Report

Preperceptual and Stimulus-Selective Enhancement of Low-Level Human Visual Cortex Excitability by Sounds

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Summary

Evidence of multisensory interactions within low-level cortices and at early post-stimulus latencies [1–6] has prompted a paradigm shift in conceptualizations of sensory organization [7–10]. However, the mechanisms of these interactions and their link to behavior remain largely unknown. One behaviorally salient stimulus is a rapidly approaching (looming) object, which can indicate potential threats [11–13]. Based on findings from humans [14] and nonhuman primates [15, 16] suggesting there to be selective multisensory (auditory-visual) integration of looming signals, we tested whether looming sounds would selectively modulate the excitability of visual cortex. We combined transcranial magnetic stimulation (TMS) over the occipital pole and psychophysics for "neurometric" and psychometric assays of changes in low-level visual cortex excitability (i.e., phosphene induction) and perception, respectively [17, 18]. Across three experiments we show that structured looming sounds considerably enhance visual cortex excitability relative to other sound categories and white-noise controls. The time course of this effect showed that modulation of visual cortex excitability started to differ between looming and stationary sounds for sound portions of very short duration (80 ms) that were significantly below (by 35 ms) perceptual discrimination threshold. Visual perceptions are thus rapidly and efficiently boosted by sounds through early, preperceptual and stimulus-selective modulation of neuronal excitability within low-level visual cortex.

Results

Studies of early auditory-visual multisensory interactions in humans have either focused on their timing (e.g., [1, 2]) or localization [3]. Similarly, intracranial studies in macaques have nearly exclusively focused on effects within auditory cortices or superior temporal regions (e.g., [4–6]). One recent exception [19] recorded from primary visual cortex and showed that response latencies could be reduced on multisensory versus visual trials during an active, but not passive, behavioral task.

Two aspects of these results are of particular relevance for theories of multisensory interactions in humans. First, they show how the behavioral relevance of the stimuli/task might dramatically impact the observed pattern of multisensory interactions (see also [20]). Second, reduced response latency to multisensory stimuli is indicative of alterations in the excitability of primary visual cortex by nonvisual (i.e., auditory) inputs. In humans, the excitability of low-level visual cortex can be probed through its direct stimulation via TMS over the occipital pole to induce illusory light sensations (phosphenes) (e.g., [21–26]). Both auditory [18] and tactile [17] stimuli have been shown to modulate phosphene induction by TMS. The present study capitalized on these observations to determine the stimulus selectivity, perceptual gain, and dynamics of sound-induced changes in this index of visual cortex excitability. We focused on sounds that gradually increased in their amplitude because such looming or approach cues have been previously shown to be both behaviorally salient across species and developmental stages [11–13, 27–29] as well as subject to selective multisensory interactions [14–16].

Looming Sounds Selectively Enhance Visual Cortex Excitability

Experiment 1 tested in 15 healthy adults whether looming sounds differentially increase visual cortex excitability relative to receding or stationary sounds. Sounds varied in a 3×4 within-subject design (Figure 1A) in their duration (250, 500, and 1000 ms) and temporal profile (i.e., looming [L], receding [R], stationary of constant low volume [S_L] that was equal to the initial and end volume, respectively, of looming and receding sounds, and stationary of constant high volume [S_H] that was equal to the initial and end volume, respectively, of receding and looming sounds). Excitability of visual cortex, probed by TMS at sound offset, differed across the sounds' temporal profiles ($F_{3,42} = 29.57$; p < 0.00001) independently of their duration ($F_{6,84} = 1.64$; p = 0.21) (Figure 2). Relative to baseline (TMS alone: phosphenes perceived in 35.8% ± 5.9% of trials; mean ± standard error of the mean [SEM]), phosphene induction was enhanced by all sound profiles (L: 70.8% ± 3.2%, $t_{14} = 5.40$, p < 0.0001; S_H: 54.9% ± 2.9%, $t_{14} = 3.10$, p < 0.01; S_L: 50% ± 3.6%, $t_{14} = 2.95$, p = 0.01) with the exception of receding sounds (R: 44.5% ± 3.2%, $t_{14} = 1.45$; p = 0.17). Notably, looming sounds doubled baseline phosphene perception (from 35.8% to 70.8%), significantly exceeding the excitability increases of all the other sounds ($t_{14} \geq 5.58$, p < 0.0001). This difference cannot be explained by differences in sound duration or end amplitude at TMS delivery, as each dynamic looming sound was equated in these parameters with a stationary control sound. Nor do the results follow the total stimulus energy; both the S_H and S_L conditions yielded similar effects. Instead, this enhanced excitability is probably due to the dynamic structure of the looming signal.

Chronometry of Sound Effects on Visual Cortex Excitability: Presence of Looming-Sensitive Changes at Short Latencies

We next investigated the time course of the looming-sensitive changes in visual cortical excitability as compared to

