Perception and neuronal coding of subjective contours in the owl

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Robust form perception and underlying neuronal mechanisms require generalized representation of object boundaries, independent of how they are defined. One visual ability essential for form perception is reconstruction of contours absent from the retinal image. Here we show that barn owls perceive subjective contours defined by grating gaps and phase-shifted abutting gratings. Moreover, single-neuron recordings from visual forebrain (visual Wulst) of awake, behaving birds revealed a high proportion of neurons signaling such subjective contours, independent of local stimulus attributes. These data suggest that the visual Wulst is important in contour-based form perception and exhibits a functional complexity analogous to mammalian extrastriate cortex.

Contours represent an essential source for form perception. Many objects can be recognized by their silhouettes alone. In natural situations in which objects occlude one another, however, boundaries may vanish; thus, the visual system would fall short if it reflected only information directly present in retinal images. Higher-order contour perception requires active organization of visual information[1] to achieve a perceptually coherent representation of shape. The ability to perceive subjective (or illusory) contours that lack a physical counterpart[2,3] is one of the most striking illustrations that the visual system contains inferences about the world[4]—whether low-level[5,6] or cognitive[7,8]—well beyond available sensory information.

The first step in form analysis is to extract low-level features, such as orientation of contrast borders, at early stages of processing[9]. Primary cortical areas are ideally suited to perform highly localized analyses of the visual scene[10]. The primary visual forebrain structures of owls and mammals, the visual Wulst and the striate cortex, respectively, have been considered functionally equivalent[11–13]. Although these structures evolved independently[11,14,15], they share important physiological properties that indicate their role in precise feature detection. Both comprise neurons with small and retinotopically organized receptive fields, a high degree of binocular interaction and selectivity for orientation and movement direction[16]. To integrate local features into a global percept, however, long-range interactions are required[17]. It was suggested that long-range connections formed by pyramidal cells of the orderly layered cerebral cortex of mammals may give rise to high-order perceptual phenomena, such as the perception of illusory contours[18]. With both behavioral and physiological investigations, we tested whether such elaborate integration tasks could be performed by an avian vertebrate with an entirely different, 'nuclear' forebrain organization[19]. It was the aim of this study, first, to examine whether barn owls (*Tyto alba*) are able to perceive subjective contours and, second, to present a neural correlate for this visual ability.

RESULTS

Psychophysics

Two owls were trained in a two-alternative-choice discrimination task to distinguish between a square and a triangle defined by contrast gradients. The baseline stimuli consisted of a white outlined square and triangle of equal size presented in the center of a CRT-screen (Fig. 1a). The owls indicated whether they perceived the square or the triangle by pecking one of two keys. Once the owls performed the baseline discrimination reliably, transfer tests were begun. Squares and triangles defined by subjective contours were occasionally inserted among ongoing trials displaying baseline stimuli. Two types of subjective contours were applied: gratings with gaps that evoke in humans the percept of a figure occluding the grating (Fig. 1b) and phase-shifted abutting gratings (Fig. 1c). The bar graphs demonstrate that both owls significantly transferred baseline stimuli with real contrast borders to corresponding geometrical figures defined by gaps in gratings (Fig. 1b) and phase-shifted abutting gratings (Fig. 1c). It is important to note that the birds had no opportunity to learn the 'correct' response to the probe stimuli, as they were randomly rewarded to probe stimuli and never reinforced. We conclude that the owls indeed exploited contour cues that evoke the percept of subjective borders in humans to discriminate these two sets of probe stimuli.

Neurophysiology

The same subjective-contour stimuli used in the psychophysical study, phase-shifted abutting gratings or gaps between gratings, were subsequently applied in neurophysiological investigations with awake, behaving birds. Recordings were obtained from two different barn owls that were trained on a visual fixation task. During the task, single-neuron activity was recorded from hyperstriatum accessorium of the visual Wulst, which receives projections from the Wulst's granular layer, which, in turn, receives input from the thalamus. We used chronically implanted microelectrodes and transmitted neural signals via radiotelemetry[20] to give the birds maximal freedom during the task. A neuron's receptive field (RF), orientation and direction preference were determined by search stimuli. Size, orientation and motion direction of stimuli were adjusted to the cells' preferences. After the optimal stimulus configuration was found, a sequence of bars (defined by contrast gradients) and subjective-contour stimuli were presented alternately. Responses from a total of 43 neurons to one or several of the applied stimulus protocols were analyzed. For 32 cells, activity elicited by a moving white bar was compared to abutting gratings that were phase-shifted by

