spatial variation in shark densities? Robbins *et al.* [10] provide two arguments for why it is unlikely that these patterns are due to asymmetric rates of movement among the various reef zones. First, the whitetip reef shark has relatively high site fidelity, typically moving less than 3 kilometres. Second, 'spillover' theory would predict higher emigration from high shark densities toward less densely populated reefs [12]. The authors note, however, that this would require invoking a less likely, reverse spillover mechanism to generate the low densities observed in the poached and fished zones.

The second question is whether shark populations on the fished reefs are coincidentally at naturally low stable densities, or whether the low densities are a result of population declines? Robbins *et al.* [10] estimated the population trajectories of both species using demographic models parameterised with the age structures and fertilities of sharks throughout the study area. Most model runs yielded negative population growth and the median annual decline rates were steep — 7% and 17% for whitetip and grey reef sharks, respectively. Such decline rates are sufficient to reduce whitetip and grey reef shark populations to 5 and 0.1% of 'virgin' abundance within 20 years.

A third question is whether no-entry zones are a suitable 'control', and are likely to represent the densities of unfished shark populations? Robbins *et al.* [10] found that shark densities in the Great Barrier Reef no-entry zones are similar to the densities found at the remote reefs of Cocos (Keeling) Islands, which lie halfway between Sri Lanka and Australia in the Indian Ocean. There is no record of commercial shark fishing and negligible shark angling at these islands.

Marine protected areas have been hailed as the silver bullet to solve the woes of declining marine biodiversity. Now, it is increasingly important to critically scrutinise the specific benefits (and costs) of marine protected areas as well as other conservation and management tools [13,14], Robbins *et al.* [10] provide persuasive evidence for ongoing and potentially threatening declines of two, charismatic reef shark species. As well as identifying a conservation problem, they also identify a potential conservation solution — strictly protected, large spatial closures may benefit reef sharks. But these no-entry zones comprise only 1% of the Great Barrier Reef area. This raises questions of whether this is sufficient habitat to ensure the long-term maintenance of viable shark populations on the Great Barrier Reef and secondly, whether the poached 'no take' zones are fit for their intended purpose? Finally, it is worth considering whether the aims of the Great Barrier Reef marine park might be better served by substantially cutting overall fishing effort on larger scales than hitherto considered.

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Ecosystem Interactions, Centre for Environment, Fisheries and Aquaculture Sciences (Cefas), Lowestoft, Suffolk NR33 OHT, UK.
E-mail: nick.dulvy@cefas.co.uk

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Cerebral Cortex: The Singular Precision of Visual Cortex Maps

A remarkable new technique, two-photon confocal fluorescence microscopy, has revealed an extraordinarily precise organization in the visual cortex. The methodology seems set to become the tool of choice for studying cortical maps.

Nicholas V. Swindale

No-one is completely certain why, but maps — spatially ordered representations of functional response properties — are a nearly ubiquitous feature of the organization of cerebral cortex. For example, motor and somatosensory cortices contain 'homunculi' in which nearby muscles or sensory receptors in

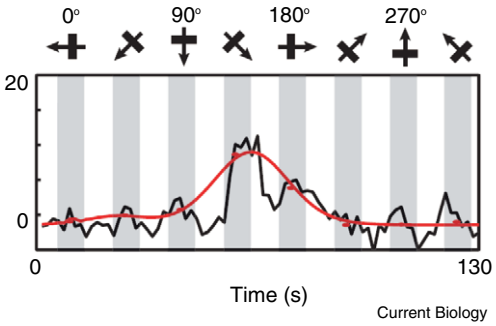

Figure 1. Example of an orientation tuning curve measured with two-photon calcium imaging from a single cell body at a depth of 230 μm below the cortical surface.