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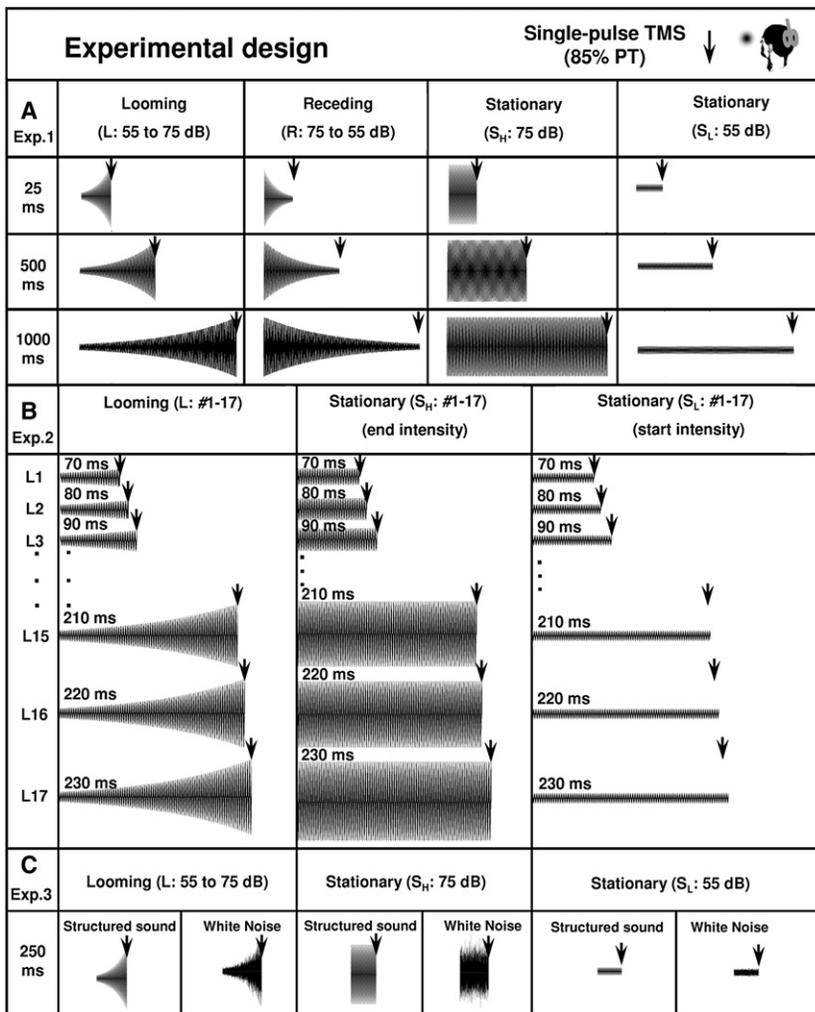[6]These authors contributed equally to this work

Figure 1. Stimuli and Experimental Design

(A) For comparisons across different sound categories (Experiment 1), looming and receding sounds of three durations (250, 500, and 1000 ms) were presented together with stationary sounds equated for start and end intensities (3 × 4 stimulus design).

(B) For evaluation of the timing of looming-sensitive changes (Experiment 2), initial portions of the 250 ms sound were presented (clipped from 70 to 230 ms) together with stationary sounds equated for start and end intensities and duration (17 × 3 stimulus design).

(C) For testing for the effects of sound structure, the 250 ms structured sounds (used in A and B) were contrasted with 250 ms of white noise of identical temporal sound profiles.

(A–C) The visual cortex was stimulated by TMS at sound offset to sample its excitability at the end of the sounds through quantification of TMS-induced illusory visual perceptions (phosphenes), in analogy to previous studies [17, 18, 21–26] (see also Supplemental Experimental Procedures). Participants were thus involved in a visual task (phosphene perception) for which the auditory stimulation was irrelevant. TMS was applied below phosphene threshold (85% PT), which was defined at baseline (i.e., in the absence of any sound).

online), we focused our chronometry study on the 250 ms sounds and a 70–230 ms interval. Also, because receding sounds failed to produce phosphene perceptions different from baseline levels, this sound profile was not investigated further so that we could maximize the number of trials and minimize individual TMS dosage. In Experiment 2A, participants (n = 11) were presented with initial portions of the looming and stationary stimuli (70–230 ms duration at 10 ms steps, randomly intermixed; Figure 1B). Looming and stationary sounds differently modulated visual cortex excitability (Figure 3B; effect of sound profile: $F_{1,10} = 35.09$; $p < 0.001$). This modulation varied with

stationary sounds. Because Experiment 1 produced selective effects with looming sounds of 250 ms duration (without evidence for a differential effect with longer sounds) and based on the results of an initial exploratory experiment (see Figure 3A and Supplemental Experimental Procedures, available
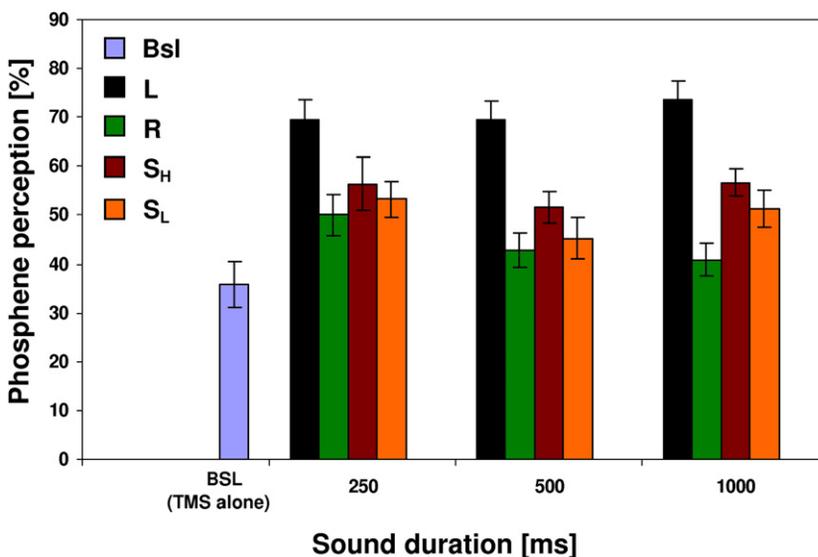

Figure 2. Modulation of Visual Cortex Excitability across Sound Categories in Experiment 1

