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Anatomy and physiology of a nociceptive modulatory system

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Although efferent control of sensory transmission is a well-established concept, a specific network for nociceptive modulation has only recently been discovered. This network includes interconnected components at midbrain, medullary and spinal levels. At the midbrain level, electrical stimulation of the periaqueductal grey (p.a.g.) inhibits spinal neurons that respond to noxious stimuli as well as nociceptor-induced reflexes and escape behaviour in a variety of species. Midbrain stimulation also produces analgesia in patients with clinically significant pain. The rostral ventral medulla (r.v.m.) has similar behavioural and physiological effects and mediates midbrain antinociceptive actions at the level of the spinal cord.

Endorphins are present at all levels of this nociceptive modulating network. Opiate microinjections at p.a.g., r.v.m. or spinal levels produce analgesia, presumably by mimicking the actions of the endorphins. The nociceptive modulatory system is diffusely organized, highly interconnected and appears to act as a unit whether activated by opiates or electrical stimulation.

There are two classes of r.v.m. neurons the activity of which is correlated with the occurrence of reflexes induced by noxious stimulation. One class (the on-cell) accelerates, the other class (the off-cell) pauses just before tail flick. Both classes project to the spinal cord and are excited by electrical stimulation of the midbrain. However, when morphine is injected either systemically or into the p.a.g., the off-cell is excited and the on-cell stops firing. The off-cell is probably the r.v.m. output cell that inhibits nociceptive transmission at the level of the spinal cord. The function of the on-cell is not clear.

The nociceptive modulatory system can be activated by a variety of stressful environmental factors, which are often, but not necessarily, noxious. The idea that the system acts as a simple negative feedback circuit is not consistent with its known properties.

Modulatory networks belong to a class of neural system that is distinct from the well-recognized motor and sensory pathways. The nociceptive modulatory system is perhaps the most completely described example of this class. Activation of this system in the absence of noxious stimulation produces neither overt behaviour nor sensation. However, activation of this system in the presence of noxious stimulation prevents the expected escape or withdrawal responses and reduces the intensity of perceived pain.

The concept of efferent or descending modulation of sensory transmission is not new. Anatomical studies have established that sensory relay nuclei such as the spinal cord dorsal horn, the dorsal column nuclei and ventrobasal thalamus receive major input from more rostral centres (Brodal 1981). Early physiological studies demonstrated that descending pathways influence afferent transmission (Hagbarth & Kerr 1954; Wall 1967; Pompeiano 1973; Satoh & Takagi 1971), but there was no direct evidence for specific control of pain sensation. In 1911, Head and Holmes postulated that the cortex inhibits thalamic responses to noxious input. They

based this conclusion on careful clinical studies that revealed the paradoxically painful sequelae of damage to somatosensory pathways. Melzack & Wall (1965), citing similar clinical observations plus a body of neurophysiological data, proposed a detailed neural mechanism to explain the variability of pain. According to their 'gate-control' hypothesis, pain sensation requires a certain balance of input from large and small diameter primary afferents to rostrally-projecting dorsal horn 'trigger cells' whose output results in withdrawal responses and pain sensation. Melzack & Wall also postulated an efferent 'central control trigger', of supraspinal origin which was activated by rapidly conducting somatosensory pathways and which could alter nociceptive transmission by shifting the effective balance of input to the dorsal horn trigger cells.

The theories of Head & Holmes and of Melzack & Wall primarily emphasized physiological interactions between the different components of the somatosensory pathway. More recent theories have instead proposed a nociceptive modulatory system which is separate from the somatosensory afferent pathways. In fact, there is direct evidence that such a specific nociceptive modulatory system contributes significantly to the variability of clinical pain. It has been shown that electrical stimulation of the midbrain periaqueductal gray (p.a.g.) in animals can reliably and selectively inhibit the tail flick reflex and other escape behaviours produced by noxious stimuli (Reynolds 1969; Mayer & Liebeskind 1974; Mayer & Price 1976). This demonstration of stimulation-produced analgesia (s.p.a.) was both unexpected and dramatic. It was important for two reasons. First, it established that selective modulation of pain is a separable function of the brain. Second, it showed that a discrete midbrain structure could be associated with that function.

