Categorical Signaling of the Strongest Stimulus by an Inhibitory Midbrain Nucleus

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The nucleus isthmi pars magnocellularis (Imc), a group of inhibitory neurons in the midbrain tegmentum, is a critical component of the spatial selection network in the vertebrate midbrain. It delivers long-range inhibition among different portions of the space map in the optic tectum (OT), thereby mediating stimulus competition in the OT. Here, we investigate the properties of relative strength-dependent competitive interactions within the Imc, in barn owls of both sexes. We find that when Imc neurons are presented simultaneously with one stimulus inside the receptive field and a second, competing stimulus outside, they exhibit gradual or switch-like response profiles as a function of relative stimulus strength. They do so both when the two stimuli are of the same sensory modality (both visual) or of different sensory modalities (visual and auditory). Moreover, Imc neurons signal the strongest stimulus in a dynamically flexible manner, indicating that Imc responses reflect an online comparison between the strengths of the competing stimuli. Notably, Imc neurons signal the strongest stimulus more categorically, and earlier than the OT. Paired recordings at spatially aligned sites in the Imc and OT reveal that although some properties of stimulus competition, such as the bias of competitive response profiles, are correlated, others such as the steepness of response profiles, are set independently. Our results demonstrate that the Imc is itself an active site of competition, and may be the first site in the midbrain selection network at which stimulus competition is resolved.

Key words: categorization; inhibition; midbrain; selection; spatial attention; superior colliculus

Significance Statement

This work sheds light on the functional properties of a small group of inhibitory neurons in the vertebrate midbrain that play a key part in how the brain selects a target among competitors. A better understanding of the functioning of these neurons is an important building block for the broader understanding of how distracters are suppressed, and of spatial attention and its dysfunction.

Introduction

For animals operating within complex environments, the ability to select the location of the highest priority stimulus is vital for adaptive behaviors. Stimulus priority is the combination of the physical salience of a stimulus as well as its behavioral relevance, and is computed at several sites in the brain (Fecteau and Munoz, 2006). Among these, the midbrain selection network has been studied for its important role in the control of stimulus

selection for spatial attention (Knudsen, 2011; Krauzlis et al., 2013). It consists of the superior colliculus [SC (in mammals)] or optic tectum [OT (in nonmammals)], which encodes a topographic map of multisensory and motor space, as well as a spatial map of stimulus priority (Knudsen, 2011; Krauzlis et al., 2013), and satellite brain areas in the midbrain tegmentum that are interconnected with the SC/OT (Graybiel, 1978; Gruberg and Udin, 1978; Sereno and Ulinski, 1987; Jiang et al., 1996; Wang et al., 2004).

Work in nonhuman primates has demonstrated that the intermediate-deep layers of the SC (SCid) are required for the selection of the target of spatial attention amid distractors (McPeck and Keller, 2004; Lovejoy and Krauzlis, 2010). In parallel, work in the avian midbrain has demonstrated that neurons in the intermediate-deep layers of the OT (OTid) signal the highest-priority stimulus categorically: they respond with a high firing rate when the stimulus inside their receptive field (RF) is of highest priority, but are suppressed abruptly to a low firing rate when it is no longer the highest priority (Mysore et al., 2011; Mysore and Knudsen, 2011, 2014). Suppression of the responses

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of SCid/OTid neurons by competing stimuli has been reported in several vertebrate species (Rizzolatti et al., 1974; Frost, 1978; Schellart et al., 1979; McPeck and Keller, 2002, 2004; Felsen and Mainen, 2008; Mysore et al., 2010; Gabay et al., 2013; Duan et al., 2015). The source of such long-range competitive inhibition that underlies the categorical signaling of OTid has been identified to be a group of inhibitory neurons in the vertebrate midbrain tegmentum, called nucleus isthmi pars magnocellularis (Imc; Gruberg and Udin, 1978; Sereno and Ulinski, 1987; Wang et al., 2004). Specifically, it has been shown in birds that inactivation of the Imc abolishes all competitive interactions in the OTid (Marín et al., 2007; Mysore and Knudsen, 2013), as well as in the cholinergic isthmi pars parvocellularis (Ipc; Graybiel, 1978; Jiang et al., 1996; Wang et al., 2006), another key area in the midbrain selection network, which serves as a point-to-point amplifier of activity across the OTid space map (Dudkin and Gruberg, 2003; Marín et al., 2007; Asadollahi and Knudsen, 2016).

Despite the importance of Imc to the signaling of the highest-priority stimulus by the OTid (Marín et al., 2007; Mysore and Knudsen, 2013), its functional properties are not well understood (Yan and Wang, 1986; Wang and Frost, 1991). Recent work in barn owls has revealed the unusual multilobed structure of spatial RFs in the Imc, which has been shown to underlie its combinatorially optimized encoding of visual space (Mahajan and Mysore, 2018). In addition, Imc neurons have been shown to exhibit global inhibitory surrounds that may serve as a substrate for stimulus competition within the Imc (Wang and Frost, 1991; Schryver and Mysore, 2019).

Here, we investigated in detail the properties of multisensory stimulus competition in, and the signaling of the most salient stimulus by, the Imc. Specifically, we examined how the responses of Imc neurons to two competing stimuli changed, as their relative strength (salience) was varied systematically. We compared them with responses of OTid neurons measured either separately, or during simultaneous paired recordings in the two brain areas. We found that Imc neurons display signatures of stimulus competition that are quantitatively different from the OTid on average, and qualitatively distinct from those of individual, spatially aligned OTid sites recorded simultaneously. Together with the finding that the Imc resolves competition earlier than the OT, our results demonstrate that the Imc is not a passive conduit of inhibition to the OT nor does it simply reflect competition occurring there. Rather, the Imc is itself an active site of stimulus competition.

Materials and Methods

Experimental design and statistical analysis. The central goals of this study were to measure the responses of Imc (and OTid) neurons to a stimulus presented inside the RF, without and with a second stimulus presented simultaneously outside the RF. In addition, with paired recordings in the Imc and OTid following the same protocol, an additional goal was to compare response properties directly at Imc and OTid sites. Each stimulus combination (either single or paired) was repeatedly tested 9–15 times in a randomly interleaved fashion. The data were analyzed, and the results were fit with sigmoids or straight lines, if necessary.

All statistical analyses were conducted within MATLAB. Statistical comparison of the time course of evolution of neural responses between two stimulus conditions was performed on a millisecond-by-millisecond basis using ANOVA. Comparison of the proportions within two distributions (see Fig. 5) was performed using the χ^2 test. All other statistical comparisons were performed using nonparametric tests (identified in the text at each instance of a statistical comparison). Correction for multiple comparisons was performed using the Holm–Bonferroni method.

Data shown as $a \pm b$ refer to the mean $\pm$ SEM, unless specified otherwise. The * symbol indicates significance at the 0.05 level (after corrections for multiple comparisons, if applicable).

Neurophysiology. Eleven adult barn owls (*Tyto alba*; male and female; shared across different studies) were used for electrophysiological recordings. Birds were group housed in an aviary with a 12 h light/dark cycle. All protocols for animal care and use followed approval by the Johns Hopkins University Institutional Animal Care and Use Committee, and were performed in accordance with National Institutes of Health *Guidelines for Care and Use of Laboratory Animals*. All experimental and surgical procedures followed previously published methods (Schryver and Mysore, 2019).