Percentage of perceived phosphenes (SEM indicated) following occipital TMS as a function of sound duration and temporal sound profile (looming [L], receding [R], stationary of high [S_H], and low intensity [S_L]).
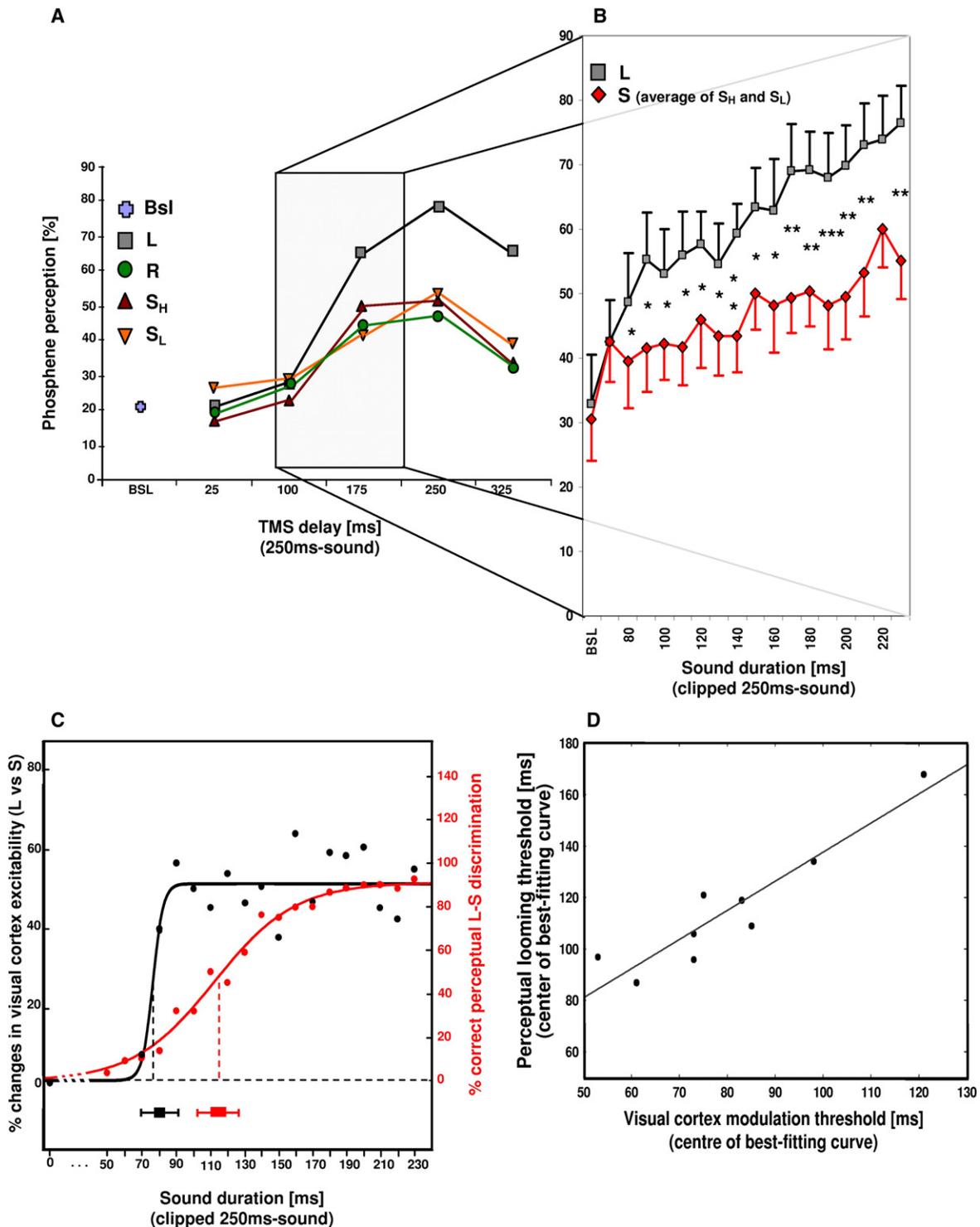
Figure 3. Timing of Sound-Induced Changes in Visual Cortex Excitability and the Comparison of Neurometric and Psychometric L versus S Sound Discriminability

Percentage of perceived phosphenes (SEM indicated) following occipital TMS in the exploratory study as a function of TMS pulse delay (25, 100, 175, 250, and 325 ms) into evolution of the 250 ms sounds (L, R, S_H, and S_L) (A) and in Experiment 2A as a function of sound duration (250 ms sounds clipped from 70 to 230 ms, in steps of 10 ms) (L, S_H, and S_L sounds only) (B). The asterisks point to significant differences between phosphene perception between L and S (mean of S_H and S_L) sounds (i.e., L > S; *p < 0.05; **p < 0.01; ***p < 0.001).

(C) Comparison between neurometric (black) and psychometric (red) sigmoid functions. Averages of curve centers from fitting in individual data are displayed below (box: ± SEM; whiskers: ± SD).

(D) Scatterplot of the correlation of participants' thresholds for visual cortex modulation (x axis) and perceptual looming experience (y axis).

stimulus duration (interaction: $F_{17,170} = 1.81$; p = 0.03). Differences between looming and stationary sounds started at 80 ms latency ($t_{10} = 2.72$; p = 0.02) and remained significant thereafter ($t_{10} \geq 2.27$; p $\leq$ 0.047; Figure 3B), except at 220 ms ($t_{10} = 2.04$; p < 0.07).