180°, 90° or 0° (Fig. 2a–d). In abutting-grating stimuli, there was no luminance difference along lines parallel to the subjective contour. Gratings with 0° phase shift (Fig. 2d) served as a control to ensure that the cell responded to the subjective contour orthogonal to the grating lines and not to other stimulus parameters, such as the orientation of the grating lines *per se*. Gratings without any shifts or gaps did not raise activity above spontaneous level ($p = 0.22$, Wilcoxon matched-pairs signed-ranks test, two-tailed). Compared to gratings without shifts or gaps, 29 of 32 cells (91%) increased activity significantly in response to subjective contours in both 180° and 90° phase-shifted abutting gratings (single-trial comparisons performed with a Mann-Whitney U-test, $p < 0.05$, two-tailed). Although not measured quantitatively, orientation tuning to bars and subjective-contour stimuli seemed to be comparable.

The responses to gaps in gratings (grating line spacing, 30 arcmin) were analyzed in seventeen cells (an example is shown in Fig. 2e–g). Because this stimulus had a luminance gradient across the putative subjective edge, it may not be regarded as a genuine subjective-contour stimulus. All single units tested responded to contours defined by gaps (10 arcmin wide) in gratings. Neurons responded well to black or white bars without any separable ON-OFF substructure, indicating that the neurons sampled contained only complex-like units. During receptive field mapping, a decrease of neuronal activity with increasing length of the bar stimulus was observed for several neurons, suggesting that these cell might be classified as 'end-stopped'.

On average, abutting gratings with a phase shift of half a cycle elicited 59% (Fig. 3) of the activity evoked by a solid bar ($p < 0.0001$, two-tailed Wilcoxon test). Interestingly, abutting gratings with a 90° phase shift that evoke a weaker contour percept in humans elicited a correspondingly weaker neural response (mean, 54%) than gratings shifted by 180° ($p = 0.03$, Wilcoxon test, two-tailed). The average response evoked by a gap in grating was only 76% compared to the situation when a bar was used as a stimulus, but the differences turned out not to be significant ($p = 0.055$, two-tailed Wilcoxon test).

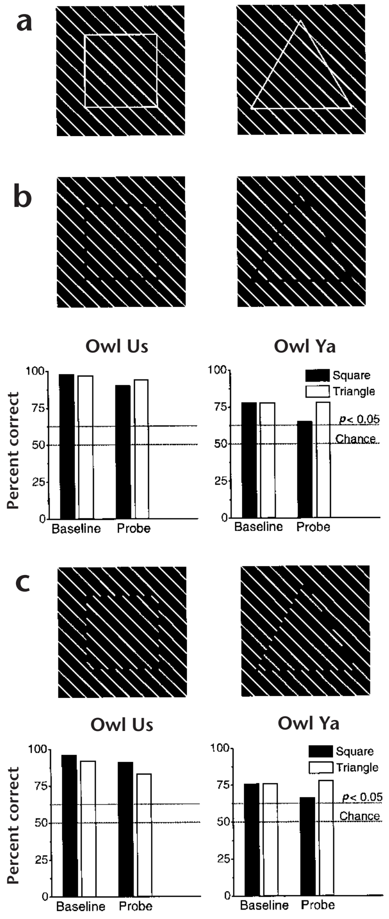


Fig. 1. Stimuli used in behavioral tests and corresponding psychophysical performance of two owls. **(a)** Baseline stimuli. The line width of the figures' outline was 1.5 arcmin to force the birds to inspect the stimuli precisely. **(b, c)** Transfer tests with geometrical figures defined by subjective contours. **(b)** Subjective contours were defined by 3 arcmin-gaps in the background grating that evoked the percept of a black figure occluding the grating (upper panel). **(c)** Probe stimuli generated by shifting abutting gratings by a half cycle. The bar graphs in **(b)** and **(c)** display the performance of two owls for the baseline stimuli ($n = 730$, left pair) and the subjective-contour figures ($n = 80$, right pair). Chance level (50%) and the confidence interval above chance ($p < 0.05$, binomial test, two-tailed, $n = 80$) are displayed. Both owls significantly transferred contrast-border figures to subjective-contour probes.