Briefly, owls were anesthetized with isoflurane (2%) and a mixture of nitrous oxide and oxygen (45:55) on experiment days, and head fixed in a sound-attenuating booth. The head fixation was calibrated following published procedures (Knudsen, 1982) such that the dorsolateral tip of the pecten oculi structures within the eyes were positioned at 7° above the horizon and ~25° lateral to the vertical midline. Isoflurane administration was ceased after birds were secured. We recorded from single and multiunit sites in the Imc and OT using epoxy-coated tungsten microelectrodes (5 M Ω at 1 kHz; A-M Systems). Recording sites in the OTid and the Imc were targeted on the basis of stereotaxic coordinates (from prior experiments) and verified on the basis of established neural signatures as described previously (Mysore et al., 2010, 2011; Mysore and Knudsen, 2013; Mahajan and Mysore, 2018; Schryver and Mysore, 2019). For Imc recordings, an electrode was positioned to enter the brain at a medial-leading angle of 5°. At a subset of sites, a second electrode was lowered into OTid to make dual recordings simultaneously. Upon positioning the recording electrodes, nitrous oxide was turned off for the duration of the data collection in some experiments; previous work has established no effect of nitrous oxide tranquilization on neural responses to competition protocols in the midbrain network (Mysore et al., 2011). Spike times were recorded using Tucker-Davis Technologies hardware and custom MATLAB software. Multiunit spike waveforms (OTid and Imc) were sorted into single neurons using the Chronux spike-sorting toolbox (Fee et al., 1996). The quality of the sorted neurons were assessed visually, and were additionally subjected to an *F* test to determine whether or not each neuron was well isolated from other neurons recorded within the same multiunit site (Mahajan and Mysore, 2018); only well isolated neurons were retained.

Stimuli. Visual stimuli were presented as black, with fixed contrast, expanding looming dots on a gray background on a 65 inch monitor. Looming dot stimuli were used as these reliably evoke strong responses in OT and Imc (Mysore et al., 2010, 2011). The strength of a looming stimulus was defined by its loom speed, with faster loom speeds typically evoking greater responses; the typical range of loom speeds used was 0 to 20°/s. Locations of visual stimuli were defined by double-pole coordinates relative to the midsagittal plane for azimuth or the visual plane for elevation (Knudsen, 1982). Auditory stimuli were presented as broadband noise bursts with equalized amplitudes delivered binaurally through earphones. Sounds were filtered with head-related transfer functions of a standard barn owl (Mysore et al., 2010). Strengths of auditory stimuli were defined by the auditory binaural level (ABL). Visual stimuli were generated using custom MATLAB scripts and psychtoolbox (PTB-3; David, 1997; Pelli, 1997), and auditory stimuli were generated using custom MATLAB scripts and Tucker-Davis Technologies hardware.

Two-dimensional RFs were collected by presenting a stimulus at various azimuthal and elevational locations. These stimuli were either a single looming dot of fixed strength or a stationary dot (radius, 3°) moving at a 45° angle over 3°. For RF measurements, stimuli were presented for five to seven repetitions, with a duration of 250 ms each and an interstimulus interval of 1000–1500 ms. Spatial locations at which a single stimulus elicited higher firing rates compared with baseline were deemed to constitute the spatial RF of the neuron, and were used to estimate RF extent [half-maximum (max) width] and center (weighted average of RF locations).

Stimulus competition protocols involved the presentation of a visual stimulus (S1) of fixed strength inside the RF, either by itself, or with a second stimulus (either visual or auditory; S2_{vis} or S2_{aud}, respectively) of

varying strengths presented at a distant location (typically, 30° away from S1). The resulting responses from paired S1 and S2 presentation were collectively called competitor strength-dependent response profiles (or competitor response profiles; CRPs; Mysore et al., 2011). Stimuli were presented for 10–15 repetitions, with a duration of 250 ms each and an interstimulus interval of 2–3 s.

Data analysis. All analyses were performed using custom MATLAB scripts. Response firing rates were determined by counting the number of spikes over a time window following stimulus onset, converting this count to firing rate (sp/s), and subtracting the baseline firing rate. The window for computing firing rates was visually estimated to capture evoked responses for each neuron; started, on average, at 120 ms (115 ms); and had a width, on average, of 170 ms for Imc (OTid) neurons. Average firing rates and error bars (SEM) were computed from the firing rates across all the repetitions of stimulus presentation, after removing outlier values. Outliers were identified as points that lay outside the range of median $\pm 1.5 \times$ interquartile range of the distribution.

To characterize the responses to the paired presentation of S1 and S2 (i.e., competitor response profiles), we calculated the correlation (Pearson, *corrcoef* command in MATLAB) as a function of the strength of S2. A significant negative correlation ($p < 0.05$) indicated that responses significantly decreased as S2 strength increased. If a neuron did not exhibit significant negative correlation, it was deemed to exhibit fixed response suppression if the suppression was significant for the majority of S2 values (one-way ANOVA on the firing rates to different competitor strengths followed by *post hoc* tests against responses to S1 alone, corrected for multiple comparisons using the Holm–Bonferroni correction). The remaining neurons were considered to not show any effect related to the presence of S2.

Negatively correlated competitor response profiles were fit with a standard sigmoidal function, and the parameters of the best fit were determined (Mysore et al., 2011). To obtain reliable estimates of the best fitting sigmoid, any S2 strength for which responses differed nonmonotonically from those of its neighbors, and did so substantially ($>150\%$ of the response difference between the neighbors), was omitted from the fitting process and subsequent analyses. We defined the transition range of each competitor response profile as the range of strengths of S2 over which responses decreased from 90% to 10% of the max response rate. The half-max of this range was defined as the transition value (i.e., the value of the strength of S2 where responses transitioned from being stronger to weaker than the half-max response). The determination of whether competitor response profiles transitioned abruptly (in a “switch-like” manner) or systematically (in a “gradual” manner) from high to low responses, we adopted previously published conventions (Asadollahi et al., 2010; Mysore et al., 2011), as follows: competitor response profiles with transition ranges that were narrower than 4°/s, or one-fifth of the physiological range of S2 strengths were considered to be switch-like responses, and others as gradual responses. Similarly, for auditory competitors, competitor response profiles with a transition range of ≤ 9 dB were considered to be switch like, and the others gradual. Incidentally, these “cutoff” values correspond closely to the dips in the bimodal distributions of transition ranges (Fig. 1F; see also Fig. 3D, respectively).

For neurons with negatively correlated competitor response profiles, we also calculated the instantaneous firing rates by first obtaining the poststimulus time histogram (PSTH) of the responses (1 ms time bins), and then smoothing the PSTH with a Gaussian kernel ($\sigma = 12$ ms). For each neuron, the instantaneous firing rates to the paired presentation of S1 and S2 (as well as to S1 alone) were normalized by the peak of the average instantaneous firing rate to S1 alone.