Changes in Visual Cortex Excitability versus Auditory Perceptual Discrimination: Evidence for Preperceptual Effects

To contrast the looming-sensitive changes in visual cortex excitability across sound durations (a "neurometric" function) with the corresponding probability of detecting looming sounds (a psychometric function), we also assessed discrimination performance in the same pool of participants (n = 11) by using the same sound portions in the absence of TMS (Experiment 2B). To allow for a comparison of the neurometric with the psychometric discriminability of looming versus stationary sounds, we expressed changes in visual cortex excitability as a percentage change (looming versus stationary sounds) across increasing sound portions. These results resembled a sigmoid function (Figure 3C, left y axis, black dots) that fits a logistic curve (Figure 3C, black line, Regression versus Corrected Total: F = 45.75, p < 0.0000001; see Supplemental Experimental Procedures). As would be expected, the psychometric function of perceptual sound discrimination also followed a sigmoid curve (Figure 3C, right y axis, red dots and line; Regression versus Corrected Total: F = 34.43, p < 0.000001) but with a rightward-shifted curve center. For statistical comparisons, nonlinear curve fitting was also conducted for each participant. The data from two participants did not fit a sigmoid function significantly. For the remaining nine participants, the mean centers of neurometric and psychometric functions were respectively estimated at 80 ± 7 ms (mean ± SEM) and 115 ± 8 ms (box plots in Figure 3C). These values not only differed significantly ($t_8 = -11.24$; p < 0.00001), but were also highly correlated across individuals ($r_7 = 0.93$; p < 0.001; Figure 3D). Differential enhancement of visual cortex excitability by looming versus stationary sounds thus occurred with sound portions of short durations that could not yet explicitly be discriminated by the participants.

Selective Enhancement of Visual Cortex Excitability by Looming Sounds Depends on Sound Structure

Experiment 3 addressed the possibility that the selective enhancement of visual cortex excitability induced by looming sounds follows from a simple monotonic intensity-response function, i.e., a simple rising sound intensity cue, rather than from the looming signal (i.e., the perception of a sound source or object approaching the participant). Prior auditory research would suggest that the perception of looming/approach is specific for structured versus broadband sounds [13]. Participants (n = 10) were presented either with structured sounds or white noise bursts (250 ms duration) that rose in intensity or remained stationary (following a 2 × 3 within-subjects design otherwise identical to Experiment 1; see Figure 1C). Each main effect and the interaction between factors were significant (effect of sound structure: $F_{1,9} = 8.35$; p < 0.05; effect of sound profile: $F_{1,9} = 50,38$; p < 0.0001; interaction: $F_{1,9} = 5.25$; p < 0.05). Relative to baseline (TMS alone: phosphenes perceived in 29.6% ± 7.5% of trials), all sounds enhanced phosphene perception (L: 70.4% ± 4.9%, $t_9 = 7.36$, p < 0.0001; S: 48.1% ± 5.5%, $t_9 = 3.46$, p < 0.01; L_{WN}: 52.1% ± 3.6%, $t_9 = 3.09$, p = 0.013; S_{WN}: 46.0% ± 3.9%, $t_9 = 2.32$, p < 0.05). Thus, as in Experiments 1 and 2, the enhancement of visual cortex excitability

induced by structured looming sounds was double that of the baseline and significantly greater than all other sounds ($t_9 \geq$ 4.08, p $\leq$ 0.01). No significant difference across white noise sounds was observed. Foremost and in contrast to structured sounds, looming white noise bursts did not enhance visual cortex excitability beyond levels observed with stationary sounds (Figure 4), consistent with previous literature [13, 15, 29, 30]. These results thus exclude the alternative explanation of a simple rising intensity mechanism.

Discussion

This is the first study to reveal stimulus-selective cross-modal interactions in low-level visual cortex, complementing existing neurophysiologic work in animals that has thus far been restricted to auditory cortices [16, 30] as well as our recent psychophysical work demonstrating selective integration of multisensory looming signals [14]. Acoustically structured looming sounds selectively enhanced visual cortex excitability (i.e., phosphene induction). The use of TMS-induced phosphenes as our principal dependent measure allowed us to specify the localization of our effects to low-level visual cortex (V1/V2), and the use of several stimulus durations allowed us to specify the onset of effects to looming sounds of very short duration (80 ms), which were below psychophysical discrimination threshold. This is strongly suggestive of a mechanism that allows for auditory-driven modulation of visual cortex at preperceptual processing stages.

We have previously shown that a brief tone induces initial changes in visual cortex excitability 60–75 ms after the sound onset [18]. Because the present looming-selective changes occur only 5–20 ms later (i.e., for TMS at 80 ms), it follows that the signal inducing the excitability changes in visual cortex probably originates from a (remote) structure that is able to differentiate between the two sound profiles within this time interval (albeit in a preperceptual manner). An alternative possibility that the auditory input is an undifferentiated signal can be discounted by the fact that simple acoustic parameters (such as intensity and temporal profile) were insufficient in selectively modulating visual cortex excitability. Regarding the potential origins of the auditory inputs into visual cortices (V1/V2), several aspects need to be considered, including (1) currently identified auditory-visual multisensory connectivity, (2) the time course of auditory signal propagation, and (3) the likely latency of brain activity discriminating looming sounds. Even considering these aspects, however, we would hasten to add that the present results (in and of themselves) provide no objective criterion for pinpointing the source(s) of auditory inputs mediating our effects.

