

Reprogramming the cortex

The neocortex of the mammalian brain has been evolving at a tremendous rate. The complex task of trying to make sense of sensory information must have been an important source of the selective pressure behind this evolution. Distinct regions of the mammalian cortex are committed to specific sensory modalities; that is, they are involved in the interpretation of signals from a particular sensory pathway. The primary visual cortex (V1), for example, gets inputs mainly from the visual stream; the primary auditory cortex (A1) from the auditory stream etc. In short, the brain 'sees' with the help of the visual cortex, 'hears' with the help of the auditory cortex, and so on. Sensory data is collated by the thalamus, a system that lies towards the middle of the brain. The thalamus acts as a relay centre. It gathers sensory information from different sensory modalities at different ports (termed nuclei) and the nuclei redirect the information to the appropriate parts of the cortex. For example, the lateral geniculate nucleus (LGN) in the thalamus gets data from the retina and projects onto V1 (figure 1).

The cortex itself is organized in a way that reflects the organization of the sensory stimulus in the world outside. If one were to look at the response of cells in V1, for example, one would find a "map" of the visual space: neighbouring cells would be responsive to neighbouring areas in the visual field. Similarly the auditory cortex has a tonotopic organization (the neurons respond to different optimal frequencies) while the somatosensory cortex contains a map of the entire body surface.

Cells in V1, one of the best studied cortical areas, have further been shown to respond best to oriented bars of light in their portion of the visual space. Different cells respond best to bars of different (preferred) orientations. If one looks at the orientation selectivity of cells in a piece of V1 tissue in a plane horizontal to its surface, one sees beautifully ordered orientation 'maps' in which the preferred orientation changes in a semi-discontinuous manner.

How do the various cortical areas come by the maps that they contain? Clearly each map reflects some property of the signal sensed via that particular sensory modality. Is the pattern exhibited by a map genetically determined – hard-wired, so to speak –, or does it arise as a consequence of some facet of the patterning inherent in a specific sensory input? The group of Mriganka Sur and his colleagues at the Massachusetts Institute of Technology in the US has been developing a technique that allows them to address this question in a remarkably direct way by rewiring the brains of ferrets (Angelucci *et al* 1998; Sharma *et al* 2000; Merzenich 2000). By certain manipulations in ferret kits, they are able to route the retinal projections (via the LGN) to the auditory cortex (A1); while normal auditory inputs to this A1 are removed. Previously they had shown that the rewired A1 had a map of visual space (Roe *et al* 1990) and that the cells in it had other properties typical of cells in a normal V1.

In a recent paper, they have extended these observations and shown that the rewired A1 is similar to the normal V1 in a deeper way (Sharma *et al* 2000). They show the existence of the orientation maps (described above) in the rewired A1, complete with the so-called 'pinwheels' (the specific arrangements of the orientation columns in the normal V1). Besides, they also demonstrate the existence of horizontal connections that connect cells in neighbouring orientation columns. These horizontal connections are virtually never seen in this anatomical area of the normal brain. The orientation maps as well as the horizontal connections are of a higher order of complexity than the simple map of visual space, and these results show that even such higher order features can be driven by the sensory inputs.

Is the animal capable of making use of the redirected sensory inputs? In other words, does it realize that the information impinging on what ought to have been its auditory cortex is in fact visual in nature? von Melchner *et al* (2000) tested this by training rewired ferrets on behavioural tasks that involved the use of both the auditory and the visual modalities (Merzenich 2000). They found that the redirected inputs to the auditory cortex were correctly interpreted by the ferrets as being visual in nature.