There has been some discussion about the representation of local and global image properties in neural responses of the primate visual system[21,22]. To test whether neural responses in the owl were sensitive to local features of the grating defining a subjective boundary, line density and line spacing of abutting gratings were varied (Fig. 4). On average, neurons responded equally well to abutting gratings of all four applied spacings of 0.25°, 0.5°, 1° and 2° (Fig. 5). This result, together with the application of a minimum grating line width of 1.5 and 3 arcmin in the present study, confirmed that the neurons responded invariantly to the subjective boundaries, not to local features of the inducing grating elements that defined the contours.

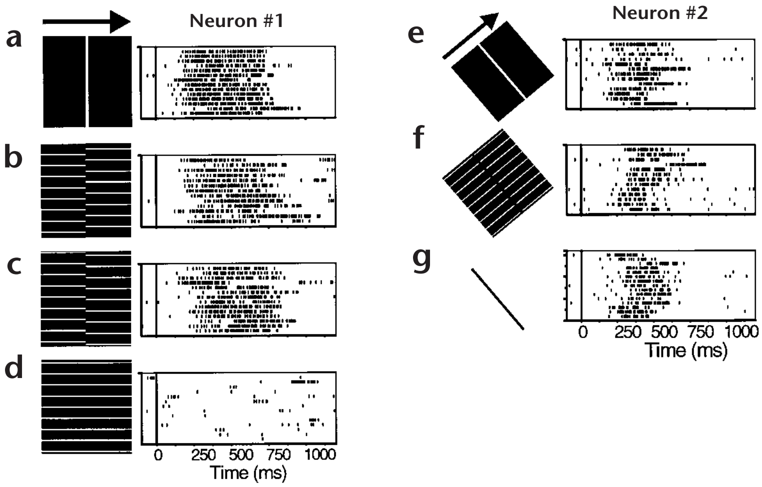
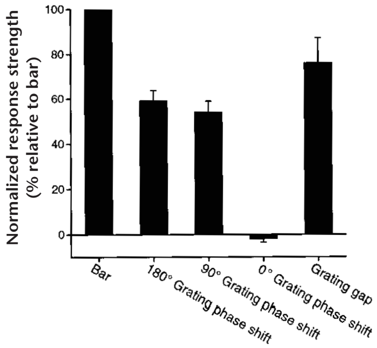


Fig. 2. Responses of visual forebrain neurons to stimuli defined by contrast borders or subjective contours. A stimulus sequence consisting of two or four different contours was repeated 15 times. The left column illustrates the contours; the right column shows corresponding dot-raster displays for several repetitions where black dots represent the occurrence of spikes. Arrows indicate motion direction of contours (motion onset at 0 ms). Gratings consisted of lines that were 1.5 arcmin wide. Neuron #1 **(a–d)** had a receptive field eccentricity of 12° and responded vigorously to a vertical bar **(a)** as well as to subjective contours defined by abutting gratings that were phase shifted by 180° **(b)** and 90° **(c)**. The neuron failed to respond to abutting gratings that were shifted by 0° **(d)**, where no contour orthogonal to the grating pattern was visible although the grating physically moved on the monitor screen. Neuron #2 (9° RF eccentricity) responded well to moving black **(e)** and white bars **(g)** and to a gap between gratings (10 arcmin wide; **f**).

Fig. 3. Normalized mean responses (± s.e.) of all units to abutting gratings and gratings with gaps relative to the activity elicited by a white bar on black background (set to 100%).



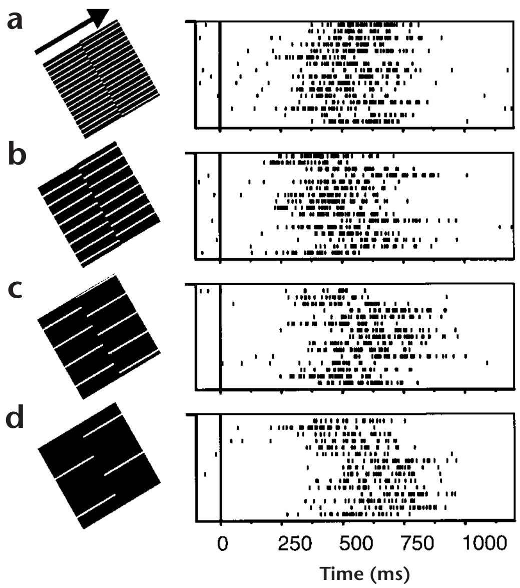


Fig. 4. Responses to subjective contours in abutting gratings of different line density and spacing. The response of a typical neuron to repeated sweeps of a subjective contour across the RF (preferred contour orientation, 40°). Grating lines were spaced by 0.25° **(a)**, 0.5° **(b)**, 1° **(c)** and 2° **(d)**. The corresponding dot-raster histograms illustrate that responses of this unit were independent of the grating line density. RF eccentricity was 10°.