The pooled population responses in Figure 2 (see also Figs. 5, 6) were obtained by binning relative strengths (S2–S1) into five bins and combining the responses of all the neurons within each bin (Mysore et al., 2011). This was done separately for OTid and Imc neurons, and separately for neurons with gradual and switch-like competitor response profiles. The time course of response suppression by S2 was determined (within each relative strength bin) by performing a millisecond-by-millisecond ANOVA, comparing the pooled instantaneous firing rates to S1 alone versus to S1 and S2 presented together (Sasikumar et al., 2018).

The time to suppression was defined as the first millisecond at which the p value of the ANOVA comparison dropped to <0.05 , remained at <0.05 for the next 25 ms, and reached 0.01 at least once in that period (Mysore et al., 2011).

Discriminability (d' -prime) between responses to two stimulus conditions was computed as the difference in mean responses over the square root of the average of the variances.

For comparison of results from dual simultaneous recordings in the Imc and OTid, we performed all analyses on data from pairs of multiunit sites, rather than on data from pairs of single neurons sorted from these multiunit sites. This is because there was no rational way of establishing which specific neurons sorted from the Imc site ought to be paired with which neurons in the OTid site; comparing all possible pairs would violate the assumption of statistical independence across samples. Notably, because multiunit sites are indeed activated as a whole upon presentation of stimuli, the comparison of Imc and OTid site responses was appropriate and meaningful.

Results

Switch-like and gradual response profiles in the Imc to competing visual stimuli

To examine relative strength-dependent stimulus competition in the Imc, we recorded extracellular responses of Imc neurons in the barn owl, using a previously published competition protocol (Materials and Methods; Asadollahi et al., 2011; Mysore et al., 2011; Fig. 1A). We presented a visual stimulus (S1) of fixed strength (loom speed; Materials and Methods) within the RF of a recorded neuron, and measured responses when a second, competing visual stimulus (S2) of varying strengths was presented far outside the RF ($>30^\circ$ away; Fig. 1A; Mysore et al., 2011). S1 and S2 were presented simultaneously and for the same duration (250 ms; Materials and Methods). The responses obtained to the paired presentation of S1 and S2 using this protocol, collectively called competitor strength-dependent response profiles (or competitor response profiles; Fig. 1B,D, bottom panels; Mysore et al., 2011), were compared with the responses to S1 presented alone (Fig. 1B,D, top panels); the two types of trials were interleaved randomly.

The majority of the recorded Imc neurons (66 of 78) exhibited competitor response profiles that were negatively correlated with the strength of S2 (Fig. 1B–E; $p < 0.05$, Pearson correlation test; Materials and Methods). Of the remaining, a small subset showed competitor response profiles with fixed response suppression, independent of the strength of S2 (1 of 78; Materials and methods), and the rest were not affected by S2 (11 of 78; Materials and Methods).

Further examination of the negatively correlated competitor response profiles revealed two distinct patterns of suppression based on how abruptly the responses transitioned from the maximum to the minimum value. In one set of competitor response profiles, the majority of response suppression was expressed over a narrow range of S2 strengths, and in the other, the response suppression increased in a graded, systematic way as a function of S2 strength. To quantify the abruptness of the response transition, we defined the competitor response profile transition range as the range of S2 strengths over which the responses dropped from 90% to 10% of the maximum response (Materials and Methods). Following a previously published convention (Mysore et al., 2011; Materials and Methods), competitor response profiles with transition ranges narrower than one-fifth the nominal range of S2 strengths (4°/s), were referred to as being switch-like (Fig. 1B,C), while those with transition ranges broader than 4°/s were referred to as being gradual (Fig. 1D,E).

Figure 1. Switch-like and gradual response suppression of Imc neurons by a competing stimulus. **A**, Schematic of experimental setup and stimulus protocol for measuring competitor strength-dependent response profiles (CRPs). Quadrilateral, Tangent monitor; dashed oval, RF of recorded Imc neuron; black dot; S1 visual looming stimulus; magenta dots, S2 visual looming stimulus. Sizes of dots denote lobe speeds. **B**, **C**, Responses of an example Imc neuron with switch-like competitor response profile (data in magenta). **B**, Rasters of spike responses to S1 alone (left; lobe speed = 3.6°/s) and to the paired stimuli (right) showing an abrupt increase in suppression with increasing S2 strength. Shaded box along the y-axis represents stimulus duration (250 ms), and dashed lines denote the time window (100–250 ms) during which response firing rates were calculated. **C**, Response firing rates corresponding to rasters in **B**. Open circle and horizontal dotted line response firing rate to S1 alone (mean $\pm$ SEM); filled circles with graded grey shading, response firing rates to paired presentation of S1 and S2 (i.e., CRP; mean $\pm$ SEM; darker colors indicating higher firing rates). Correlation coefficient of responses versus S2 strength = -0.74 ($p = 0.04$, Pearson correlation test). Solid line, Best fitting sigmoid to the competitor response profile ($r^2 = 0.95$); vertical dashed lines, transition range of this competitor response profile (0.17°/s; Materials and Methods); black arrowhead, strength of S1 (3.6°/s). **D**, **E**, Responses of an example Imc neuron with gradual competitor response profile (data in green). Conventions are as in **B** and **C**. Lobe speed of S1 = 5.6°/s, correlation coefficient of responses versus S2 strength = -0.94 , $p = 0.0002$, Pearson correlation test; $r^2 = 0.99$ for the best fitting sigmoid; transition range = 5.75°/s. **F**, Histogram of transition ranges of competitor response profiles that exhibited a negative correlation with the strength of S2 ($n = 66$ neurons/78 total). Vertical line: “cutoff” transition range of 4°/s (see “Materials and Methods”). The median strength of S1 was 7°/s with 95% CI of 6.3°/s, 7.7°/s; median distance of S2 from S1 = 43° with 95% CI of 40°–46°. Inset, RF locations (in double-pole coordinates) of Imc neurons at which competitor response profiles were recorded; colors correspond to whether the neurons had switch-like (magenta) or gradual (green) competitor response profiles.

Across the population of 66 Imc neurons that exhibited negatively correlated competitor response profiles, the majority (39 of 66; 59%) of neurons exhibited switch-like responses, and the rest (27/66; 40%) exhibited gradual competitor response profiles (Fig. 1F). Switch-like and gradual competitor response profiles were recorded at neurons both encoding for locations within the frontal portion of visual space (within $\pm 15^\circ$ in azimuth and elevation), as well as in the more peripheral portion (Fig. 1F, inset), indicating that switch-like or gradual modulation of response suppression did not depend on the spatial location of the RF.

Time course of stimulus competition in the Imc

The time course of response suppression was different between Imc neurons with gradual versus switch-like competitor response profiles. For each neuron with a gradual competitor response profile, we first calculated the instantaneous firing rate responses to S1 alone, and to the paired presentation of S1 and S2 for every strength of S2 (Materials and Methods). We then binned the relative competitor strength (S2–S1) values into five bins (Fig. 2A–H, columns), and, for each bin, grouped across neurons the normalized instantaneous firing rate responses to S1 alone, and separately, to paired S1 and S2 (Fig. 2, compare A, B; Materials and Methods). We quantified the emergence of response suppression for each relative strength bin by comparing the pooled responses to S1 and the pooled responses to paired S1 and S2 (Fig. 2C, gray vs green, respectively) using a millisecond-by-millisecond running ANOVA procedure (Fig. 2D; Materials and Methods). The time to suppression was defined as the first instant at which the responses diverged significantly (Fig. 2D; dashed vertical arrows; Materials and Methods).

