

tunic. The large funnel-like structure filled with a layer of sediment on the top is interpreted as the oral siphon, as is found in all solitary ascidians. To the lower right of the main body, presumably representing the original dorsal side, a narrow but distinct extension is interpreted as the excurrent (or cloacal) siphon, and is consistent with a rather marked change in level in this area. Lower and outside to the cloacal siphon there is an arcuate area, where there is a recognizable recurvate dark ridge. This structure is difficult to interpret, but when compared to the last stage of metamorphosis of living ascidians²⁵, it could represent the remnant of a larval tail.

A prominent rectangular dark area, covered with numerous lighter dots, is located in the upper part of the body cavity. The lower ventral corner of the dark area extends downward as a 'tail-like' structure. The dark area, compared to modern ascidians, with its dextral configuration, large size and appropriate location in the body, is consistent with its identification as the pharynx. Whether the lighter dots represent gill openings remains to be confirmed.

Two interesting structures are located above and below the oral siphon. The area between the siphon and the pharynx is preserved in dark grey. Observation shows a longitudinal alignment of structures suggestive of buccal tentacles. Above the oral siphon, another set of short tentacle-like filaments is recognizable. They are superficially similar to siphonal fringe or oral lobes of some extant ascidians, but are also reminiscent of the tentacles of extant lophophorate phoronids and the Lower Cambrian lophophorate *Cambrotentacus*⁴. We suggest that this Cambrian tunicate was a suspension feeder, with water entering the oral siphon and being expelled through the cloacal siphon after filtration.

The main body and upper two-thirds of the supporting stem lie laterally on the same bedding plane, but the lower third of the stem is bent steeply into the sediment and attached to a free cheek of a trilobite. This arrangement could indicate burial of the tunicate *in situ*. The presence of agglutinated quartz grains, substantially coarser than the surrounding matrix, on the lower stem suggests, however, that the animal inhabited a higher-energy, sandy sea floor and was transported to its point of burial. During deposition the heavier trilobite sclerite sank first, so tethering the tunicate in the rapidly accumulating sediment. The three-dimensional preservation and remains of the delicate tentacles are indicative of its suffering little decomposition.

Urochordata are believed to represent the most basal chordate branch within Chordata^{11,26}; however, whether the ancestral chordates were free-swimming or sessile has been a long-standing question^{18,26,27}. Traditional hypotheses hold that vertebrates evolved by pedomorphosis from a urochordate-like larval stage, and that the ancestor of chordates would have resembled a sessile lophophorate^{12,13,27}. Recent models, supported by molecular data, posit a free-swimming ancestry of chordates, including urochordates^{28–30}.

Fossils may preserve combinations of characters not seen in extant groups, and so are crucial for testing schemes of how characters were acquired in the origin of new body plans. The interpretation of the present specimen, as possessing oral tentacles comparable to those seen in lophophorates, is consistent with traditional views—if not modern, molecule-based hypotheses—but a single example is far from being conclusive. Further palaeontological and molecular work is needed to investigate the problem. □

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Orientation-selective adaptation and tilt after-effect from invisible patterns

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Exposure to visual patterns of high contrast (for example, gratings formed by alternating white and black bars) creates after-effects in perception. We become temporarily insensitive to faint test patterns that resemble the pre-exposed pattern (such as gratings of the same orientation), and we require more contrast to detect them. Moreover, if the test pattern is slightly tilted relative to the pre-exposed one, this tilt may be perceptually exaggerated: we experience a tilt after-effect^{2,3}. Here we show that these visual after-effects occur even if the pre-exposed grating is too fine to be perceptually resolved. After looking at a very fine grating, so high

in spatial frequency that it was perceptually indistinguishable from a uniform field, observers required more contrast to detect a test grating presented at the same orientation than one presented at the orthogonal orientation. They also experienced a tilt after-effect that depended on the relation of the test pattern's tilt to the unresolvable orientation of the pre-exposed pattern. Because these after-effects are due to changes in orientation-sensitive mechanisms in visual cortex⁴⁻⁶, our observations imply that extremely fine details, even those too fine to be seen, can penetrate the visual system as far as the cortex, where they are represented neurally without conscious awareness.