The vertical axis indicates percentage change in the fluorescence signal. Grey areas indicate 8 second periods of visual stimulation with a square wave grating moving at the orientations and directions indicated at the top. The visual display was blank for 8 second periods between epochs of stimulation. The black line shows the average response to 10 stimulus repetitions. The red line is a smoothed tuning function fit to the peak responses. From this, the cell's preferred orientation, maximal response and tuning width can be determined. (Reproduced with permission from [4].)

the skin make connections with correspondingly close regions of cortex; auditory cortex contains a frequency map consisting of neighbouring bands of neurons which respond to neighbouring sound frequencies; and the visual cortex of higher mammals contains maps of retinotopic position (visual space) and stimulus orientation, among others. In addition to local continuity in the representation of response features, a common feature of all the maps is the tendency for cells having the same tangential position in the cortical sheet, but positioned at different depths below the surface, to have similar response properties. This property, known as columnar organization, means that cortical maps can, for the most part, be considered to be two-dimensional entities.

How precisely organized are these maps? To what extent do the responses of individual neurons conform to the overall layout? This issue is important because precision in organization at a cellular level would suggest that maps have a correspondingly important function. Characterizing map structures accurately might suggest what that function is, and also help to constrain theories of cortical development.

Until recently, little was known about the organization of maps on a cellular scale. The pioneering studies of Mountcastle [1] and Hubel and Wiesel [2] used extracellular microelectrode recording, which has the limitation of being able to record the responses of only very small numbers of neurons at a few locations. Optical imaging, where responses are measured with a camera focussed just below the surface of the cortex, can record responses over relatively large areas of cortex (2–5 mm is typical), but has a spatial resolution of only about 0.1 mm and cannot determine the responses of individual neurons. Functional magnetic resonance imaging has been used to study maps in a variety of species, but it has an even more limited spatial resolution than optical imaging and similarly cannot determine the responses of individual cells. However, a stunning new technique, two-photon confocal fluorescence imaging, introduced by Ohki *et al.* [3,4], combines the complementary advantages of microelectrode recording and optical recording and seems set to become the tool of choice for studying cortical maps for the forseeable future.

The way two-photon imaging works is fundamentally simple. A beam of low-wavelength laser light is brought to a focus at a point in the tissue being imaged. If the beam is intense enough, a fluorescent molecule can be excited exclusively by the near-simultaneous arrival of two photons. The excitation is a very non-linear function of laser intensity and the strength of the fluorescent signal falls off rapidly with distance from the focal point. Thus, almost all the light emitted by the fluorophore comes from the single focal point of the laser. A two-dimensional image can be obtained by scanning the focussed laser across the tissue at a single depth and measuring the intensity of the fluorescent signal at each point. This image has a very narrow depth of focus and is relatively little affected by light scattered from layers above and below the focal plane. Because cortex is relatively transparent to low wavelength light it is possible to obtain detailed cellular resolution (in the micron range) at depths of several hundred microns into the tissue. The responses of individual neurons can be measured by injecting a calcium sensitive dye. This dye gets taken up by cells and emits a fluorescent signal when intracellular calcium levels rise as a result of cells firing action potentials.

Ohki *et al.* [4] used the technique to measure the orientation tuning curves (Figure 1) of thousands of neurons within structures known as pinwheels in the visual cortex (area 18) of the cat. Pinwheels are of particular interest because they are regions where large numbers of cells with different preferred stimulus orientations come together at a point, known technically as a singularity. This results in a situation where nearby cells can have very different orientation preferences (Figure 2). Although this would seem to conflict with the principle of smoothness in cortical mapping, it can be shown, mathematically and

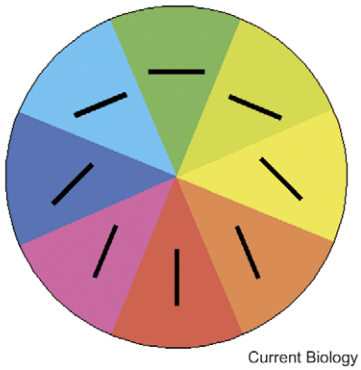

Figure 2. Diagram of an orientation pinwheel, with a singularity at the centre.