We found that among the neurons with gradual competitor response profiles, there was a systematic reduction in the time to suppression as a function of relative strength, as follows: from 253 ms at relative strength of -7° /s to 124 ms at relative strength of $+10^\circ$ /s (Fig. 2I, green data; slope = -6.6 ; 95% CI, -12.4 , -0.72 ; linear regression). We repeated the above analysis for neurons with switch-like competitor response profiles and found that the times to suppression also decreased as a function of relative strength (from 370 to 91 ms), but with a much steeper slope than for

Figure 2. Time course of response suppression for Imc neurons with switch-like versus gradual competitor response profiles (CRPs). **A–D**, Analysis of response time courses of Imc neurons with gradual competitor response profiles; columns, responses binned by the relative strength of S1 and S2 (S2-S1). **A**, **B**, Instantaneous firing rates (IFRs) of neurons to S1 alone (**A**) or to S1 and S2 presented together (**B**), computed by smoothing PSTHs (1 ms time bins) with a Gaussian kernel (SD = 12 ms; Materials and Methods). For each neuron, instantaneous firing rates are normalized by the peak firing rate of that neuron to S1 alone. Neurons in **A** are sorted by the half-peak firing rate; neurons in **B** are in the same order as in **A**. **C**, Pooled average firing rates to S1 alone (gray) or to S1 and S2 (green) of all neurons within each bin. Translucent bands indicate SEM. **D**, Time course of p values obtained by performing ANOVA between the responses to S1 versus to S1 and S2 at each millisecond. Vertical dashed arrows (and colored text), time to suppression (defined as the first instant at which responses to paired S1 and S2 diverge significantly from responses to S1 alone; Materials and Methods); horizontal dashed lines, p value thresholds used in determining time to suppression (bottom line, $p = 0.05$; top line, $p = 0.01$). **E–H**, Same as **A–D**, but for Imc neurons with switch-like competitor response profiles. **I**, Comparison of time to suppression for neurons with switch-like (magenta) versus gradual competitor response profiles (green). **J**, Plot of average amount of response suppression ($\pm$ SEM) for switch-like versus gradual neurons for the two relative strength bins on either side of the selection boundary. Text reports the effect size (η^2).

Figure 3. Switch-like and gradual response suppression of Imc neurons by an auditory competitor. **A**, Schematic of experimental setup and stimulus protocol for measuring auditory competitor response profiles (conventions are as in Fig. 1A). Black dot, S1 visual looming stimulus; magenta sound symbol, S2_{aud}; size of symbol represents binaural intensity of S2_{aud}. **B**, **C**, Switch-like auditory competitor response profile measured at an example Imc neuron. **B**, Response rasters. Dashed lines, time window (100–250 ms) during which response firing rates were calculated. S1 loom speed = 6°/s. **C**, Spike counts. Correlation coefficient of versus S2_{aud} strength = -0.82 ($p = 0.04$, Pearson correlation test); $r^2 = 0.98$ for the best fitting sigmoid, transition range = 2.9 dB. All other conventions are as in Figure 1, B and C. **D**, **E**, Gradual auditory competitor response profile measured at an example Imc neuron. **D**, Rasters. Dashed lines, Time window (100–300 ms) during which response firing rates were calculated. S1 loom speed = 5.6°/s. **E**, Spike counts. Correlation coefficient of responses versus S2_{aud} strength = 0.99 ($p = 0.00008$, Pearson correlation test), $r^2 = 0.99$ for the best fitting sigmoid, transition range = 31 dB. All other conventions as in Figure 1, D and E. **F**, Histogram of transition ranges of competitor response profiles recorded in Imc that exhibited a negative correlation with the strength of S2_{aud} ($n = 20$ neurons/35 total). Vertical line: “cut-off” transition range of 9 dB (Materials and Methods). The median strength of S1 was 5.6°/s; 95% CI: 5.1°/s, 6.1°/s. Grad, Gradual; Switch, switch-like.

neurons with gradual competitor response profile (Fig. 2I, magenta data; slope = -17.4 ; 95% CI, -28.1 , -6.8 ; linear regression). There was, however, no systematic difference in the response latency of neurons with gradual versus switch-like competitor response profiles (latency of response to S1 alone: gradual:

median = 136 ms; 95% CI, 113, 159; switch-like: median = 110 ms; 95% CI, 95, 125; $p = 0.11$; sign test).

Notably, the times to suppression for switch-like competitor response profiles exhibited a large, abrupt change across the relative strength of zero (i.e., the “selection boundary”), compared with gradual competitor response profiles (Fig. 2I; switch-like, 200 ms change from 301 to 101 ms; gradual, 37 ms change from 174 to 137 ms). This large change was accompanied by a “flip” in the values of time to suppression for switch-like versus gradual response profiles across the selection boundary, as follows: longer times to suppression for switch-like competitor response profiles when $S2 < S1$ (Fig. 2I, magenta vs green data at negative relative strength values), but shorter times to suppression for switch-like competitor response profiles when $S2 > S1$ (Fig. 2I, magenta vs green data at positive relative strength values). This flip was accounted for by intrinsic differences in the properties of switch-like versus gradual competitor response profiles in the Imc and, specifically, by the relative magnitudes of suppression. Neurons with switch-like response profiles showed weak suppression when $S2 < S1$, weaker than for gradual response profiles, predicting later times to suppression, but showed strong suppression when $S2 > S1$, stronger than for gradual response profiles (Fig. 2I), predicting earlier times to suppression.

Multisensory stimulus competition in the Imc

The occurrence of gradual and switch-like competitor response profiles was not restricted to just the visual sensory modality. We measured “auditory” competitor response profiles using a visual S1 (of fixed strength) and an S2_{aud} (of varying strengths). S2_{aud} stimuli were broadband noise bursts, and S2_{aud} “strength” was varied by changing the binaural sound level (Materials and Methods).

We recorded auditory competitor response profiles of 35 Imc neurons, and, of these, 20 exhibited competitor response profiles that were negatively correlated with the strength of S2_{aud} (Materials and Methods; $p < 0.05$, Pearson correlation test). Further examination of the responses of these neurons revealed two distinct patterns of suppression as a function of the strength of S2_{aud} (gradual or switch-

like; Fig. 3B–E). Switch-like auditory competitor response profiles were defined as those for which the transition range was narrower than 9 dB (one-fifth the full range of S2_{aud} strengths; just as in the case of visual competitor response profiles, and

consistent with previously published literature; Asadollahi et al., 2010; Mysore et al., 2011). Of the 20 neurons with correlated competitor response profiles, 10 were found to be gradual and the rest, switch-like (Fig. 3D). Thus, consistent with the role of Imc in enabling within-sensory as well as cross-sensory stimulus competition in the OTid (Mysore and Knudsen, 2013), Imc neurons themselves exhibit signatures of multisensory stimulus competition (Schryver and Mysore, 2019).