Regarding multisensory interconnectivity, several accounts have been advocated to explain multisensory interplay within low-level cortices (e.g., [8–10, 31, 32]). These include (1) subcortical influences, e.g., superior colliculus (e.g., [33]) or thalamic (e.g., [34, 35]), (2) direct cortico-cortical connections (e.g., [36–38]), and (3) indirect and/or feedback connections (e.g., [8, 10]). As to direct cortico-cortical connectivity between auditory cortices (primary and nonprimary) and posterior visual areas (V1/V2), retrograde tracing showed there to be monosynaptic projections from auditory cortices as well as areas of the superior temporal sulcus to V1 [36]. More specifically, caudal parabelt regions accounted for 70% of the projections from auditory cortices to V1. Interestingly, projections from primary auditory cortices to V1 seem to preferentially terminate in portions representing the peripheral visual
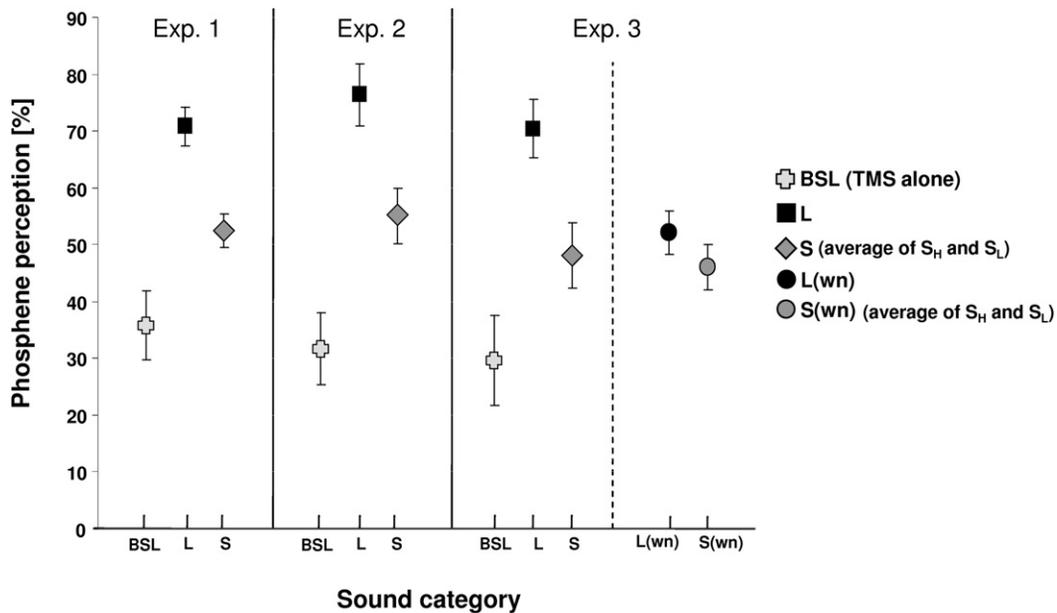
Figure 4. Looming-Sensitive Changes Depend on Structured Sounds

The looming-sensitive changes observed in Experiments 1 and 2 (L sound > S sounds [mean of S_H and S_L]) were clearly replicated in a third experiment (SEM indicated) when the same (structured) sounds were used (compare Experiments 1 and 2 with left panel of Experiment 3). No such effect was observed with white noise (wn), as shown by the identical response profiles to rising and stationary intensity white noise (Experiment 3, right panel; L sound = S sounds [mean of S_H and S_L]).

field [36], though less dense projections and projections from other auditory regions onto foveal visual field representations cannot be excluded at this stage. As such, it would be interesting for future investigations to examine whether and how sounds affect phosphene perception at specific visual field locations, as this might provide insights (albeit indirect) as to the potential auditory origins.

In terms of the time course of signal propagation within auditory cortices, responses within human primary auditory cortex have been documented at ~15 ms (e.g., [39]). Responses in monosynaptically connected regions have been recorded as early as 2–5 ms later (e.g., [40]; see also [41] for anatomic data concerning the connectivity of primate auditory regions). It is therefore plausible and likely for there to be at least a rudimentary analysis/processing of the looming (and other) sounds in nonprimary auditory cortices before our signal elicits its effects in visual cortices.

Regarding the latency at which the brain might discriminate between looming and other categories of sounds, no neuroimaging or neurophysiologic data currently exist. Rather, prior work on looming sound processing in humans has been conducted only with fMRI [27, 28]. This work has shown that lateral portions of the superior temporal cortex, the superior temporal sulcus, and intraparietal sulcus as well as the amygdala are sensitive to looming cues (see also [16, 30, 42] for comparable results in monkeys). It is therefore likely that one or several of these regions is mediating the present effects, making it incumbent for future studies to investigate the spatiotemporal brain dynamics of looming sound discrimination.

More generally, our results suggest that the mechanism for visual cortex excitability modulation is sensitive to very weak changes in the dynamic sound profile, which in turn can lead to dramatic modulations of perception. Our findings that thresholds for differential modulations in visual cortex excitability are systematically shorter than the corresponding

thresholds for sound discrimination would seem to link the processes mediating changes in visual cortex excitability and explicit sound discrimination. One possibility is that both are shaped by a common process that occurs during early periods of sound analysis within low-level auditory cortices (e.g., see [16, 27, 28] for evidence concerning auditory structures involved in processing looming sounds). These data would then favor the looming sensitivity of sound-induced changes in visual cortex excitability to be driven by low-level features of the looming sound, rather than by highly processed auditory information.

Alternatively, the effects on visual excitability reported here might have been brought about by stimulus-driven (reflexive) attention, akin to those previously demonstrated for voluntary visual spatial attention shifts instructed by symbolic attention cues [22]. Although visual spatial attention was not varied in the present study (invariant phosphene position) and participants were instructed to ignore any sounds during the TMS experiments while reporting phosphene presence/absence, looming sounds might have nonetheless driven cross-modal attentional mechanisms more than any other sound category. To date, the predominant interpretation of the preferential processing of looming sounds is that they serve as a salient warning cue [11–13] that might in turn bias overt auditory attention [29] and orienting [28] so as to facilitate auditory object detection and identification. Current models of auditory saliency maps [43] would indeed predict that the structured looming sounds would have produced the largest effect. Still, to the extent that looming structured sounds are preferentially (and in our case preperceptually) processed by the auditory system, our results would constitute the first demonstration of a cross-modal attention effect that is both stimulus selective and evident at preperceptual latencies (for further discussion see Supplemental Results and Discussion).