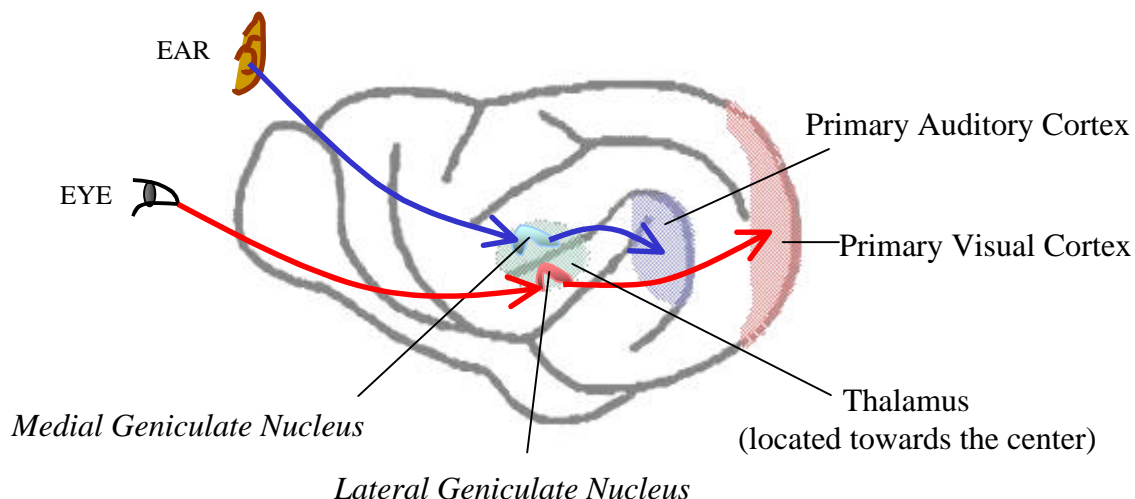


Figure 1. A sketch of a ferret brain showing relevant neuronal connections in the auditory and visual pathways. Nerve fibers from the ear carry auditory information to the *medial geniculate nucleus* (MGN) in the thalamus. The MGN projects to the primary auditory cortex (A1). Projections from the eye carry visual information to the *lateral geniculate nucleus* (LGN) in the thalamus. The LGN in turn projects to the primary visual cortex (V1).

These experiments demonstrate the role of the incoming inputs, guided by the thalamus, to programme the cortex. Besides, they point to some underlying similarities in different sub-areas of the cortex with respect to their computational capabilities. After all, if the so-called auditory cortical tissue can process visual information, it could mean that the different cortical areas are carrying out similar kinds of computations; and in that sense are not inherently very different. However, this is not strictly true. The orientation map in the rewired A1, though it shares several features of the normal V1, also has differences, which appear to derive from some inherent feature of the anatomical area that would have been the normal A1. Thus, a complete understanding of cortical development would need to balance the roles of both the extrinsic as well as the intrinsic cues in the cortex.

A further implication seems to be that it is the thalamus that plays the crucial role in assigning different roles to different areas of the sensory cortex. Metaphorically speaking, one might say that the thalamus is a smart input/output port that treats the cortex as a programmable chip. And, during the course of development, it makes use of various (differently structured) sensory inputs to ‘write in’ the appropriate algorithms that aid the brain as a whole in interpreting the precise information contained in the inputs.

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Rett and ICF syndromes: methylation moves into medicine

Two human genetic disorders, Rett and ICF syndromes, have recently been shown to be caused by mutations in genes encoding proteins involved in gene silencing through DNA methylation. Rett (RTT) syndrome is a progressive childhood neurodevelopmental disorder that affects females exclusively with an incidence of 1 in 10,000–15,000 female births. Patients with RTT syndrome appear to develop normally until 6–18 months of age, then gradually lose speech and purposeful hand use and display a whole range of symptoms. These include microcephaly, seizures, autism, jerky truncal ataxia, scoliosis (abnormal lateral curvature of the vertebral column), vacant stare, severe dementia, intermittent hyperventilation, spastic paraparesis (increased muscle tone and weakness of the lower extremities), and vasomotor disturbances of legs. Another typical symptom of RTT patients is constant hand-wringing.

The genetic basis of RTT has been difficult to determine, as most RTT cases are sporadic. The simplest model is that RTT is an X-linked dominant trait with lethality in hemizygous males. Non-random (skewed) X-inactivation in asymptomatic mothers of RTT patients supports this model. A small number of familial cases have helped to locate the putative RTT gene to Xq28 by analysis of shared haplotypes in affected sisters and half-sisters.

