1.81 octaves for InsP

168 (IQR = 1.48) and 1.73 octaves for InsA (IQR = 0.75). There was no significant difference 169 in bandwidth between InsP and InsA (*p* = 0.656), nor between either region and HGPM 170 (*p* = 0.432 and 0.960, respectively), highlighting that the tuning properties to tones were 171 similar across the three regions.

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172 **Discussion**

173 Our results demonstrate that neurons in the human insula significantly modulate their 174 activity in response to auditory stimuli that are passively presented, in the absence of 175 any obvious behavioral or emotional salience. Responses in insula had similar latencies 176 and bandwidths to HGPM, but were more transient. The highest proportion of these 177 responses were in InsP, though a smaller minority of neurons in InsA were also 178 modulated, albeit more weakly. Overall, these results suggest that processing of basic 179 properties of acoustic stimuli contribute to the postulated role of the insula.

180 The insula, particularly InsA, is known to be part of a salience network [49, 50] which 181 detects and marks how behaviorally relevant the stimulus/events in the environment 182 are. Depending on the degree of saliency, the insula mediates further processing by

183 directing attention or allocating working memory by coordinating between different brain 184 networks (e.g. activating a fronto-parietal network for attention and deactivating the 185 ‘mind wandering’ default mode network for a highly salient event). How does InsA 186 receive sensory inputs for marking their saliency? Theoretical models of insula function 187 [1, 50, 51] suggest that InsP receives sensory inputs which are then re-represented in 188 InsA. There is now ample evidence to support the idea that interoceptive signals from 189 the body do indeed reach InsP [1]. Whether this is also the case for exteroceptive 190 signals such as auditory, however, has historically been less certain. Our high spatial 191 temporal resolution single neuron data recorded from the insula highlights that – like 192 signals from the body – auditory signals from the external environment are received in 193 InsP. It should be noted that in the saliency model of insula function, stimuli need not be 194 salient or behaviorally relevant to reach InsP, and they were not in the case of the 195 presence study. The saliency of the stimuli is proposed to be marked in InsA. This 196 model may then explain why in our data the proportion of modulated neurons is less in 197 InsA compared to that in InsP when passively listening to sounds, as these sounds were 198 not required to be salient.

199 Evidence showing that insula activity is modulated even during passive listening may 200 explain – at least in part – why lesions to the insula can cause altered auditory 201 processing, such as in cases of acquired amusia, temporal processing deficits or 202 hyperacusis after a stroke [52, 53]. The results of the current study are also consistent 203 with neuroanatomical studies showing that the insula receives direct projections from 204 auditory cortex [54, 55] and medial geniculate body [56], although as highlighted by 205 Remedios et al. [14], anatomical results do not give insight into the nature of the 206 involvement of the insula in auditory processing without corresponding physiological 207 data, which we have provided here.

208 These results may also explain why stimulation of insula can produce auditory 209 perceptual experiences [35-37]. Contrasting with these other studies, Duong et al. [38]

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210 did not find auditory responses from stimulation of the insula, which the authors 211 suggested was due to previous studies stimulating structures adjacent to rather than 212 within the insula proper. In the current study, our electrode locations were confirmed to 213 be in the insula based on individual participants’ MRIs, as well as plotted on a template 214 using MNI coordinates. While it is plausible that some studies may have stimulated 215 regions adjacent to the insula to produce auditory perceptual experiences, another 216 possibility is that an absence of behavioral response may be due to the still relatively 217 small fraction of neurons in this region being involved in auditory processing (about 1/3 218 in the current study). Thus, if particular neurons were not stimulated by electrodes within 219 the insula, then an auditory percept would likely not be produced. Further studies 220 utilizing both stimulation mapping and auditory presentation in the same neurons would 221 be invaluable for elucidating the dynamics of these auditory-responsive insula neurons.

222 Reports of the tuning properties of single neurons in HGPM are rare, with only a handful 223 studies to date examining this [57-59]. In the first systematic study of individual neurons 224 in human auditory cortex, the bandwidth measured in a similar manner to the current 225 manuscript was at least 1 octave [58], consistent with what we found here. Contrastingly 226 though, the bandwidth of neuronal responses to tones here was considerably larger 227 than what was demonstrated previously when using an approach that estimates based 228 on spectro-temporal receptive fields derived from random-chord stimuli [59]. It is 229 therefore likely that basic tonal stimuli – although widely used historically to study the 230 function of the auditory system [60] – do not reveal the full extent of the precision of 231 stimulus encoding, and future studies should examine this with refined stimuli for both 232 the HGPM and the insula. Moreover, while this study represents a rich and unique 233 dataset, the spatial extent of electrode coverage was still somewhat limited, as 234 determined solely by the clinical needs of the patients. This work lays the foundation for 235 further, more comprehensive, studies aimed at understanding the full extent of auditory 236 related single neuron activation throughout the insula.