Imc signals the strongest stimulus accurately and flexibly

The observation of abrupt response suppression in switch-like competitor response profiles led us to ask whether the strength of S2 at which the transition occurred from high to low response values was meaningful. Because this transition was also well defined (albeit less so) in gradual competitor response profiles, we asked this question more generally of switch-like as well as gradual competitor response profiles in the Imc. Specifically, we wondered whether the strength of S2 at which the transition occurred was related to the (fixed) strength of the stimulus inside the RF, S1 (because this comparison is only meaningful when both stimuli are of the same sensory modality, we restricted our analysis to visual competitor response profiles). To this end, we first defined as the competitor response profile “transition value” the strength of S2 at which the responses were half-way between the maximum and minimum values, and quantified it as the midpoint of the transition range (Fig. 4A; Materials and Methods). We then compared the transition value of each competitor response profile to the strength of S1 used to measure the competitor response profile, and defined this difference as the competitor response profile “bias” (bias = transition value – strength of S1; Fig. 4A).

Across the population of Imc neurons with correlated visual competitor response profiles ($n = 66$), we found neurons with a range of competitor response profile biases (Fig. 4B). Some had a negative bias, indicating that these competitor response profiles transitioned from a high to a low value when S2 was less than S1, and others had a positive bias, indicating that those competitor response profiles transitioned from a high to a low value when S2 was greater than S1. However, across the population, the competitor response profile bias was distributed around zero (Fig. 4B; median competitor response profile bias = -0.52 , $p = 0.39$; sign test against 0). This indicated that, on average, Imc neurons responded at a high level when S1 (RF stimulus) was the strongest stimulus, but transitioned to responding at a low level when S1 was no longer the strongest stimulus (i.e., just when S2 exceeded S1 in strength). This was true separately both for switch-like as well as for gradual competitor response profiles (Fig. 4C, top and bottom panels, respectively; switch-like: median bias = -0.68 ; $p = 0.52$; sign test against 0; $n = 39$ neurons; gradual: median bias = -0.36 ; $p = 0.7$; sign test against 0; $n = 27$ neurons).

These results indicated that Imc may signal the strongest of the competing stimuli without any bias (i.e., “accurately”), and suggested the interesting possibility that transition values of Imc competitor response profiles are not fixed quantities, but are coupled “flexibly” to the strength of S1. To test this hypothesis that competitor response profile transition values depend on the strength of S1, we measured two competitor response profiles for each of a subset of Imc neurons. One competitor response profile was measured with a weaker S1, another with a stronger S1 ($S1 + 6^\circ/s$), with S2 varying over the same range of loom speeds in both cases (Figure 4D,G; Mysore et al., 2011). The stimuli corresponding to the two competitor response profiles were presented in a randomly interleaved manner. We found that the

competitor response profile transition value shifted with S1 strength in the predicted way: a stronger S1 produced a right-shifted competitor response profile (Fig. 4E,F, data from example neuron with switch-like competitor response profile showing a right shift in transition value; Fig. 4H,I, data from example neuron with gradual competitor response profile showing a right shift). Across the population of tested neurons, we found that the magnitude of the shift in competitor response profile transition value matched, on average, the magnitude of the change in S1 strength [Fig. 4H; median shift ratio (shift in transition values/change in S1 strength) = 0.89 ; $p = 0.26$; sign test against 1; $n = 27$ neurons].

Together, these results established that Imc neurons report dynamically an online comparison between the strengths of the two competing stimuli. They signal accurately (with average bias indistinguishable from 0) the strongest of the two competing stimuli, and do so flexibly (with transition values coupled to the strength of S1).

Comparison of signatures of stimulus competition in the Imc and OTid

The findings of switch-like and gradual competitor response profiles in the Imc, as well as accurate and flexible signaling of the strongest stimulus, parallel previous findings in two other key nuclei in the midbrain selection network, namely the OTid (Mysore et al., 2011; Mysore and Knudsen, 2011, 2014) and the cholinergic Ipc (Asadollahi et al., 2010). These nuclei are interconnected as follows: Imc receives focal input from layer 10 of the OT (OT₁₀), but projects back broadly across the OTid as well as the Ipc (Wang et al., 2004), and Ipc receives focal input from OT₁₀ and projects back focally to the OT (Wang et al., 2006), serving to amplify OTid responses (Marín et al., 2007; Asadollahi and Knudsen, 2016). Additionally, Imc is the source of competitive inhibition in the OTid and Ipc, as follows: focal inactivation of Imc abolishes competitive interactions in both areas (Marín et al., 2007; Mysore and Knudsen, 2013). Considering this interconnectedness, we were interested in whether the signatures of stimulus competition in the Imc simply reflect computations occurring previously (for instance, in the OT), or if the Imc itself serves as an active site at which computations related to stimulus competition occur. We focused on the comparison between Imc and OTid (rather than Ipc as well) because the OTid is the “output” hub of the midbrain selection network, involved in controlling behavior as well as relaying information from the midbrain to cortical areas (Shipp, 2004; Knudsen, 2018).

To address this question, we compared the metrics of stimulus competition in Imc and OTid. To minimize the impact of any idiosyncratic differences in spike sorting, selection of count windows, or other analysis choices on this comparison, we measured competitor response profiles in the OTid as well (Materials and Methods; Mysore et al., 2011).

First, we compared the accuracy with which Imc versus OTid neurons, on average, signal the strongest stimulus. We found that the competitor response profile bias distributions were not distinguishable (Fig. 5A; red vs blue data; $p = 1.0$; sign test), indicating that Imc and OTid neurons signaled the strongest stimulus with comparable accuracy.

Next, we compared the relative proportions of neurons that exhibited switch-like, gradual, or uncorrelated competitor response profiles in the Imc versus OTid (Fig. 5B). The proportions were different between Imc and OTid (Imc: $n = 78$ neurons; 50% switch like; 34.6% gradual; and 15.4% uncorrelated competitor response profiles; OTid: $n = 53$ neurons; 37.7% switch like;

Figure 4. Dependence of transition value of competitor response profile on S1 strength for lmc neurons. **A**, Definition of transition value of competitor response profile and competitor response profile bias for switch-like visual competitor response profile in Figure 1C (see Materials and Methods). S1 = 3.6°/s (black triangle); transition value = 5.9°/s; bias = 2.3°/s. **B**, Distribution of competitor response profile bias for visual competitor response profiles; $n = 66$ lmc neurons. Median = -0.52, $p = 0.39$; sign versus 0.

30.2% gradual; and 32.1% uncorrelated competitor response profiles; χ^2 statistic = 5.21, $p = 0.02$, followed by Holm–Bonferroni correction for multiple comparisons; see below). Indeed, the proportions of competitor response profile types measured in the OTid in this study were not significantly different from those previously published, confirming the veracity of our results (OTid published data; Mysore et al., 2011; $n = 169$ neurons; 30.2% switch like; 33.1% gradual; and 36.7% uncorrelated competitor response profiles; χ^2 statistic = -90.96; $p = 1.0$ between measured and published OTid followed by Holm–Bonferroni correction for multiple comparisons).

