SENSORY SYSTEMS

Ecstatic about RAVE

We are all naturally dependent on opioids for our emotional health, and some of us indulge in their recreational use. Both internally generated endorphins and drugs exert their action by interacting with specific membrane receptor proteins on neurons. Opioid painkillers, such as morphine, also exert their effect through the μ-opioid receptor (MOR). Use of morphine is hindered in the long-term by the development of tolerance to the painkilling effects of the drug. In contrast to the prevailing view, Whistler and colleagues provide *in vivo* evidence that endocytosis of MOR can reduce, rather than increase, the development of tolerance to morphine.

Opioid receptors belong to the large superfamily of G-protein-coupled receptors (GPCRs). The mechanisms for mediating the development of tolerance and dependence to morphine are controversial. The ability of MOR agonists to promote endocytosis of MOR is not related linearly to the agonist activity. The net amount of a signal that is transmitted to the cell is a function of both processes, a relationship termed 'relative activity versus endocytosis', or RAVE. Morphine has a high RAVE value because of an inability to promote endocytosis. Endorphins and other opiate drugs have similar signalling efficacies, but have lower RAVE values because they induce endocytosis.

Whistler and colleagues reasoned that if prolonged signalling at MOR contributes to the development of tolerance, then lowering the RAVE value by reducing that prolonged signalling would reduce the unwanted side effects. Consistent with this hypothesis, they showed that rats treated chronically with morphine plus the enkephalin, DAMGO (D-Ala^2-MePhe^4-Gly^5-ol), which facilitates MOR endocytosis, had reduced development of analgesic tolerance compared with rats treated with morphine alone. The present study shows that MOR can dimerize, as seen with other GPCRs, and proposes that this dimerization is mechanistically important in influencing the endocytic properties of the receptor, thereby reducing the development of tolerance to morphine.

The current view that endocytosis of MOR contributes directly to

DEVELOPMENT

Sonic goes ballooning

The development of the brain depends on the precise coordination of growth and patterning mechanisms. Although the patterning mechanisms are becoming well understood, less is known about the factors that influence the overall shape and size of the brain. However, some studies have indicated that the signalling molecule sonic hedgehog (Shh) might be important in controlling growth and cell survival during the later stages of development. Now, as reported in *Nature Neuroscience*, Britto *et al.* propose that Shh also controls a much earlier event in brain morphogenesis, namely the expansion, or 'ballooning', of the forebrain and midbrain vesicles.

In the 1950s, Källén showed that the embryonic chick brain collapses if the notochord and anterior hindbrain are separated from the neuroepithelium. At the time, the resulting convoluted appearance of the brain was attributed to overexpansion of the tissue, and this seemed to be borne out by an increase in the number of mitotic figures. Because of this, the phenomenon became known as 'experimental overgrowth'. However, it was later shown that although more cells were in mitosis, their cell cycle was longer, so the net outcome was actually cell loss.

This result indicated that the notochord might secrete a trophic factor that is important for expansion of the brain vesicles. Could this be Shh? To investigate this issue, Britto *et al.* recreated Källén's collapse phenotype by separating the notochord from the midbrain. They found that this caused a drop in Shh levels, both in the notochord itself and in the floor plate. The expression of patterning genes was largely unaffected, indicating that the main effect was on growth rather than regional patterning.

To confirm that Shh was required for vesicle expansion, Britto *et al.* then tried grafting Shh-expressing COS cells into the region of the ventral midbrain of the manipulated embryos. They showed that the ballooning of the forebrain and midbrain vesicles was restored in a dose-dependent fashion. They also showed that injection of the Shh inhibitor cyclopamine into intact embryos produced an overall reduction in the size of the head, providing further evidence that Shh controls growth in this region.

This is the first direct demonstration that Shh can regulate the ballooning of the forebrain and midbrain vesicles. This study not only provides a long-overdue explanation for an old phenomenon, but also gives us an intriguing insight into the role of Shh in early brain growth.

Heather Wood

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tolerance by decreasing the number of functional receptors on the cell surface has discouraged drug discovery programmes from investigating new agonists to promote endocytosis. However, this study indicates that agonists that promote endocytosis of MOR might provide analgesics with improved tolerance profiles. In the meantime, administration of drugs that promote endocytosis of MOR with morphine might produce less tolerance than morphine alone.

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opiates | opiate receptors

ELECTROENCEPHALOGRAPHY

Brain waves in phase

Cognitive neuroscience is making increasing use of techniques such as functional magnetic resonance imaging and electroencephalography (EEG) to visualize changes in brain activity that occur in response to particular stimuli. Individual EEGs are typically averaged to give a stimulus-evoked event-related potential (ERP). It is often assumed that the ERP represents the sum of individual events that occur with a fixed latency and polarity, independently of the rest of the EEG, and that the averaging process removes background EEG activity, which is considered to be noise. An alternative explanation, however, is that the ERP arises because of 'phase resetting' of ongoing EEG activity that results from the stimulus presentation.

It is becoming clear that if we are to interpret this kind of study correctly, we need to know more about the origin of the EEG and ERP. Makeig and colleagues have attempted to resolve the question of what the ERP actually represents by undertaking a detailed analysis of EEG and ERP data from a straightforward experiment in which subjects viewed simple visual stimuli that required no response.

First, they compared the averaged ERP in the period immediately after stimulus presentation with the individual EEG readings (epochs) from which it had been obtained. There were large post-stimulus increases in the alpha band (between 8 and 12 Hz) in the ERP waveforms that were not paralleled by similar increases in the individual EEGs. In fact, the amplitude of the ERP, at a range of frequencies below 20 Hz, was much larger than would be expected if the phases of the individual EEG epochs were randomly distributed.

Makeig *et al.* then looked at the intertrial coherence, which measured how consistent the EEG phase was across trials. Before and after the stimulus presentation the phase distribution was uniform across trials, but during stimulus presentation a dominant phase distribution emerged, indicating that the phases of the EEGs had been reset by the stimulus. This phase resetting was sufficient to account for the peristimulus ERP.

Further analysis of the individual trials showed that for those trials with the lowest post-stimulus signal in the alpha band, the average ERP was also low. This is inconsistent with the commonly made assumption that the ERP sums fixed-latency, fixed-polarity events that occur independently of the rest of the EEG.

Makeig *et al.* next used independent-component analysis, which can separate out the different electrical activities that contribute to the EEG recording, to find out which portions of the EEG signals were phase reset. The results indicated that multiple independent processes, probably occurring within compact cortical domains, contribute to the recorded EEGs. The waveforms seem to result from the sum of these ongoing, independent processes, which are partially reset by the occurrence of a visual stimulus to produce the averaged ERP.

The authors comment that, in general, averaged ERPs might result from a combination of phase resetting of ongoing EEG activity with concurrent energy increases, but point out that phase resetting explains many features of the data. Given the recent surge of interest in the role of brain dynamics and correlated activity in cognitive processes, studies such as this will make an important contribution to our understanding of cortical function.

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