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237 **Methods**

238 *Participants*

239 Extracellular single neuron recordings were obtained from 11 adult neurosurgical 240 patients, implanted with electrodes for the purposes of monitoring epileptiform activity 241 prior to potential treatment. Research was conducted under approval of the University of 242 Iowa Institutional Review Board and written informed consent was obtained from all 243 participants prior to data collection. Recordings were made while subjects were reclined 244 in a hospital bed, in a custom-designed dedicated electromagnetically-shielded facility 245 within the University of Iowa Clinical Research Unit.

246

247 *Electrodes and recording system*

248 Stereo electroencephalography (sEEG) depth electrodes (Ad-Tech Medical, Oak Creek, 249 WI) were placed in brain locations based solely on a clinical need to identify seizure foci 250 [61]. For the purposes of recording single neurons, sEEG electrodes included here in 251 the insula and HGPM were of a hybrid design [62]. These consisted of eight 39 µm 252 diameter platinum-iridium high-impedance microwires that were insulated plus one 253 uninsulated microwire. These microwires protruded from the end of the macro recording 254 probe and were prepared with a cut length between 2 to 4 mm, depending on the 255 distance of the most distal macro contact to the appropriate brain target. Each of these 256 microwires was individually separated in a splay pattern in the operating room 257 immediately prior to implantation. Electrode locations were confirmed based on post 258 operative MRI scans, preprocessed using Freesurfer [63] (see below for further details). 259 All neurophysiological data were recorded using a Neuralynx Atlas System (Neuralynx, 260 Bozeman, MT). High impedance recordings were first passed through a preamplifier 261 located on top of the patient’s head (ATLAS-HS-36-CHET-A9, Neuralynx, Bozeman, 262 MT) prior to interfacing with the ATLAS acquisition system. These were subsequently 263 recorded with a 32000 Hz sampling rate, filtered between 0.1 – 8000 Hz and referenced 264 online to the uninsulated microwire.

265

266 *Stimuli and procedure*

267 Experimental stimuli were trains of acoustic clicks (used previously in [64]) and pure 268 tones (used previously in [65]). Clicks were digitally generated as rectangular pulses 269 (0.2 ms duration) and were presented in trains of five at a rate of 100 Hz (train duration 270 40 ms, 2 s inter-train interval). Tones were presented at six frequencies between 250 271 and 8 kHz in 1-octave steps (300 ms duration, 5 ms rise–fall time, 2 s interstimulus 272 interval). The six tones were presented 50 times each in a random order. The stimuli

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273 were presented at a comfortable level, approximately 50-60 dB above hearing 274 threshold. Stimuli were delivered to both ears via insert earphones (ER4B, Etymotic 275 Research, Elk Grove Village, IL) that were integrated into custom-fit earmolds or foam 276 ear tips (for two participants). In each participant, the intensity difference between click 277 trains and pure tones was within 20 dB and varied depending on comfort. Inter-stimulus 278 intervals were chosen randomly within a Gaussian distribution (mean interval 2 s; SD = 279 10 ms) to reduce heterodyning in the recordings secondary to power line noise. 280 Stimulus delivery was controlled by a TDT RP2.1 and RZ2 real-time processor (Tucker 281 Davis Technologies, Alachua, FL).

282

283 *Imaging*

284 A T1-weighted structural MRI scan of the brain was conducted for each participant both 285 before and after the implantation of electrodes. The images were captured using a 3T 286 Siemens TIM Trio scanner and a 12-channel head coil. MPRAGE images had a spatial 287 resolution of 0.78 × 0.78 mm, a slice thickness of 1.0 mm, and utilized a repetition time 288 (TR) of 2.53 s and an echo time (TE) of 3.52 ms. To locate the recording positions on 289 the preoperative structural MRI scans, these images were aligned with post 290 implantation structural MRIs. This alignment was achieved using a 3D linear registration 291 algorithm (Functional MRI of the Brain Linear Image Registration Tool; [66]) and custom 292 written MATLAB scripts (MathWorks, Natick, MA). Included microwire bundles were 293 verified to be within gray matter for either the insula or HGPM. Electrode locations were 294 co-registered for each participant to a template brain, in order to derive MNI coordinates 295 for the purposes of visualization. Coordinates of microwire locations were then 296 visualized on an fsaverage MRI template showing the insula and neighboring region of 297 HGPM, using custom-written MATLAB scripts.