The larger fraction of switch-like competitor response profiles in the lmc than the OTid (50% lmc vs 37.7% OTid) suggested that lmc ensembles may be able to signal the strongest stimulus more categorically than the OTid (Mysore and Knudsen, 2011) or, in other words, that the signaling of the strongest stimulus may be more “precise” in the lmc (Fig. 5C). To test this directly, we quantified a metric of categorical signaling: the discriminability (d') across the selection

C, Distributions of biases of competitor response profiles, separately for lmc neurons with switch-like competitor response profiles (top) and gradual competitor response profiles (bottom). Switch-like: median bias = -0.68, $p = 0.52$, sign test against 0 followed by Holm–Bonferroni correction for multiple comparisons, $n = 39$ neurons; gradual: median bias = -0.36, $p = 0.7$, sign test against 0 followed by Holm–Bonferroni correction for multiple comparisons, $n = 27$ neurons. **D**, Schematic of experimental protocol to measure two competitor response profiles with S1 stimuli of two different strengths, indicated by magenta and blue dots. S1 = 11.6°/s, magenta data; S1 = 17.6°/s, blue data. **E**, **F**, Two competitor response profiles (shown in magenta and blue, respectively) measured using protocol in **D** at an example neuron with switch-like competitor response profile. **E**, Rasters. **F**, Firing rate plots (conventions are as in Fig. 1). Transition values: 6.95°/s (when S1 = 11.6°/s; magenta data) and 12.3°/s (when S1 = 17.6°/s; blue data). Shift ratio (shift in transition value/change in S1 strength) for this example neuron = 0.88. **G–I**, Schematic (**G**) and two competitor response profiles (**H**, **I**) measured at an example neuron with gradual competitor response profile. Transition values: 3.22°/s (when S1 = 7.6°/s; green data) and 9.01°/s (when S1 = 13.6°/s; orange data). Shift ratio for this example neuron = 0.96. **J**, Distribution of the shift ratio across $n = 27$ lmc neurons. Median shift ratio = 0.89, $p = 0.26$; sign test against 1.

Figure 5. Imc signals stimulus competition with greater precision, and earlier than OTid. **A**, Distributions of biases of competitor response profiles of OTid neurons (blue; $n = 36$ neurons with correlated competitor response profiles; median = -0.83 ; $p = 0.62$; sign test against 0 and Imc neurons (red; data reproduced from Fig. 4B; $n = 66$; median = -0.52). No significant difference between Imc and OTid medians ($p = 1.0$, sign test after Holm–Bonferroni correction for multiple comparisons). **B**, Proportions of switch-like (S), gradual (G), and uncorrelated (U) competitor response profiles measured in Imc (stacked bar on left; $n = 78$ neurons at which competitor response profiles were measured) and OTid (middle bar; $n = 53$ neurons at which competitor response profiles were measured). Previously published OTid proportions ($n = 169$ neurons at which competitor response profiles were measured; data adapted from Mysore et al., 2011) are also shown for completeness (right bar; unfilled). * (n.s.): χ^2 statistic = 5.21, $p = 0.02$ ($p = 1.0$), χ^2 test between Imc and OTid proportions measured here followed by Holm–Bonferroni correction for multiple comparisons. **C**, Comparison of d' -prime (Materials and Methods) computed from pooled competitor response profile responses measured in OTid (blue) versus Imc (red). * $p = 0.01$, permutation test (Materials and Methods). Inset, Schematic of pooled competitor response profile responses across neurons illustrating d' -prime computation across the selection boundary (vertical gray line). **D**, Pooled instantaneous firing rates (IFRs) of OTid neurons with gradual competitor response profiles in response to S1 alone (gray) or paired S1 and S2 (green), binned into five relative strength bins (columns). **E**, Millisecond-by-millisecond running ANOVA to determine time to suppression (vertical dashed line): the first instant at which responses to paired S1 and S2 diverge significantly from responses to S1 alone (Materials and Methods). OTid responses never diverge significantly for the first relative strength bin ($S2-S1 = -7^\circ/\text{s}$; left-most panel). **F**, **G**, Same as **E** and **F**, but for OTid neurons with switch-like competitor response profiles (magenta data); OTid responses never diverge significantly for the first relative strength bin ($S2-S1 = -7^\circ/\text{s}$; left-most column). **H**, Population summary: Scatter plot of time to suppression for OTid neurons versus Imc neurons for the different relative strength bins (i.e., scatter plot of green numbers in **D** versus magenta numbers in **F**). Dots, (Imc, OTid) time to suppression pairs for the different relative strength bins; magenta data, switch-like competitor response profiles; green data, gradual competitor response profiles; progressively larger dots indicate increasing relative strength ($S2-S1$). For plotting purposes, time to suppression values corresponding to cases in which the responses to paired S1 and S2 never diverged from those to S1 alone, are coded as 450 ms. Dashed line: line of equality. Inset, Boxplot of differences between OTid and Imc time to suppression values; * $p = 0.02$, sign test against 0.

boundary (relative strength = $0^\circ/\text{s}$; $S1 = S2$; Mysore et al., 2011; Materials and Methods). To compute this metric, we pooled competitor response profile responses across all recorded Imc neurons (gradual, switch-like, and uncorrelated competitor response profiles) binned into five relative strength bins, and then calculated the d' -prime between the pooled responses in the relative strength bin of $-3.5^\circ/\text{s}$ versus the relative strength bin of $+3.5^\circ/\text{s}$ (straddling the boundary; Fig. 5C, inset; Materials and Methods). We repeated this across all OTid neurons. This approach allowed us to estimate the ability of a downstream neuron of Imc (or OTid; ideal observer) to decode the strongest stimulus from population activity in the Imc (OTid). We found that d' across the selection boundary ($S1 = S2$) was nearly twice as high in the Imc as the OTid (Fig. 5C; Imc = 1.02; OTid = 0.46; $p = 0.01$, permutation test). This result established that the Imc

signaled the strongest stimulus more categorically (with greater precision) than the OTid.

Finally, we compared the time course of stimulus competition in the Imc versus the OTid. To this end, we computed the instantaneous firing rates of OTid neurons to S1, and to paired S1 and S2, for each competitor response profile and each strength of S2 (Materials and Methods). Following the procedure used for analyzing Imc time courses, we binned paired S1 + S2 responses into five bins. Within each bin, we pooled the instantaneous firing rates across switch-like (and separately, across gradual) OTid neurons (Fig. 5D,F; Materials and Methods) and compared the pooled responses to S1 alone with those of S1 + S2 using a millisecond-by-millisecond ANOVA procedure. We quantified separately for OTid neurons with gradual competitor response profiles (Fig. 5E) and switch-like competitor response profiles

(Fig. 5G) the time to suppression at the different relative strength bins (Fig. 5E,G; Materials and Methods). We found that times to suppression were consistently faster in the Imc than the OTid (Fig. 5H; Imc faster than OT by median value of 18 ms; $p = 0.02$, sign test).

Together, these quantitative differences in precision and time course of responses to paired S1 and S2 indicate that the Imc is itself a site where computations related to stimulus competition occur, and also that the presence of a competitor is signaled first in the Imc followed by the OTid.

Simultaneous, paired measurements of stimulus competition in the Imc and OTid

As a final step, we wanted to go beyond comparisons between Imc and OTid populations recorded independently, and directly compare signatures of competition in simultaneously recorded Imc and OT sites encoding for the same portion of sensory space. This approach can reveal how stimulus competition in the two brain areas unfolds at the same time during exposure to the same competing stimuli.