In conclusion, TMS in combination with psychophysics shows there to be auditory inputs that alter visual neuronal excitability at short latencies, in a preperceptual manner, and with dependence on the qualitative features of the sounds (both in terms of looming versus receding and also of structured sounds versus white noise). The results provide novel information on the nature of the auditory signal modulating visual cortex excitability and on the dynamics of these effects both with respect to stimulus delivery and to psychophysical discrimination. The pattern of results supports a prominent role of preperceptual auditory-driven signals in multisensory interactions within low-level visual cortex.

Supplemental Data

Supplemental Data include Supplemental Results, Supplemental Discussion, and Supplemental Experimental Procedures and can be found with this article online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)01707-2.

Acknowledgments

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Supplemental Data

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Supplemental Results and Discussion

Looming-sensitive changes on phosphene report: Perceptual or response bias?

Several aspects of our data clearly argue against our results being explained by a response bias, rather than a visual perceptual bias of the participants to looming sounds. First, response biases should equally apply to both tested looming sound conditions (structured sounds versus white noise). However, there were clear differences in the report of induced phosphenes between structured rising intensity sounds and rising intensity white noise (Fig. 4), speaking clearly against this interpretation. Second, we found that reaction times (RTs) were not significantly faster (biased) for structured looming sound vs. all the other sounds across all experiments. In Experiment 1, the RTs to looming sounds were too slow (1692ms; SEM=140.6ms) to be attributable to a response pattern that might have been triggered by an automatic avoidance mechanism to looming sounds, and there was no significant RT difference across sound durations ($F_{2,28}$=1.22; p=0.31) or sound profiles ($F_{3,42}$=1.02; p=0.39), nor a significant interaction ($F_{6,84}$=0.83; p=0.55), which should have occurred with such a mechanism. Likewise, in Experiment 3, the ANOVA (2x2) on RT with the factors Sound Structure (structured sound vs. white noise) and Sound Profile (looming vs. stationary (SH and SL collapsed)) showed no main effects (all p>0.25) nor significant interactions (p>0.39). This represents a further argument against a response bias. Third, an independent, more

stringent argument against a response bias with respect to looming sounds is that we found a difference between looming and stationary sounds relative to V1/V2 excitability for sound durations (80ms) well below the psychophysical looming-vs.-stationary discrimination threshold. This result rules out any residual possibility that a strategy could have been adopted by the participants with respect to the looming sound.

A role for oscillations?

Of importance here is our finding that the latency of sound-induced changes in visual cortex coincides (occurs in synchrony) with the initial feed-forward visual processing in visual cortex (if sounds and visual stimuli were to co-occur in the environment). This timing raises a possible, albeit currently speculative, manner in which auditory inputs, especially those signalling approach, could boost processing of visual (and perhaps also multisensory) stimuli. Recent work by Lakatos et al. [1] introduces a potential mechanism that may also be occurring here. They found that somatosensory inputs to primary auditory cortex alter the phase of ongoing oscillatory activity and as a consequence amplify neuronal excitability and thus reactivity to auditory signals. While their effects involved phase resetting of low-frequency oscillations (delta and theta), investigations of integrative responses to auditory-visual looming signals within lateral belt regions of auditory cortex and the superior temporal sulcus highlight the role of enhanced gamma oscillations [2]. One possibility is that there are complex interactions between phenomena observed at these different frequency bands [3]. Alternatively, phase resetting and coupling might enhance simple detection of multisensory events versus binding of multisensory signals for integration into an unified percept, depending on the frequency band. While the present data do not allow us to specify whether (and if so which) specific frequency bands are mediating the observed excitability changes in visual cortex, we would speculate that the auditory inputs into visual cortices are resulting in a

similar phase reset (and likely other effects as well), though combined TMS-EEG experiments would be required to substantiate this speculation.

Differential adaptation to stationary and looming sounds with differential alerting potential?

Note that any effects of cross-modal attention have to be dissociated from differential alerting effects across the different sound types around the time of TMS pulse delivery. It could be argued that despite one control sound being replayed from its onset at the maximum sound intensity (high intensity stationary sound), this sound might not have had maximum alerting potential. This is because there might have been stronger adaptation to stationary (especially for long sounds) than looming sound. However, this is unlikely as visual excitability steadily increased (never dropped) over increasing sound duration across all conditions (see Fig. 3 a,b) and because this steady increase was identical for stationary sounds of low and high intensity (all t-tests between S_L- and S_H-sounds n.s. for all sound durations/TMS-delays ranging from 70-230ms, Expt. 2). In addition, differential effects were observed for rising intensity structured sounds versus white noise bursts, further ruling out an explanation based on simple alerting mechanisms based on sound intensity. Note also that we have chosen to use a sound intensity (max 75dB) well below the threshold for evoking a startle response [c.f. 4 for discussion].

Supplemental Experimental Procedures

Participants. A total of twenty-seven healthy volunteers with normal hearing participated in the study (ten women; mean age, 28.5 years; range, 19–41 years). The experiments were performed in the Functional Brain Mapping Laboratory, University of Geneva and the Centre

for Cognitive Neuroimaging, University of Glasgow. All participants gave written informed consent to the study that was approved independently by the local Ethics Committees (Geneva University Hospital, University of Geneva and the FIMS, University of Glasgow). Fifteen participants took part in Experiment 1, 11 in Experiment 2a and 2b (including psychophysical testing), and 10 in Experiment 3 (with 9 of them participating in each of the first two experiments).