DISCUSSION

The data presented in this study demonstrate that barn owls perceive subjective contours and interpret them as 'real' borders. Single-neuron recordings from the visual Wulst of behaving owls revealed a substantial proportion of neurons that coded subjective contours independent of edge-inducing elements. This suggests that an orderly layered cortical architecture is not a prerequisite for neuronal long-range integration and the resulting high-order perceptual phenomena.

Psychophysics

If the detection of edges in the absence of contrast gradients reflects fundamental visual constraints and not just an artifact of visual processing, one would expect subjective-contour perception or related capabilities in various species that rely heavily on vision[23–25]. The large, frontally oriented eyes of barn owls and their prey-catching skills suggest elaborate binocular vision[26]. We demonstrated in behavioral tests that owls can indeed exploit contours that humans perceive as 'subjective'. Even more important, although owls were not trained to discriminate abstract figures defined by subjective contours, they interpreted subjective contours as 'real' borders to perform the discrimination task in transfer tests. The ability to perceive subjective contours may, therefore, provide an 'anti-camouflage device'[4], evolved primarily to detect occluded objects.

Neurophysiology

The hyperstriatum accessorium of the owl's visual Wulst seems to be specifically adapted to tasks requiring long-range computations[17]. Both the proportion of neurons sensitive to subjective contours as well as the response characteristics support this notion. We found that 91% of the cells responded significantly to subjective contours defined by phase-shifted abutting gratings. This is a very high proportion compared to mammalian visual cortex. In the cat, 42% of V1 neurons and 60% of V2 units conveyed information about orientation of subjective contours defined by abutting gratings[27]. In primates, equivalent types of subjective borders are first represented in area V2 of extrastriate cortex[28,29], whereas neurons in the primary visual cortex of monkeys are still sensitive to local contrast attributes, such as the edge of a grating line tip[21,22]. The 59% average strength of responses to abutting gratings in the owl visual Wulst mirrors the 60% found in V2 of the monkey[22].

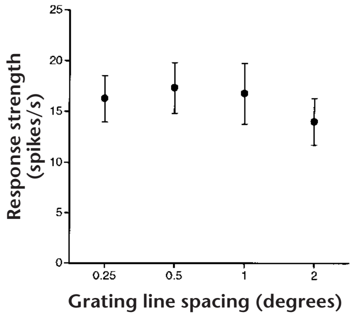
Response invariance to abutting gratings of different line spacing is probably one of the most remarkable features of Wulst neurons (Fig. 4) and represents the strongest evidence for global contour coding. Similar effects have been reported only for V2 units in the awake monkey[29], whereas changing inducing line density decreased responses of V2 neurons to subjective gratings in the anesthetized cat[27].

From a functional point of view, the hyperstiatum accessorium is analogous to extrastriate cortical areas rather than to striate cortex of mammals. Because binocular vision has evolved independently in mammals and birds[11,14], forebrain organization may have adopted different strategies to cope with environmental constraints. The neurophysiological results derived from relatively early processing levels in the owl present further evidence that many aspects of subjective-contour coding deal with low-level processes[5,6], although top-down modulations might have been active in these awake birds[30]. Low-level aspects in contour perception are also emphasized by computational studies[31]. Future work will be needed to establish a direct relationship between neuronal responses to subjective borders and subjective-contour perception[32].

METHODS

Psychophysics. Tame owls from the institute's breeding stock rested on a perch 57 cm from a CRT-screen located inside a sound-attenuated and darkened booth. Behavioral performance was controlled and monitored by custom-written software running on a Silicon Graphics workstation that also delivered the visual stimuli. Whenever the owl oriented its gaze toward the

Fig. 5. Mean responses of a total of 13 neurons were unaffected by variable density or spacing of the grating lines. Neurons responded equally well to all four applied spacings of 0.25°, 0.5°, 1° and 2° (p = 0.21, Friedman two-way ANOVA, two-tailed, n = 13).



screen, a trial was automatically initiated, and a fixation target was displayed (see detailed description below). After a variable waiting period, a stimulus was presented, upon which the bird responded by pecking one of two keys. Rewards (chicken meat) were delivered by an automated feeder.