Our continued progress in understanding nociception can in part be attributed to the fact that noxious stimuli elicit stereotyped withdrawal reflexes in animals which can be correlated with pain sensation in man. Likewise, correlation of the blockade of these reflexes with human pain relief has opened the way for analysis of the neural mechanisms underlying analgesia. For example, the tail flick reflex in the rat is produced at temperatures that are painful in man. The rank order of potency of opiate analgesics for inhibiting this reflex is the same as their rank order of analgesic potency for alleviating clinically significant pain in man (D'Amour & Smith 1941; Grumbach & Chernov 1965). Similarly, p.a.g. stimulation, which inhibits responses to noxious stimulation in animals, reduces the severity of clinical pain in humans with no other consistent disturbance of neural function (Hosobuchi *et al.* 1977; Richardson & Akil 1977).

1. Anatomy of nociceptive modulating networks

Studies mapping the effective sites for s.p.a. in a variety of species have concentrated on the medial mesodiencephalic and the rostroventral pontomedullary regions, in particular the midbrain periaqueductal grey (p.a.g.) and the medullary nucleus raphe magnus (n.r.m.). It is clear, however, that the functionally significant brainstem regions for s.p.a. do not respect the cytoarchitectonic boundaries of these two nuclei. The anatomy of these two interconnected brainstem regions has recently been reviewed in detail (Basbaum & Fields 1984) and will be briefly described below.

Inhibition of spinal reflexes and neuronal responses induced by noxious stimuli can be elicited from sites throughout the p.a.g. Because of problems with current spread, the resolution of available mapping studies does not permit an exact delineation of the behaviourally significant

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midbrain regions. In any case, cell somata and not fibres of passage are clearly critical for s.p.a. because inhibition of nocifensor reflexes such as tail flick and paw withdrawal can also be obtained by glutamate (Behbehani & Fields 1979) or opiate (Yaksh 1978) microinjection.

Although the inhibitory effects of p.a.g. stimulation on spinal reflexes are blocked by lesions of the dorsolateral funiculus (d.l.f.) (Basbaum *et al.* 1976), the great majority of p.a.g. cells do not project directly to the spinal cord via the d.l.f. (Basbaum & Fields 1979; but see Mantyh & Peschanski 1982). The p.a.g. does, however, provide the major input to the rostroventral region of the medulla, which is the largest brainstem source of descending d.l.f. axons. Furthermore, lesions of (Behbehani & Fields 1979; Prieto *et al.* 1983) or local anaesthetic injections into (Gebhart *et al.* 1983) this medullary area block the antinociceptive action of p.a.g. stimulation. Thus, the rostral medulla is a necessary element in the nociceptive modulatory circuit activated by stimulation of the p.a.g. The midbrain cells that project to the medulla are present throughout the tegmentum but are concentrated in the dorsomedial p.a.g. and in a larger cell group that includes the ventrolateral p.a.g. and the adjacent nucleus cuneiformis (Abols & Basbaum 1981). In the rat, there is also a projection from the dorsal raphe nucleus (Beitz 1982*a*; Gallager & Pert 1978). The significance of these neurons for nociceptive modulation is indicated by the observation that the lowest thresholds for midbrain s.p.a. (Dostrovsky *et al.* 1982) are obtained from regions that are largely coextensive with the ventral group of cells that projects to the medulla (figure 1).

In the rostral medulla, very low currents are effective for s.p.a., and in contrast to the midbrain, a high resolution map of this region has been constructed (Zorman *et al.* 1981). The lowest threshold sites are the nucleus raphe magnus and the adjacent reticular formation ventral to the nucleus reticularis gigantocellularis and medial to the facial nucleus. In the rat, the rostrocaudal extent for s.p.a. is approximately from the caudal facial nucleus to the caudal superior olive. This region will be referred to as the rostroventral medulla (r.v.m.).