Colour is conventionally used to code for orientation, because like orientation it is a cyclic variable. Note that there is no way of smoothly interpolating between the orientations shown without a discontinuity at which unlike orientations meet.

by computer simulations, that singularities are probably an inevitable result of trying to map a cyclic variable smoothly onto a two-dimensional surface [5]. Because the optical imaging technique used to visualize pinwheels averages signals from large numbers of neurons, however, the singularities might be an artefact of averaging and not really exist. For example, neither extracellular recording or optical imaging would be able to distinguish the arrangements shown in Figure 3A and 3B, although only one of them contains a singularity. It is also possible that cells simply become unresponsive or unselective for orientation in pinwheel centres, although there are results suggesting that this does not happen [6].

What Ohki *et al.* [4] found was that orientation tuning remains sharp and systematically related to position right up to the pinwheel centre, indicating that the arrangement is as close to being a singularity as possible, given the discrete spacing of cell bodies (Figure 3C,D). Analysis also indicated little variation of properties with depth, confirming the precision of columnar organization. The responses of neurons close to the singularities were not identical to those elsewhere however. Among cells more than 65 μm from a singularity, only 7% were unresponsive or non-selective for orientation (in itself a remarkable finding, because it has never been certain from microelectrode studies what percentage of cells in the visual cortex is actually visually responsive). Within 65 μm of a singularity, however, the fraction of non-selective or unresponsive cells rose to about 21%. Average responses were also weaker and the tuning curves of responsive cells were broader. These effects, although not large, are of interest because many models qualitatively predict them; however, the new data are precise enough to constrain more detailed models capable of making quantitative predictions about the shapes of tuning curves.

These results are important in showing that cortical maps can be organized with single cell precision. This scale is not only well beyond the resolution of conventional optical recording, but also largely inaccessible to extracellular microelectrodes, most of which are unable to localize the positions of recorded cells with a precision better than ±50 μm, which is larger than the spacing between cells. Naturally, many questions remain unanswered. Some we may hope to have answers to soon, such as whether or not orientation maps in primates are similarly precise in their organization and whether or not the map of retinotopic position is also orderly on a cellular level.

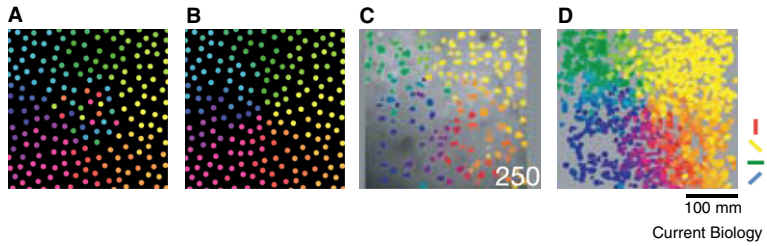

Figure 3. Orientation preferences at a singularity.

Possible (A,B), and actual (C,D), arrangements of orientation preferences around a singularity. Colour codes for orientation as in Figure 2. In (A) the pinwheel organization is present in the periphery but close to the centre orientation preferences vary randomly. In (B) the pinwheel is ordered right into the centre. (C) shows orientation preferences measured optically at a depth of 250 μm. Each coloured spot is a single cell body in the plane of focus of the imaging microscope. (D) is the sum of the preferences of cells taken over a series of depths from 130 to 290 μm. (C,D) are reproduced with permission from [4] and (A,B) are taken from supplementary material to the same paper at www.nature.com/nature.

Others issues may be harder to resolve. In the visual cortices of lower mammals, such as rats and squirrels, well organized retinotopic maps are present, but orientation maps are absent [3,7,8]. Instead, preferences seem to vary randomly from cell to cell. Yet squirrels have well developed visual abilities that, superficially at least, do not seem very different from those of cats. This would seem to give weight to the argument that columns and maps are epiphenomena of development and not essential for the cortex to function well [9]. It is tempting to argue, however — especially given Ohki *et al.*'s [4] recent findings in the cat — that, because precisely organized maps are present in higher mammals and absent in lower ones, there must be some evolutionary advantage in having precise maps.

This argument would be strengthened if some significant qualitative difference in the visual abilities of animals with precise orientation columns could be demonstrated. Comparisons of visual abilities among different mammals are mostly limited to simple abilities such as spatial resolution, orientation discrimination and stereoscopic depth perception. But there is much more to vision than these simple tests reveal (ask any artist), so it may be that more sophisticated comparisons will be needed. It would certainly not be surprising if the visual worlds of cats and primates turned out to be fundamentally different in some way from those of rats and squirrels.