Baseline stimuli consisted of a white outlined square and triangle presented on a black monitor background displaying a line grating with lines (1.5 arcmin wide) oriented at 45° relative to the vertical. Background grating lines were spaced 0.25° apart. This background later served to construct subjective contours. Baseline stimuli were presented pseudo-randomized in blocks of twenty trials with a probability of $p = 0.5$ for both triangle and square. Errors were followed by correction trials. Once performance reached 75% correct responses, rewarding was gradually reduced to 85% to habituate the birds to occasional absence of reward following correct responses.

Probe-trial probability in transfer tests was set to $p = 0.10$, that is, one square and one triangle defined by subjective contours appeared in each block of twenty trials. Subjective figures were generated by either introducing a gap (3 arcmin) to the background grating or by shifting abutting gratings by half a cycle. Responses to subjective-contour figures were randomly rewarded at $p = 0.5$, independent of the owl's response. For probe stimuli, no correction trials were applied. Performance on probe trials was determined by a binomial test (two-tailed) based on 80 observations for each probe figure averaged over successive daily sessions.

Neurophysiology. The fixation target consisted of two small parallel lines (0.8° long, 0.1° wide, separated by 0.2°) that appeared whenever the bird oriented its gaze to the screen. After a variable time delay (2–5 s), the fixation target turned 90° for 400 ms, upon which the bird had to peck a key to get a reward. Gaze orientation was detected automatically by means of an infrared reflex photoelectric device in combination with a light-reflective foil attached to the top of the bird's head. A trial was interrupted whenever the birds made head movements larger than ± 1.5°. During training, owls learned to avoid head movements while fixating. Fixation was additionally controlled by observing the gaze and eyes under infrared illumination at high magnification on a television monitor. Eye movements were not measured, as they are virtually absent in owls[33].

For electrode implantation, owls were given diazepam (1 mg per kg) for sedation and were anesthetized with ketamine (15 mg per kg per h). After exposing the dura, three to four microdrives supplied with one or two microelectrodes were fixed to the skull with dental cement, and the wound was sutured closed and treated with antibiotic ointment. Electrodes were moved up and down by manually turning microdrives. Tungsten microelectrodes (10 MΩ, F. Hear) were used to record from the hyperstriatum accessorium, representing the binocular visual field adjacent to the area centralis implanted according to stereotactic coordinates[12].

A custom-built miniature two-channel FM-stereo radiotransmitter attached to the skull transmitted neuronal activity. The transmitter output was received by a dipole antenna and fed to an FM tuner, which demodulated the signal. After filtering and amplification, the signals' waveforms were digitized at a sampling rate of 32 kHz and stored to disk using a PC-based recording system (Datawave Discovery). Preliminary cluster cutting was performed on-line to estimate the response characteristics during the experiment. Single-unit isolation was repeated off-line. Care and treatment of the owls were in accordance with the guidelines for animal experimentation as approved by the Regierungspräsidium Köln, Germany.

Stimulation and data analysis. The color monitor (ELSA 17H96, 16 inch) had a spatial resolution of 1280 × 1024 pixels and was refreshed at a frame rate of 76 Hz. Orientation and motion-direction preference of a cell was determined by on-line inspection of the responses elicited by a moving bar that could be rotated in 10° steps. All contour stimuli were swept across the RF for 1 s at a constant velocity of 7° per s. The width of the solid bar stimulus was 10 arcmin; gratings consisted of lines that were 3 or 1.5 arcmin wide, respectively. Spikes were counted during stimulus presentation in a one-second time window. Spontaneous activity was determined from 100-ms intervals preceding motion onset.

ACKNOWLEDGEMENTS

We are grateful to R. v.d.Willigen for introduction to graphics programming. B. Gaese provided a clocked-sequence program for data collection. We thank B. Gaese and H. Luksch for suggestions on an earlier version of the manuscript and M. Schäfer for help with data analysis. Supported by a grant from the DFG to H.W. (WA 606/6).

RECEIVED 12 APRIL; ACCEPTED 17 MAY 1999

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