The most effective medullary sites for s.p.a. are coextensive with the distribution of r.v.m. neurons that project to the cord via the d.l.f. (figure 1). The d.l.f. projection of these r.v.m. cells terminates most densely in dorsal horn laminae containing neurons responding to noxious stimuli (I, II, V) (Basbaum *et al.* 1978; Basbaum & Fields 1978). Glutamate (Satoh *et al.* 1983) and opiate (Azami *et al.* 1982; Dickenson *et al.* 1979) microinjections in r.v.m. also produce analgesia, thus implicating r.v.m. somata. Furthermore, this suggests that the output neuron that is inhibitory to nocifensor reflexes is excited by the opiate microinjection. Moreover, the fact that glutamate and electrical stimulation of the p.a.g. are predominantly excitatory to medullospinal neurons in r.v.m. (Behbehani & Fields 1979; Vanegas *et al.* 1984*a*) strongly supports the idea that r.v.m. is necessary for p.a.g. nociceptive modulating actions at the cord. It is unlikely, however, that r.v.m. functions as a simple relay, since recent studies have shown that the connection between p.a.g. and r.v.m. is reciprocal (Beitz 1982*b*; Mantyh 1982).

Electrical stimulation of r.v.m. inhibits dorsal horn neurons responding to noxious stimulation (Fields *et al.* 1977), including identified spinothalamic tract cells (Willis *et al.* 1977). This effect is blocked by d.l.f. lesions. The synaptic mechanism of the inhibition is unclear. Although n.r.m. stimulation produces inhibitory postsynaptic potentials in spinothalamic tract cells (Giesler *et al.* 1981), it also causes increases in the electrical thresholds of the spinal terminals of C-nociceptors suggesting that presynaptic actions also occur (Hentall & Fields 1979).

There is little detailed information on the dorsal horn circuitry that underlies the descending inhibitory control of nociceptive transmission. The r.v.m. is a major source of serotonin (5HT)

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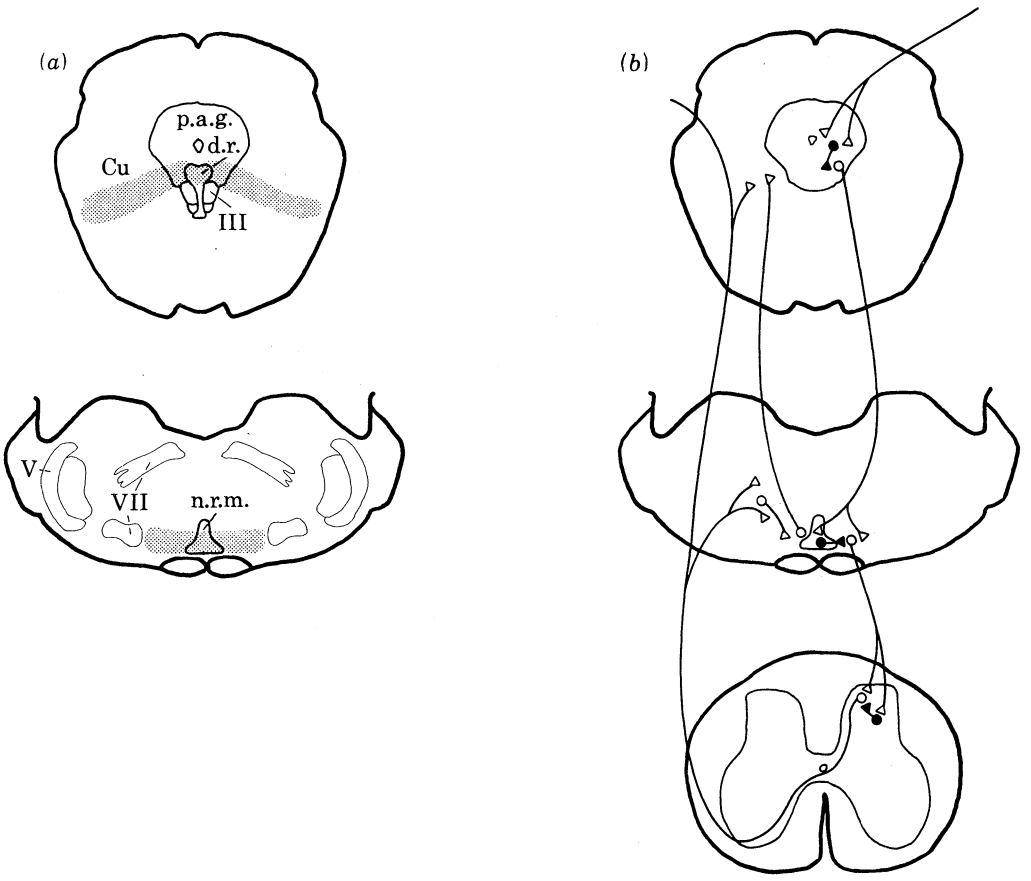