Vision is not perfect. When grating patterns are used as resolution targets, the highest spatial frequency resolvable by humans is about 50 cycles per degree of angle subtended at the observer's eye⁷. The higher spatial frequencies are lost partly because of diffraction and other sources of optical blur. These optical losses can be bypassed if the targets are presented as interference fringe patterns generated directly on the retina, but when this is done the resolution limit is only slightly increased⁷⁻¹⁰. It follows that there are neural, as well as optical, limitations on visual resolving power; but the roles of the retina and the brain in imposing these limitations have not been worked out. Here we report observations suggesting that perceptually unresolved patterns can penetrate to the visual cortex, and selectively modify visual sensitivity and pattern perception there. Our failure to perceive these patterns consciously is therefore partly

caused by limitations in cortical processing.

Orientation information is first analysed at the primary visual cortex. Neurons in the primary visual cortex respond selectively to their preferred orientation⁶. Moreover, prolonged exposure of such neurons to a grating in one orientation will selectively reduce their population sensitivity to gratings in the same orientation^{4,5,11}. This property of the cortical neurons made it possible for us to test psychophysically whether a perceptually unresolvable grating could still be represented in visual cortex. The logic is simple: if an unresolvable grating can produce an orientation-selective perceptual after-effect, then its spatial structure, including its orientation, is registered at least at the earliest stages of cortical processing.

Using a He-Ne laser interferometer, subjects in our first experiment viewed a horizontal or vertical adapting grating at full contrast for one minute. Next, their ability to detect horizontal or vertical test gratings of various contrasts was assessed (Fig. 1a). Every 6 s, the continued presentation of the adapting grating was interrupted by a 1-s trial in which either a horizontal or a vertical test grating was presented, the orientation being randomly varied from trial to trial. During each trial, the test grating was presented during either the first or (randomly) the second of two successive 250-ms intervals demarcated by audible clicks. After each trial, the observer had to indicate whether it was the first or the second interval that had contained the test grating. The 500-ms pair of test intervals were preceded and followed by a 250-ms period during which the field

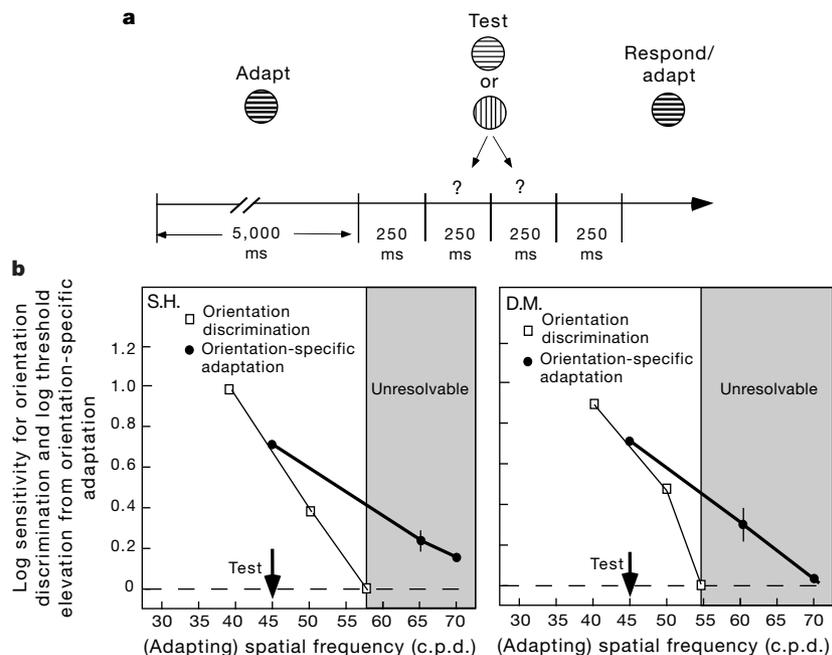


Figure 1 Orientation-selective adaptation experiment. **a**, Experimental procedure. After viewing a horizontal or vertical adapting grating at full contrast for 1 min, observers' sensitivity to briefly presented testing gratings were measured in a 2AFC staircase procedure. The procedure searched for threshold contrasts for a 45-c.p.d. horizontal and vertical testing grating in two independent but interleaved staircases. Both adapting and testing gratings were 3° in diameter with a mean luminance of 1,000 trolands. Shown is the sequence of one 6-s trial, during which the test grating was presented during either the first or (randomly) the second of two successive 250-ms intervals demarcated by audible clicks. The test grating could be either horizontal or vertical—the orientation was varied randomly from trial to trial. After each trial, the observer had to indicate whether it was the first or the second interval that had contained the test grating. The 500-ms pair of test intervals were preceded and followed by a 250-ms period in which the field was uniform; during the remaining 5 s of the cycle time, the field was again occupied by the adapting grating. **b**, Comparison of the effect of orientation-specific adaptation with the sensitivity of orientation discrimination, both plotted as functions of spatial frequency. Open squares plot the sensitivities of the two observers discriminating between two