To this end, we first positioned an electrode in the Imc, mapped the RF, and then positioned a second electrode in the OTid such that the OTid RF overlapped with the Imc RF (Fig. 6A, spatially “aligned” OTid and Imc RFs, dashed ovals). We simultaneously recorded OTid and Imc responses while presenting S1 and S2 per the competitor response profile stimulus protocol: S1 was presented within the overlapping portion of the RFs, and S2 was presented far outside [$>30^\circ$ away; Fig. 6A, S1 and S2 (both were visual looming dots); Materials and Methods].

Responses from a pair of simultaneously recorded, aligned Imc and OTid sites (distance between RF centers = 8° ; Materials and Methods) showed that the nature of the competitor response profile was different in the two areas for this example pair (Fig. 6B,C, switch-like at the Imc site and gradual at the OTid site). However, both competitor response profiles exhibited negative bias (Fig. 6C, vertical arrows to the left of black arrowheads).

We quantified these properties for each aligned site pair in our population ($n = 26$ pairs) for which both Imc and OTid competitor response profiles were negatively correlated with the strength of S2 ($n = 17$ pairs). Across these 17 pairs of Imc-OTid sites (average difference in centers of RF = $6.3^\circ \pm 0.95^\circ$), we found that competitor response profile biases in Imc were not different from those in OTid (Fig. 6D; $p = 0.33$, sign test of bias differences between Imc and OTid). Notably, competitor response profile biases at paired Imc and OTid sites were positively correlated (Fig. 6D; Pearson's $\rho = 0.6$, $p = 0.011$). By contrast, competitor response profile transition ranges at paired Imc and OTid sites were uncorrelated (Fig. 6E; Pearson's $\rho = -0.37$, $p = 0.15$). These results regarding competitor response profile biases and transition ranges did not depend on the degree of alignment between the paired Imc and OTid sites (Fig. 6F; bias vs alignment: Pearson's $\rho = 0.07$, $p = 0.8$; transition vs alignment: Pearson's $\rho = 0.03$, $p = 0.9$). Thus, for Imc and OTid sites encoding for the same portion of sensory space, the accuracy of signaling the strongest stimulus was correlated, but precision of the signaling was not.

Finally, we examined the speed at which paired Imc and OTid sites signaled the presence of a competing stimulus. We compared the time course of response suppression by calculating (as before) the time to suppression within each relative strength bin for OTid sites (Fig. 6G,H) as well as paired Imc sites (Fig. 6I, J). The times to suppression at paired Imc and OTid sites were highly correlated (Pearson's $\rho = 0.99$, $p = 0.0007$, correlation

test), with Imc sites signaling the presence of the competitor earlier than paired OTid, consistent with our findings from independent recordings (Fig. 6K; best fit line has positive intercept (intercept = 52.8; 95% CI, 16.2, 89.4), with slope not different from 1 (slope = 0.84; 95% CI, 0.66, 1.01), indicating that OTid time to suppression are significantly above line of equality).

Discussion

This study elucidates the properties of multisensory, salience-dependent stimulus competition in a pivotal nucleus in the mid-brain selection network in vertebrates, namely the Imc (Graybiel, 1978; Gruber and Lettvin, 1980; Sereno and Ulinski, 1987; Jiang et al., 1996; Wang et al., 2004). This small group of GABAergic neurons (Wang et al., 2004; Mahajan and Mysore, 2018), which supplies inhibition in a combinatorial manner to all parts of the OT space map (Wang et al., 2004; Mahajan and Mysore, 2018), serves a critical function: without it, competitive interactions and selection in the OTid are abolished (Marin et al., 2007; Mysore and Knudsen, 2013). Considering the critical role of the intermediate and deep layers of the SCid in target selection for spatial attention (McPeck and Keller, 2004; Lovejoy and Krauzlis, 2010), the Imc appears to occupy a spot of central importance within the vertebrate midbrain selection network.

One manner in which the Imc might control competition and selection in the OTid is by serving as a relay of inhibition, converting the input excitatory drive from OT₁₀ into powerful inhibition that is distributed broadly across the OTid space map. Together with the anatomic projection patterns of Imc, this implementation would allow Imc to facilitate computations in the OTid. However, another possibility is that the Imc is itself a site at which computations relating to stimulus competition occur actively (i.e., one at which information about competing stimuli is compared), with this processed information then being relayed to downstream targets (Ipc and OTid). Our results directly support the latter hypothesis.

We found that most Imc neurons ($\sim 85\%$) responded to a visual RF stimulus (S1) with decreasing firing rates as the strength of a distant visual competitor (S2) was systematically increased. The responses transitioned from a high to a low value in an abrupt (switch-like) manner in the majority of these cases (60%), and gradually in the others. Notably, the strength of S2 at which the transition from high to low responses occurred was coupled to the strength of S1, and was, on average, equal to it. These results demonstrated that Imc neurons perform an online comparison of the strengths of the competing stimuli, and signal the strongest one. The large proportion (60%) of switch-like response profiles resulted in the Imc signaling the strongest stimulus. (We have shown in previous work that a population of neurons in which $\geq 30\%$ exhibit switch-like competitive response profiles produces categorical signaling at the level of the entire ensemble (Mysore et al., 2011)).

Imc neurons also exhibited multisensory stimulus competition. When Imc was tested with competing stimuli of different sensory modalities, we found results qualitatively similar to those when both stimuli were visual. These results indicated that Imc signals the strongest stimulus, independently of the sensory modalities. Notably, building off of findings that the average transition value of Imc response profiles is equal to the strength of S1, in the auditory case, the average transition value (-71 dB) across neurons presents an estimate of the binaural level of an auditory competitor that the Imc deems to be equivalent in strength to the average loom speed of S1 ($6.9^\circ/\text{s}$).

Figure 6. Signatures of stimulus competition at simultaneously recorded, aligned lmc and OTid sites. **A**, Schematic of experimental setup for simultaneous, paired recordings in lmc and OTid. S1 and S2, Stimulus protocol for measuring competitor response profiles; colored icons to the left, recording electrodes, positioned in OTid (blue) and lmc (red); dashed ovals, RFs of recorded site. **B**, Raster plots of responses for example paired, aligned lmc (left panels) and OTid (right panels) sites; distance between RF centers = 8° (Materials and Methods). Top panels, Responses to S1 alone. Bottom panels, Responses to S1 and S2 presented together. Strength of S1 = 9.6°/s. All other conventions similar to Figure 1B. **C**, Firing rate responses corresponding to rasters in **B**. Conventions are as in Figure 1C. Competitor response profile correlation values: lmc = -0.81, ($p = 0.009$); OTid = -0.99, ($p < 0.05$ ($p = 0.0000004$)). Competitor response profile transition ranges: lmc = 2.8°/s (switch-like competitor response profile); OTid = 15.3°/s (gradual competitor response profile). Competitor response profile transition values: lmc = 4.1°/s; OTid = 6.2°/s. **D**, Scatter plot of competitor response profile biases measured at paired lmc and OTid sites ($n = 17$ pairs; average distance between RF centers = $6.3^\circ \pm 0.95^\circ$). Dashed gray line, line of equality; solid purple line, best fit line to data; Pearson's $\rho = 0.6$, $p = 0.01$. **E**, Scatter plot of transition ranges of competitor response profiles measured at paired lmc and OTid sites. Dashed gray lines, cutoff value of transition ranges (4°/s) for switch-like versus gradual competitor response profiles. **F**, Plot of difference in transition ranges of competitor response profiles (OTid - lmc; top) or biases of competitor response profile (OTid - lmc; bottom) for paired lmc-OTid sites as a function of distance between lmc and OTid RFs. Pearson's $\rho = 0.03$, $p = 0.9$ (competitor response profile transition ranges); $\rho = 0.07$, $p = 0.8$ (competitor response profile biases). **G**, **I**, Response time courses for OTid (**G**; blue) and lmc sites (**I**; red) recorded simultaneously; shown are the pooled averages across OTid sites, and separately, across lmc sites (conventions are as in Fig. 2). **H**, **J**, Results from the millisecond-by-millisecond AVOVA are