Procedure, occipital TMS and auditory stimuli. Illusory visual percepts (phosphenes) were induced by single-pulse TMS over the occipital pole via a 70mm figure-of-eight TMS coil (connected to a Magstim Rapid[2] Transcranial Magnetic Stimulator, Magstim Company, Spring Gardens, UK). This type of protocol has been extensively used to probe visual cortex excitability [e.g. 4-11], based on findings of phosphenes to originate from early visual areas (V1/V2) [e.g. 6,7].

All participants underwent a training session prior to the experiments. This session included careful determination of the site from which the occipital TMS pulse induced a phosphene, at the minimum intensity needed to evoke a phosphene on approximately 50% of trials (Phosphene Threshold; PT). This session also served to evaluate the consistency of phosphene perception through repeated stimulation over time. The optimal TMS coil position over the occipital pole as well as the shape, size, and position of the perceived phosphenes varied somewhat across participants, but was constant for each participant across the different TMS blocks. The phosphenes consistently appeared within the lower visual field quadrant opposite to the stimulated occipital cortex, corresponding to stimulation of the dorsal part of the occipital pole representing the central part of the lower visual field, and in accordance with previous reports (e.g. [4,5,7-10]).

During the experiments, TMS was applied at individual sub-threshold intensity of 85% PT always targeting the same optimal stimulation site localized on average (±SEM) 3.24±0.1cm above the inion and 0.25±0.32cm to the left of the midline. The 85% sub-PT intensity was chosen, because it was previously shown to induce phosphene perception in approximately 50% of trials when single-pulse TMS is paired with brief auditory stimuli [4] and thus to be optimized for the prevention of both floor and ceiling effects. With mean PT across participants being 64.81% of maximum stimulator output (SEM=2.42), stimulation intensity amounted to 55.11% (SEM=2.28).

During all TMS blocks, participants were blindfolded. Participants were instructed to make a button-press with their right index finger whenever a phosphene was perceived and with the right middle finger whenever no phosphene was perceived. In addition, the blindfold mask was removed in between TMS blocks to prevent systematic drifts in PT [12] by adaptation to darkness or drowsiness [for more details regarding the procedures see 8,9].

Auditory stimuli consisted of pure tones with 0.4kHz carrier frequency, composed of triangular waveforms and generated in Cool Edit Pro Software (Syntrillium software Corp, www.syntrillium.com). Sounds rose exponentially in intensity from 55 to 75dB or fell exponentially from 75 to 55dB sound pressure level (SPL) at the ear (44,100kHz sampling rate) and changed 20dB in intensity from start to end irrespective of sound duration (250, 500 and 1000 ms). The slopes of the rising and falling sound amplitudes were exponential as exponential changes more closely approximate the changes in intensity that occur because of constant source velocity in natural environments than do linear changes [13]. The maximum sound pressure level (75 dB) measured at a distance of 75cm was well below those inducing a startle response as previously shown [4]. Sounds were presented through two loud speakers at 75cm distance from the participant and were thus perceived centrally. It is worth mentioning that studies in humans that have documented non-linear neural response interactions between

auditory and visual stimuli at ~50ms post-stimulus onset have used centrally-presented [14-16] or parafoveally-presented stimuli within 4° eccentricity [see Figure 1 in ref. 17]. Similarly, integrative effects on BOLD response slope and peak latency have been observed within the more caudal portions of primary visual cortex that include central visual field representations [see Figure 3 in ref. 18].

Sensitivity study with sound categories of different temporal profiles (Experiment 1): TMS probe of visual cortex excitability

The aim was to study the sensitivity of visual cortex excitability to looming relative to receding and stationary sounds. The sounds were of 250, 500 and 1000ms duration and either rose in intensity from 55 to 75dB (looming sound, L) or fell from 75 to 55dB (receding sound, R), and hence changed by 20dB from start to end irrespective of sound duration, or remained stationary at 55dB (low intensity stationary sound, S_L) or 75dB (high intensity stationary sound, S_H). TMS was applied at sound-offset, i.e. either at 250, 500 and 1000 ms. Sound conditions and trials without sound presentation (TMS alone) were randomly intermixed at a rate of 2 to 4 sec following the participant's manual response. Each participant completed 4 blocks of 78 trials, with each block lasting approximately 4min. There was a total of 312 trials resulting in 24 repetitions per condition (4x3+1, sound-profile x sound-duration + TMS alone=baseline).

Chronometry study (Experiment 2a): TMS probe of visual cortex excitability

The aim of this experiment, which included an exploratory pilot study, was to test when in time the different sound categories started to modulate visual cortex excitability differentially.

Exploratory study. As in Experiment 1, four sound categories (L, R, S_H, S_L) were presented but only for one sound duration (250ms). TMS (85% of PT) was applied at 4

different time points into sound evolution and 1 time point after sound-offset (25, 100, 175, 250, 325ms). We used this wide range with large TMS sample steps in order to approximate the temporal window where sounds modulate visual cortical excitability. TMS-pulse delays and trials without sound presentation (TMS alone) were randomly intermixed within blocks. Each participant completed 4 blocks of 126 trials, with each block lasting approximately 7min. There were a total of 504 trials resulting in 24 repetitions per stimulus condition (4x5+1, sound-profile x TMS-samples + TMS alone). All other experimental conditions were identical to Experiment 1. The exploratory data from four participants (Fig. 3a) suggest a sound profile-independent increase of visual cortex excitability at 100ms (relative to baseline), further rising to peak-levels at 250ms and dropping back towards baseline-values at 325ms, just 75ms after stimulus offset. Corroborating our results of Experiment 1, looming sounds showed greater modulation of visual cortical excitability than the remaining sounds, although all modulation-curves exhibited similar temporal profiles (Fig. 3a). Based on these exploratory data, we chose an interval of 70-230ms as the time-window of interest (with 10ms steps) for further evaluation in Experiment 2a.