grating orientations. For both observers, this sensitivity drops rapidly as the spatial frequency approaches 50 c.p.d.; S.H. could not discern the orientation of gratings beyond 57 c.p.d.; D.M. could not discern that beyond 55 c.p.d. In comparison, the orientation-specific adaptation effect decreases more slowly as the spatial frequency increases, still measurable beyond the point at which the grating is no longer resolved. For both subjects, adaptation to a grating had different effects on test gratings of horizontal and vertical orientations, depending on the orientation of the adapting grating. The threshold contrast for the test gratings of the same orientation as the adapting grating was elevated relative to that for perpendicular test gratings. The magnitude of the orientation-selective adaptation after-effect was expressed as the difference in relative sensitivity between the two test orientations when horizontal and vertical adaptation were compared. Filled circles are the logarithmic difference between the relative sensitivities for horizontal and for vertical pre-adaptation. Thresholds were elevated for the same adapt/test orientations, and reduced for the orthogonal adapt/test orientation. This is true even when the adapting frequency far exceeds the conventional resolution limit (shaded areas). Error bars, ± 1 s.e.

was uniform; during the remaining 5 s of the cycle time, the field was again occupied by the adapting grating. Space average intensity remained constant throughout at 1,000 trolands.

The test grating was always 45 cycles per degree (c.p.d.), a spatial frequency just below the resolution limit. The spatial frequency of the adapting grating was varied in different runs from the visible to the invisible range; at adapting frequencies higher than about 55 c.p.d., the subjects were unable to tell the orientation of the adapting grating in a forced-choice discrimination task (Fig. 1b).

For both subjects tested (S.H. and D.M.), pre-adaptation to a grating had different effects on test gratings of horizontal and vertical orientations. The threshold contrast for the test gratings of the same orientation as the adapting grating was elevated relative to that for perpendicular test gratings. Figure 1b shows the magnitude of the orientation-selective adaptation after-effect, expressed as the difference in relative sensitivity between the two test orientations when horizontal and vertical adaptation are compared. The figure plots the logarithmic difference between the relative sensitivities for horizontal and for vertical pre-adaptation.

Orientation-selective adaptation using resolvable gratings has been shown long ago¹, but it is surprising that gratings at and even beyond the resolution limit can still produce orientation-selective adaptation effects: conscious perception is unresponsive or poorly responsive to such spatial frequencies, with visual sensitivity declining as the fifth to sixth power of spatial frequency in this range¹⁰. But our 45-c.p.d. interference fringe patterns produced effects comparable in magnitude to those documented for easily visible spatial frequencies; and even invisibly fine adapting frequencies (60–70 c.p.d.) produced measurable effects. Unexpectedly, in comparison with pre-adaptation to uniform fields, pre-adaptation to unresolvably fine patterns actually improved contrast sensitivity for test gratings very near the limit of resolution; this effect, not yet well understood, was not orientation selective and is factored out in Fig. 1b. A more detailed description of the results can be found in Supplementary Information.

These results imply that gratings too fine to be resolved can nevertheless selectively activate orientation-selective functional units (neurons or synapses) in the cortex. Orientation information must be registered at least at the cortical stage of processing where orientation-specific adaptation arises; and it must then be lost to perception owing to cortical limitations on spatial resolution.

As a further test of this idea, we asked whether a tilt after-effect is experienced after adaptation to unresolvable gratings. It is generally thought that perception of local orientation is based on the distribution of activity among many orientation-selective neurons in visual cortex, and that adaptation at a particular orientation selectively depresses the sensitivity of neurons sensitive to that orientation. When these neurons are subsequently stimulated with a slightly different orientation than the adapting one, the activity distribution is shifted away from the adapting orientation; as a result, the test grating appears to be tilted further away from the adapting orientation—the tilt after-effect¹². In this view, the tilt after-effect implies that the functional units whose sensitivity is modulated by adaptation are not only orientation selective but are actually used for representing orientation in perceptual experience¹³.