The advance in technique represented by these studies also deserves comment. Being able to 'see' into the brain and record the activities of many neurons simultaneously and non-invasively is probably the dream of most neurophysiologists. Two-photon imaging comes close to this although there are still limitations, including the need to inject dyes and the fact that the calcium signal is slow and indirectly related to the pattern of firing of action potentials. My guess is that it will soon be possible to overcome these problems, for example by genetically engineering cells to express voltage sensitive dyes in their membranes, so that action potentials can be resolved. If (or when) this happens, the age of microelectrode recording may

finally come to an end and a new era in neuroscience will begin.

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Department of Ophthalmology and Visual Sciences, University of British Columbia, 2550 Willow St., Vancouver, V5Z 3N9, British Columbia, Canada.
E-mail: swindale@interchange.ubc.ca

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Recalibrating Time: When Did I Do that?

A recent study has shown that illusory inversion of temporal order can be induced by the 'intentional binding' of an action with its consequence, and that this is associated with increased activation in a brain area implicated in conflict monitoring.

Elizabeth Liddle[1] and Stephen R. Jackson[2]

Most actions require precise timing: for example, we calibrate our sensorimotor system in such a way that the end point of an action occurs at the time we intend. This is true for the tennis player or musician and was likely true for our hunting ancestors — the salient moment is not when we initiate an action, or even when the motor action itself is completed, but the time at which its goal is achieved. Changing circumstances, however, require that this system be continuously recalibrated so as to provide an accurate estimate of sensorimotor delays. For instance, different materials, lighting conditions or acoustic environments will each modulate the time that elapses between the initiation of an action and the arrival of sensory feedback. Moreover, the brain must be selective about what, from a barrage of sensory input, is worthy of further investigation, and one such filter is likely to involve the computation of a 'forward sensory model' that provides an estimate of the sensory consequences of our own actions [1,2]. If this estimate is confirmed by afferent sensory information, then we need not spend additional resources on further processing. But if there is a mismatch between the model's prediction and afferent sensory information, then we may be alerted to investigate further [3].

Stetson *et al.* [4] recently investigated the effect of the 'intentional binding' of an action to a consequent stimulus on the perception of temporal order. Haggard *et al.* [5] had earlier shown that, as an action and its apparent result become causally bound, the time period perceived to elapse between the two events is compressed: intention and goal appear to become one. In contrast, arbitrary pairings of action and stimulus produce no such temporal compression. Intentional binding would seem, therefore, to involve the temporal elision of action and goal.

While it is relatively easy to see how such elision might arise from processes that prioritise the temporal precision of the endpoint of action, it is less obvious how such elision might be represented neurally. One model of this process might be that the intention to act 'resets' a single 'clock', bringing the representation of the action into temporal alignment with the representation of its result. An alternative model might be that this recalibrated timeline is maintained alongside a 'baseline' timeframe representing a more veridical sequence of events.

Stetson *et al.* [4] sought to quantify the magnitude of temporal sensorimotor recalibration observed in a simple motor learning task, and to determine, using functional magnetic resonance imaging (fMRI), whether the brain activation associated with sensorimotor recalibration supported the hypothesis of a single 'clock' that was recalibrated, or whether it favoured the existence of multiple time estimates. The authors asked participants to make a key-press following a cue, under two conditions (Figure 1).

In a baseline condition, for the majority of trials, a brief visual stimulus ('flash') was presented very shortly after the key-press; in a second condition, an additional interval of 100 milliseconds was 'injected' between the response and the flash. In both conditions, in the remaining minority of trials, the flash occurred randomly. Participants were asked to report which had occurred first: their key-press, or the flash. From their responses, the authors computed the 'point of subjective simultaneity' — the point in time at which each participant experienced the flash occurring at the same time as the key press — for each condition.

In the baseline condition, the point of subjective simultaneity tended to be at around the time at which most of the flashes were presented. In other words, participants appeared to assume