Main experiment. Initial portions of the original 250ms (55-75dB) looming-sound (Experiment 1) were presented (clipped from 70ms to 230ms in steps of 10ms to cover a narrower time-window with smaller TMS sample steps). Additional stationary (control) sounds were created that matched the dynamic sounds in start- or end-amplitude and duration. Sound conditions and trials without sound presentation (TMS alone) were randomly intermixed and TMS was applied at the sound offsets, as in Experiment 1. There were 7 (to 8) blocks with 108 trials per block (each block lasting approximately 7min) giving rise to a total of 756 (to 864) trials, resulting in 14 (to 16) repetitions per stimulus condition (3x17+1, sound-profile x time-sample + TMS alone=baseline).

Psychophysical assessment (Experiment 2b): Sound discrimination task

Our aim was to assess the perceptual threshold for discriminating between looming and stationary sounds for comparison with the threshold at which visual cortex excitability starts to dissociate between the two sounds (L vs. S).

The clipped 250ms looming sounds of Experiment 2a (ranging from 50 to 230 ms in 10ms-steps and generated from the initial 55-75dB looming sound, Experiment 1) were represented. Additionally, we included initial portions of the 250ms 75-55dB receding sound (Experiment 1), and stationary sounds equated to the dynamic sounds' start amplitudes (S_L: 55dB, S_H: 75dB), all clipped in chunks of increasing length of 50 to 230ms (10ms steps). Sounds were presented in pairs of equal duration and equal start-intensity at an inter-stimulus interval of 1000ms using the following possible pairings: L-L, L-S_L, S_L-L, R-R, R-S_H, S_H-R (allowing the determination of the threshold for L- vs. S-sound discrimination, R vs. S discrimination not reported). Participants were asked to classify the sounds of one pair as same or different by button-press (right index finger for "same" and right middle finger for "different"). Each new pair of stimuli was delivered 2 sec after the participants' manual response.

Participants underwent 4 blocks with 152 (x2) trials per block (lasting approximately 8min) giving rise to a total of 608 (x2) trials with 8 repetitions per stimulus condition.

Sensitivity study with sound categories of different sound structure (Experiment 3): TMS probe of visual cortex excitability

With the aim of ruling out several confounds (see text), we presented white noise of 250ms duration which rose in intensity from 55 to 75dB (looming sound, L), or remained stationary at 55dB (low intensity stationary sound, S_L) or 75dB (high intensity stationary sound, S_H), in addition to the corresponding conditions of structured sounds. TMS was applied at sound-

offset, i.e. at 250ms. Sound conditions and trials without sound presentation (TMS alone) were randomly intermixed at a rate of every 2-4 sec following the participant's manual response. Each participant completed 3 blocks of 56 trials, with each block lasting approximately 5min. There was a total of 168 trials resulting in 24 repetitions per condition (3x2+1, sound-profile x sound-duration + TMS alone=baseline).

Data Analyses

For the sensitivity study (Experiment1), percentages of phosphene perception (across trials) were submitted to a repeated-measure ANOVA using temporal sound profile (L, R, S_H, S_L) and sound duration (250, 500 and 1000ms) as the within-subject factors. Planned paired t-tests were performed to examine differences between the baseline probe (TMS alone) and the effect of different temporal sound profiles (L, R, S_H, S_L) on phosphene perception. The same ANOVA was performed on reaction times of perceived phosphenes to compare across sound duration and sound profile (see Supplemental Results and Discussion above).

For the chronometry study (Experiment 2a), percentages of phosphene perception were submitted to a repeated-measure ANOVA using sound-duration/TMS-delay (baseline, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220 and 230ms) and sound profile (L, S) as the within-subject factors. S_H- and S_L-curves were averaged as not showing significant within-condition differences nor differential contrasts with the L- curve. Planned post-hoc comparisons included paired t-tests across conditions per sound duration/TMS-delay (to explore significant interaction of sound profile by sound duration/TMS-delay).

A relative difference curve (percentage change L- relative to S-probe) was calculated for each participant to quantify looming-sensitive changes in visual cortex excitability. The resulting curves were fitted with a sigmoid function $y=a/(1+exp(-1*(x-b)/ c))$ using nonlinear regression analysis and least-square estimation implemented in STATISTICA software

(StatSoft, Inc., version 8.0. www.statsoft.com). In the function, y represents the average percentage increase of cortical excitability induced by looming relative to stationary sounds , x is the sound offset where TMS was applied and a, b, and c are constants (a is the amplitude, b the centre, c the width of the curve).

For the psychophysics experiment (Experiment 2b), data of each participant were fitted using the same sigmoid function (and analysis) to allow the comparison between 'neurometric' and psychometric curve characteristics. Curve centres were then compared between both data sets using paired t-tests and correlation analysis (visual cortex modulation threshold vs. perceptual thresholds), including only participants in which the sigmoid function significantly fitted both 'neurometric' and psychometric data (2 out of 11 participants excluded due to no significant fit).

For the final sensitivity study (Experiment 3), percentages of phosphene perception (across trials) were submitted to a repeated-measure 2x2 ANOVA using Sound Structure (structured vs. white noise) and Sound Profile (L vs. S) as the within-subject factors. S_H- and S_L-curves were averaged as neither showed significant within-condition differences. Planned paired t-tests were performed to examine differences between the baseline probe (TMS alone) and the effect of different sound-profiles (L, L_{WN}, S, S_{WN}) on phosphene perception. The same ANOVA was performed on reaction times of perceived phosphenes to compare across the same conditions (see Supplemental Results and Discussion above).

Supplemental References

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