To determine whether unresolvable gratings can activate these orientation-selective and orientation-signalling cortical units, subjects were asked to set a briefly presented 48 c.p.d. grating to a subjectively horizontal orientation after adapting to a full-contrast grating with a spatial frequency at least 12% higher than the resolution limit (66 c.p.d. for S.H., and 60 c.p.d. for D.M.). The adapting grating was tilted away from horizontal by 15° clockwise or anti-clockwise (see Fig. 2a for the procedure). With visible gratings, the perceived orientation of a test grating close in orientation to the adapting orientation is pushed away from the adapting orientation.

If an unresolvable grating similarly induces a tilt after-effect, for the test grating to be perceived as horizontal, it will need to be slightly tilted clockwise after adapting to a 15° clockwise-tilted grating, and slightly anti-clockwise after adapting to an anti-clockwise grating. This is exactly what we observed. Figure 2b shows the mean tilt after-effect (offset from horizontal) after adapting to an unresolvable grating. The magnitude of the tilt after-effect is comparable to what has been observed with a high-contrast resolvable grating³. This effect was maintained at an adapting frequency of 66 c.p.d., which yielded a tilt after-effect of $1.2 \pm 0.28^\circ$ (mean \pm s.e.m.) in each direction. In these experiments, the two pre-adapting tilts were presented in randomly interleaved blocks in each run, so that the subject had no knowledge of the adapting tilt when the adapting stimulus was unresolvable.

Our results indicate that gratings of high spatial frequency may be represented in the cortex (and generate after-effects) even when not consciously perceived¹⁴. The cortex could limit perceptual resolution either by blocking high-spatial-frequency signals after the stage at which the after-effects are generated, or by blocking weak signals of any spatial frequency. To decide between these possibilities, we tried a low-spatial-frequency adapting grating equated in contrast with the unresolvable one relative to their respective thresholds. Near the resolution limit, the threshold contrast was found to vary at least as the fifth power of frequency¹⁰. On that basis, the unresolvable adapting gratings had roughly half the (extrapolated) threshold contrast. For comparison, we chose an adapting grating of 36 c.p.d., placed roughly symmetrically with the unresolvable one

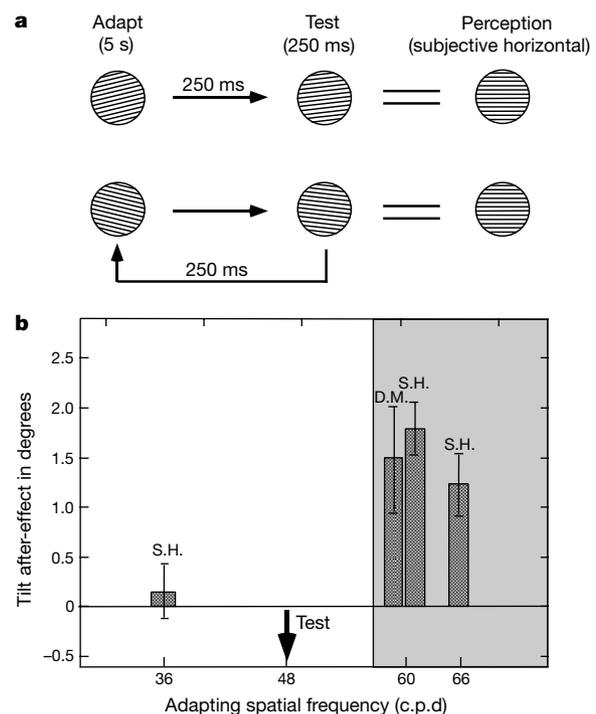


Figure 2 Tilt after-effect experiment. **a**, Experimental procedure. The observers' task was to set the test grating to the perceptual horizontal after adapting to a grating that was tilted either clockwise or anti-clockwise 15° from horizontal. With a resolvable adapting grating, observers set the test grating slightly clockwise when the adapting grating was clockwise, and anti-clockwise when the adapting was anti-clockwise. Here, unresolvable gratings also produced a tilt after-effect. See also Supplementary Information. **b**, Settings for perceptual horizontal after adapting to slightly tilted unresolvable gratings (in the shaded frequency range), or to a subthreshold resolvable grating equated for detectability as a control. Observers' settings for horizontal were consistently tilted towards the adapting orientation, even though the adapting grating was invisible, and observers had no idea what its orientation was. For the control condition at 36 c.p.d., observers set the horizontal veridically. Error bars, ± 1 s.e.

about the test grating (48 c.p.d.) on the frequency scale. The contrast of the 36 c.p.d. adapting grating was set to half of its threshold contrast, 3.5%. No measurable tilt after-effect was found under this condition (Fig. 2b).