Our results also showed distinct time courses of responses for neurons with switch-like versus gradual competitor response profiles. When S2 was weaker than S1, switch-like neurons signaled the presence of a competitor slower than gradual neurons, but when S2 was stronger than S1, switch-like neurons flipped over to being faster. By contrast, in the OTid, it has been reported that neurons with switch-like profiles are slower to signal the strongest stimulus at all stimulus strengths (Mysore et al., 2011). We found that in the Imc, this potentially puzzling flip was accounted for by differences in the intrinsic nature of switch-like versus gradual neuron response profiles in the Imc, which resulted in greater amounts of response suppression for gradual competitor response profiles when the competitor was weaker than the stimulus in the RF ($S2 < S1$), but resulted in greater amounts of suppression for switch-like competitor response profiles when the competitor was stronger ($S2 > S1$). Thus, compared with neurons with gradual competitor response profiles, neurons with switch-like competitor response profiles quickly and effectively signal response suppression when a competing stimulus is stronger. This effect is potentially consistent with circuit mechanisms necessary for producing switch-like response profiles. Recently, it was shown that a “donut-like” pattern of inhibition (Serenio and Ulinski, 1987; Wang et al., 2004) controls the categorical nature of switch-like response profiles by selectively enhancing response differences for stimuli that are on either side of the selection boundary (Mahajan and Mysore, 2020). Such a mechanism could, in principle, account also for the time course effects reported here, although this remains to be tested.

The signatures of competition in the Imc are quantitatively and qualitatively different from those in the OTid. Imc and OTid neurons both signaled the strongest stimulus accurately, with almost no bias in estimating when the two competing stimuli were equal in strength. However, with respect to another key aspect of stimulus competition, namely, the precision with which neurons signal the strongest stimulus—either in a binary-like, explicitly categorical manner, or in a more analog, gradual manner—Imc differed quantitatively from the OTid: it signaled the strongest stimulus much more accurately ($2\times$ better). These results first came to light from data collected independently (in different experiments) in the Imc and OTid, but using the same experimental setup, stimulus protocols, and analysis pipelines. Subsequently, paired simultaneous recordings in spatially aligned portions of the Imc and OTid not only confirmed these findings, but extended them. They revealed that some aspects of stimulus competition occurred in a coordinated manner in portions of Imc and OTid that encode for the same region of sensory space (i.e., that are active at the same time in a bird experiencing the competing stimuli). Specifically, the bias of neurons in estimating whether a competing stimulus was weaker or stronger than their RF stimulus was highly correlated. This suggests the presence of a shared or mutually dependent mechanism in setting the bias of competition. By contrast, the precision with which neurons signaled the strongest stimulus was not correlated between Imc and

OTid neurons encoding for overlapping regions of sensory space. This suggests the presence of independent mechanisms in these two areas involved in setting the precision of competition, providing further support for Imc being an active, independent locus of competition, rather than a simple inhibitory relay.

In interpreting comparisons between functional properties of Imc and OTid neurons, it is important to note that there is much more heterogeneity of cell types (anatomic and molecular) in the OTid than in the Imc. As a result, the observed differences may, in part, be due to nonuniform sampling of the different cell types in the OTid. This concern is somewhat mitigated by two aspects. First, that because the electrodes used in the two areas were identical, any bias in the sizes of the cells recorded is similar in both areas. Second, and perhaps more importantly, the paired Imc-OTid recording experiments (Fig. 6) yielded results similar to those of the independent recording experiments (Figs. 1–5). This is pertinent because in the paired recordings, multiunit sites were analyzed (necessarily so, to lawfully define pairs for comparison), whereas in the independent recordings, single units were analyzed. The finding of similar results from both approaches adds confidence to our conclusions.

Analysis of response time courses in both the separate as well as the paired Imc-OTid experiments demonstrated that the Imc reports stimulus competition and signals the strongest stimulus earlier than the OTid. Considering that the Ipc, the other key nucleus in the midbrain selection network, is also a downstream target of the Imc (just as the OTid is), it is plausible that the Ipc will also be slower than the Imc at signaling the strongest stimulus. This hypothesis needs to be tested directly. Consequently, the Imc emerges as the site within the heavily interconnected midbrain selection network at which stimulus competition is potentially resolved first. In any case, the finding above, along with the categorical signaling and anatomic connectivity of Imc, reveals that the Imc sends differentially (categorically) enhanced competitive inhibition to OTid and Ipc sites encoding the weaker versus the stronger competing stimuli.

In summary, not only does the Imc actively perform computations of relative strength-dependent stimulus competition, and does so earlier than the OTid, some aspects of these computations differ qualitatively from paired OTid sites. Overall, the Imc is more categorical in its signaling of the strongest stimulus.

The mechanism by which competition within the Imc may occur is yet to be demonstrated directly. Clues come from modeling and slice experiments, respectively predicting (Mysore and Knudsen, 2012) and demonstrating (Goddard et al., 2014) the presence of long-range inhibitory projections between Imc neurons. These projections could serve to deliver the competitive inhibition (Li et al., 2007) necessary for the functional signatures reported here.

Considering that the midbrain selection network is conserved across vertebrate species (Graybiel, 1978; Gruberg and Udin, 1978; Serenio and Ulinski, 1987; Jiang et al., 1996; Wang et al., 2004), our findings have the power to guide understanding of the midbrain mechanisms underlying spatial selection in other vertebrates. More generally, these findings have implications for unpacking how key computations underlying various forms of selection such as perceptual categorization and value-based decision-making, may be implemented in neural circuits (Mysore and Kothari, 2020). The ubiquity of selection in adaptive behavior, coupled with our current lack of understanding of the precise neural mechanisms that underpin it, highlight the importance of the barn owl midbrain selection

also shown for OTid (H; blue) and Imc sites (J; red), respectively. Columns, Relative strength bins; IFR, instantaneous firing rate. Horizontal dashed lines, p value cutoffs (0.05, bottom line; 0.01, top line); vertical dashed arrows (and colored text), time to suppression; Materials and Methods). K, Scatter plot of time to suppression measured at aligned Imc versus OTid sites recorded simultaneously. Each dot, time to suppression pair for a different relative strength bin; data correspond to the colored numbers in G and I. Dashed line: line of equality. Pearson's $\rho = 0.99$, $p = 0.0007$.

circuit as a gateway for generating hypotheses about viable circuit solutions for selection and decision-making in general.

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