Mouse methyl-CpG-binding protein 2 (*Mecp2*) gene is located in a 40 kb interval between the *L1Cam* and *Rsvp* genes on the X chromosome. This region is syntenic to human Xq28 and, with the exception of the F8A gene, locus order is conserved between two species. *Mecp2* has been shown to bind specifically to methylated DNA *in vitro*. The human *MECP2* gene has several isoforms including a 10.1 kb transcript abundant in fetal brain and a 5 kb transcript abundant in adult brain. The gene contains three coding exons and a giant 3'UTR; the MECP2 protein is 486 amino acids long. It contains an 85 amino acid methyl-CpG-binding domain (MBD) which binds to 5'mCpG, and a 104 amino acid transcription repression domain (TRD) which interacts with histone deacetylase and a transcription corepressor, SIN3A. This leads to deacetylation of core histone proteins and transcription silencing. Gene-targeting experiment to mutate the *MECP2* gene in male mouse embryonic stem (ES) cells have shown that ES cells grow like parental lines and are capable of differentiation, but there is no fetal development. Thus, like DNA methyltransferase, *MECP2* is dispensable in stem cells but essential for embryonic development.

Using heteroduplex and sequencing techniques, Amir *et al* (1999) have shown that mutations in the *MECP2* gene cause RTT syndrome. They examined 21 sporadic and eight familial RTT cases and found 3 *de novo* missense mutations in MBD, and one frame-shift and one nonsense mutation in the TRD in 5/21 sporadic cases. In one familial case (out of 8), a missense mutation was detected in half-sisters which was not present in their mother, implying that the mother was either a germ-line mosaic or had a very low level of mosaicism in her blood lymphocytes which was beyond the resolution of detection. Recent observations suggest that mutations in *MECP2* account for most of the typical cases of RTT syndrome (Cheadle *et al* 2000). All these mutations are predicted to interfere with MECP2 function. Thus, RTT syndrome is the first human genetic disorder that is caused by mutations in a gene encoding a *trans*-acting factor – a factor, in other words, that has a role in the epigenetic regulation of gene expression. Cheadle *et al* (2000) have carried out a genotype-phenotype correlation analysis and have found that milder disease phenotypes are present in patients carrying missense mutations as compared with those with truncating mutations.

ICF (immunodeficiency, centromeric instability and facial anomalies) syndrome is a rare disorder with an autosomal recessive mode of inheritance. Patients with ICF syndrome have growth and developmental abnormalities, and facial dysmorphism such as hypertelorism (abnormal distance between two eyes), flat nasal bridge, epicanthal folds (skin folds at the corner of the eyes), micrognathia (small jaws), low set ears and tongue protrusion. Other features of ICF patients include otitis media, mental retardation, recurrent and prolonged respiratory infections, diarrhea, and infection of the skin and digestive system. Metaphase chromosome preparations from cultured peripheral blood from ICF patients show a remarkable tendency of chromosomes 1, 9 and 16 to form “windmill” multiradials by interchange within decondensed heterochromatin regions. Interestingly, this feature is seen only in

lymphocytes of ICF patients. Normal cells treated with the demethylating agent 5-azacytidine have the same effect, suggesting that demethylation of heterochromatin on chromosomes 1, 9 and 16 causes its decondensation and leads to the formation of windmill multiradials. Two years ago genetic mapping localized the ICF trait to the long arm of chromosome 20. A candidate gene, DNA methyltransferase 3b (*DNMT3b*), was localized to the same area in 1999. Given the role of demethylation in the windmill phenotype in ICF patients, the *DNMT3b* gene became a good candidate gene for the syndrome. Soon disease-causing mutations were found in the gene in ICF patients (Xu *et al* 1999). These mutations do not result in a complete absence of *DNMT3b* activity; this makes sense as the complete lack of this enzyme in mice is lethal *in utero*. Kondo *et al* (2000) have recently investigated the methylation abnormalities in CpG islands of B cell lines from ICF patients and their unaffected parents. They found that the methylation abnormality in ICF patients is restricted to a small portion of the genome.

With the discovery of genes for RTT and ICF syndromes, prenatal diagnosis for these syndromes in families at risk has become a reality. However, there are several questions that remain unanswered. For example, if both genes are involved in methylation-mediated transcription silencing, why do mutations in them lead to diseases with completely non-overlapping phenotypes? Could the disease phenotype of RTT patients be due to excessive non-specific 'transcriptional noise' owing to a silencing defect? And, if general transcriptional noise is the culprit, why is the brain the primary target? Demethylation leads to decondensation of heterochromatin on chromosomes 1, 9 and 16, but could it cause immunodeficiency in ICF patients? What is the relationship between demethylation and craniofacial abnormalities in ICF patients? Why is the windmill phenotype seen only in blood lymphocytes? One hopes that with increasing information about the structure and function of the *MECP2* and *DNMT3b* genes and their other family members, these questions will get answered.