Similarly, in measurements of the elevation in contrast threshold produced by pre-exposure to a range of adapting grating contrasts, at three spatial frequencies spanning the resolution limit, we found that adapting gratings of frequency greater than the resolution limit were more effective than correspondingly subthreshold gratings of lower frequency (although subthreshold gratings in the high but resolvable frequency range were not entirely ineffective). Thus, the cortical requirement for conscious perception seems to depend on spatial frequency, and not only on contrast or signal strength represented at the cortical input.

The idea that limits on visual resolution are partly imposed at the cortical level is supported by evidence that cortically projecting thalamic relay neurons in macaque often respond well to spatial frequencies far above the human resolution limit, in some cases exceeding 100 c.p.d. (ref. 15). If the projection from thalamus to cortex were as precise as the one from retina to thalamus, this would allow the visual system to form a representation of unresolvable patterns at the cortical site of pattern adaptation. The lower spatial-frequency limits for cortical after-effects (70 c.p.d.), as compared with thalamic neurons (100 c.p.d.), may reflect imperfect precision in the projection from thalamus to cortex¹⁶.

In normal vision with incoherent light, diffraction markedly reduces the retinal image contrast for spatial frequencies near the resolution limit. Why should the cortex have orientation-selective mechanisms (or frequency-selective ones¹⁷) that respond to high spatial frequencies that are normally only faintly represented in the retinal image? One answer derives from the view¹⁸ that neural mechanisms might compensate for optical blur: frequency components that have been optically attenuated might even require a reciprocal enhancement of neural sensitivity for their appropriate representation.

But on our evidence, activation of orientation-selective units at the stage of cortical pattern adaptation is not sufficient for perceptual awareness of the pattern orientation. The nature of the added requirement is not clear: one possibility¹⁹ is that information must be relayed from primary visual cortex to another region of the brain to be represented in conscious experience. □

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Disruption of neurotransmission in *Drosophila* mushroom body blocks retrieval but not acquisition of memory

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Surgical, pharmacological and genetic lesion studies have revealed distinct anatomical sites involved with different forms of learning. Studies of patients with localized brain damage and work in rodent model systems, for example, have shown that the hippocampal formation participates in acquisition of declarative tasks but is not the site of their long-term storage^{1,2}. Such lesions are usually irreversible, however, which has limited their use for dissecting the temporal processes of acquisition, storage and retrieval of memories^{3,4}. Studies in bees and flies have similarly revealed a distinct anatomical region of the insect brain, the mushroom body, that is involved specifically in olfactory associative learning^{5,6}. We have used a temperature-sensitive *dynamitin* transgene, which disrupts synaptic transmission reversibly and on the time-scale of minutes⁷, to investigate the temporal requirements for ongoing neural activity during memory formation. Here we show that synaptic transmission from mushroom body neurons is required during memory retrieval but not during acquisition or storage. We propose that the hebbian processes underlying olfactory associative learning reside in mushroom body dendrites or upstream of the mushroom body and that the resulting alterations in synaptic strength modulate mushroom body output during memory retrieval.

The mushroom body is a central control neuropil that receives multimodal input^{8–10}. In *Drosophila*, one hemisphere of the mushroom body consists of around 2,500 kenyon cells, whose primary afferents convey olfactory input through the antennal-glomerular tract (AGT; Fig. 1). The AGT projects from the antennal lobe, which itself receives olfactory input from the antennae. Mushroom body efferents project to other neuropil regions that are ultimately involved in motor output. Mushroom body neurons are believed to integrate multimodal information, including olfactory stimuli, and to modulate behavioural responses through motor output. Consistent with this anatomical view of the mushroom body, genetic and pharmacological disruptions of mushroom body