298

299 *Data processing and analysis*

300 Data from high impedance electrodes localized to the insula or HGPM were first 301 extracted using Matlab and denoised with an implementation of the demodulated band 302 transform [67]. These data were downsampled to 12 kHz and common average re 303 referenced to all high impedance contacts on the same assembly prior to spike sorting. 304 Spike sorting was performed using an automated procedure with manual curation, 305 utilizing an algorithm implementing high-order spectral decomposition to aid in pattern 306 recognition and identify individual features [68]. Briefly, filters were estimated for 307 potential candidate single neuron waveforms for each channel through a process 308 related to blind deconvolution. Extracted features were clustered using a gaussian 309 mixture model in Matlab (R2022a, Mathworks Inc) and spike times from these clustered

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310 features were used to plot separate candidate waveforms. Single neurons were then 311 manually curated and defined based on classical waveform shapes, uniformity of 312 waveforms across different spike times for each cluster, and interspike interval 313 distributions that did not violate refractory periods (<1% of interspike intervals occurring 314 within 1 ms). See **Supplementary Figure 1** for summarized curation details. Putative 315 single neuron spike times were then epoched around the stimuli for each trial (500 ms 316 before stimulus onset to 1000 ms post-stimulus onset). Raster plots were created to 317 show neuronal activity for each trial and spike density functions were estimated by 318 convolving single neuron spike times with a gaussian kernel (1 ms resolution, generally 319 5 ms standard deviation, though a standard deviation of 15 ms was used for display 320 purposes in **Figure 1**).

321

322 *Statistical analysis*

323 Single neuron modulation to auditory stimuli was determined using a two-tailed paired *t* 324 test against baseline, wherein spike rates across trials between 0 to 350 ms post 325 stimulus were compared to baseline firing rates from a pre-stimulus window of the same 326 duration (-400 to -50 ms). This was only considered for neurons that had a mean firing 327 rate ≥1 Hz across the baseline and post-stimulus windows. The *p*-value alpha for this 328 test was set at 0.05 for click stimuli and Benjamini-Hochberg false discovery rate (FDR) 329 correction [69, 70] was applied to tonal response *p*-values to account for multiple 330 comparisons (based on the six different tone frequencies presented). Magnitudes for all 331 responses were determined by averaging the z-scored firing rate across the post 332 stimulus window. The latency of significantly modulated neurons was then determined 333 according to the first peak in the baseline z-scored spike density function from 10 ms to 334 350 ms post-stimulus, wherein this peak was a minimum value of half of the maximum 335 peak within this time window. For neurons that suppressed their activity in response to 336 auditory stimuli, spike density functions were first inverted prior to finding the peak. For 337 tone stimuli, the best frequency of each neuron was defined as the tone that elicited the 338 maximum absolute value of the z-scored average, wherein this response was 339 significant. Bandwidth in octaves was determined based on the full width at half 340 maximum of this frequency, which was obtained after interpolation of both frequencies 341 and magnitudes to create 10000 discrete sampling points. Where the lowest or highest 342 frequencies were the maximum absolute value (i.e. showed the strongest modulation), 343 these were taken as the low or high cutoff for this calculation, respectively. Response 344 durations were determined based on the time after the first peak wherein the spike 345 density function was reduced to 5% of the maximum peak value. Regional differences in 346 response properties were examined using a linear mixed effects modeling approach. 347 Output (response magnitude, latency or duration) was modeled using region of interest

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348 (ROI, i.e. HGPM, InsP, or InsA) as a categorical fixed effect, with random intercept of 349 channel nested within participant:

350 Output ~ ROI + (1 | Participant : Channel) + (1 | Participant) 351

352 **Data and code availability**

353 Data and analysis scripts used in this manuscript are available upon reasonable request 354 from the authors.

355

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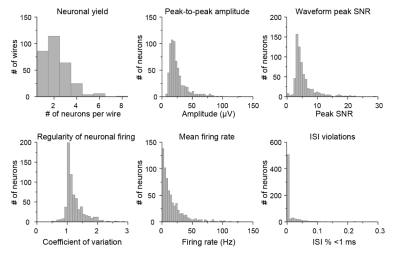
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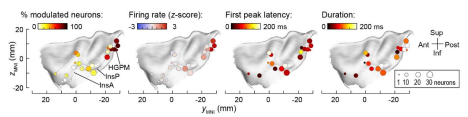
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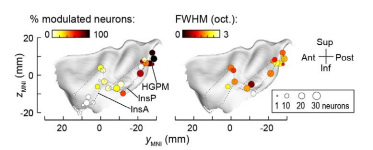
527 **Supplementary Figure 1.** Curation plots for all isolated putatitive single neurons. ISI: inter 528 spike interval. SNR: signal-to-noise ratio, calculated as the ratio of the peak of each single 529 neuron waveform divided by the median absolute value of the signal for that channel, based on 530 the method implemented in SpikeForest [71].

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533 **Supplementary Figure 2.** Template reconstruction of insula showing for clicks (left to right) the 534 proportion of modulated neurons, mean magnitude of firing rate changes, latency of modulation 535 and duration of modulation.

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536 

537 **Supplementary Figure 3.** Template reconstruction of insula showing for tones the proportion of 538 modulated neurons (left panel) and mean bandwidth of tuning within a location (right panel).