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Feverish honeybees

The therapeutic benefits of fever have been recorded since the time of Hippocrates. At the turn of the 20th century, syphilis was a dreaded disease whose neuro-degenerative effects were terrible and many mental institutions were occupied with patients suffering from it; there was no effective treatment available then. In 1905, Karl Landsteiner showed that fever was able to kill syphilis-causing spirochaetes. Twelve years later, in a discovery that was to earn him the Nobel Prize, Julius Wagner-Jauregg discovered that infecting syphilitic patients with a mild form of fever-causing malaria resulted in remission of the neuropsychiatric condition in 30–50% of patients; the malarial fever apparently arrested the growth of the spirochaetes (the rate of natural remission of the disease was only 1%). The malaria itself was treatable with quinine. Since then, the understanding of the role of fever in combating infection took a new turn. Fever is now considered to be an evolutionary adaptation to fighting infection and is a result of active thermoregulation (Nesse and Williams 1994). It continues to be accepted as a basic evolutionary response by many clinicians (Romanovsky and Szekely 1998; Marik 2000). Kluger (1979) has shown that even poikilothermic lizards actively seek out warm places when infected. Amazingly, it now turns out that honeybee colonies too are capable of mounting a systemic response to infection by developing fever.

Larvae of honeybees (*Apis mellifera*) are often infected by the heat-sensitive fungus *Ascosphaera apis* which causes “chalk brood disease”. The disease derives its name from the chalky mummified masses that the dead larvae resemble. The normal temperature within the brood portion of the hive (brood-comb) is 33–36°C and, within this range, is correlated closely with ambient temperature. Starks *et al* (2000) have found that combs infected with *Ascosphaera* display an elevated temperature; in effect, they get fever. In replicated experiments, they recorded temperatures of the brood-comb in pre-treatment, treatment and post-treatment periods. The pre-treatment period consisted of a pre-feed period of ten days in which colonies were unfed and a feed period of five days in which sugar-water was provided. In the treatment period, colonies were fed for two days on sugar-water containing macerated sporulating mummified larvae; these were sufficient to cause infection. In the post-treatment period, colonies were unfed. The experiment ended on the 30th day. Brood-comb temperatures were significantly higher in the treatment period compared to the pre-treatment period while there was no significant difference between brood-comb temperatures of the pre-feed period and the post-treatment period. Moreover, *A. apis* apparently needs only a slight chilling of the larvae to cause disease. It is clear then that honeybees elevate brood-comb temperatures, which they achieve by increased flight muscle activity, as a defensive response to infection. According to the authors, this prevents larvae from succumbing to the disease. The authors suggest that either honeybee workers detect infestation before symptoms are visible or that the larvae somehow communicate the ingestion of the fungus. In any event, the infection is detected fairly early and triggers the appropriate febrile response.

Here is evidence that the honeybee colony is responding just as a unitary organism would to infection. Starks *et al* (2000) refer to the phenomenon as “a striking example of convergent evolution between this ‘superorganism’ and other fever-producing animals”. In honeybee colonies a single queen does all the egg-laying and the hegemony of the queen is generally accepted, part of the reason being that the workers are often more closely related to each other than to the queen owing to the haplo-diploid system of sex determination. This can lead to an extremely close level of integration at the colony level, along with division of labour. In consequence, colonies of honeybees and other social insects have been described as superorganisms (one colony = one organism), and it appears that colony-level selection rather than individual-level selection is in operation (Seeley 1997; but see Keller and Reeve 1999). The ability of the colony to develop a fever is yet another indication of an organism-like physiological response. Honeybees exhibit other regulatory mechanisms to control elevated nest temperature such as fanning, tongue-lashing (water-evaporation from extruded fluid droplets) and partial evacuation of the nest to prevent overheating (Seeley and Heinrich 1981). They also use temperature as a lethal defense against predatory wasps: the wasps are surrounded by workers and get virtually overheated to death (Ono *et al* 1995). And now fever!

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