FIGURE 1. Anatomy of nociceptive modulatory systems. (*a*) Regions of low threshold s.p.a. and origin of descending pathways. At the top, midbrain stipple indicates region of overlap between low threshold sites for s.p.a. and somata of cells projecting to the rostral ventral medulla (r.v.m.). Below, in the rostral medulla, stipple indicates overlap between lowest threshold site for s.p.a. and somata of cell projecting to the spinal cord through the dorsolateral funiculus. (*b*) Connectivity of component nuclei of the nociceptive modulatory system (Basbaum & Fields 1984). The p.a.g. (top) receives input from cortex and projects to the r.v.m. (middle) which in turn projects to the dorsal horn of the spinal cord (below). At each level of the system there are enkephalin-containing interneurons (small black neurons). The r.v.m. also projects to the midbrain region (p.a.g. and c.u.), which provides its major input. Cord neurons project rostrally to terminate at medullary and midbrain regions, including parts of the nociceptive modulating system. Abbreviations: c.u., nucleus cuneiformis; d.r., dorsal raphe nucleus; n.r.m., nucleus raphe magnus; III, oculomotor nucleus; V, trigeminal complex; VII, facial nerve nucleus.

in the superficial dorsal horn and there is a body of evidence indicating that medullospinal 5HT neurons are involved in nociceptive modulation (for review, see Basbaum *et al.* 1983). Serotonin-containing synapses contact several classes of dorsal horn cells, including presumed nociceptive dorsal horn projection cells (Dubner *et al.* 1983) and interneurons, some of which contain enkephalin (Glazer & Basbaum 1983). Enkephalin is present in the presynaptic elements of axodendritic synapses and there are enkephalinergic synapses onto identified dorsal horn projection cells (Ruda 1982). Enkephalin has not been observed in axo-axonic synapses (Glazer & Basbaum 1983) despite the evidence that there are opioid binding sites upon the spinal branches of primary afferents (Fields *et al.* 1980) and that such terminals are sensitive to opiates (Carstens *et al.* 1979). Figure 2 illustrates our present knowledge of the relevant circuitry of the superficial dorsal horn.

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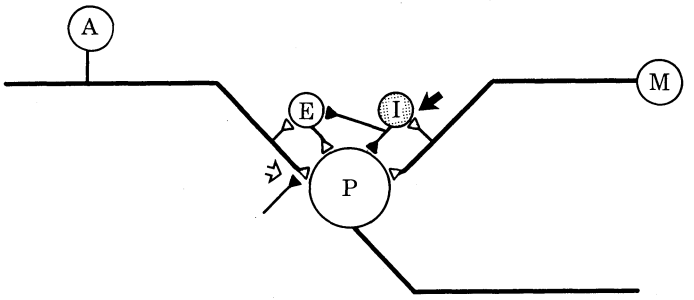

FIGURE 2. Diagram of dorsal horn circuitry relevant to nociceptive modulation. The primary afferent nociceptor (A), sends a process into the spinal cord dorsal horn which contacts an excitatory interneuron (E) as well as the projection cell (P) (i.e. a spinothalamic tract cell). The modulating cells (M), presumably arising in the rostral ventral medulla and projecting to the dorsal horn of the cord, terminate on and directly inhibit the projection cell. A serotonin to enkephalin synapse has been found (closed arrow) raising the possibility that the descending system acts in part through an inhibitory endorphinergic interneuron (I). The inhibitory interneurons may inhibit either the excitatory interneuron or the projection cell. The open arrow indicates that there are opiate receptors on central processes of primary afferent nociceptors, raising the possibility of opiate mediated presynaptic control of transmitter release. However, enkephalin-like immunoreactivity has not yet been found in the presynaptic element of axoaxonic synapses in the superficial dorsal horn.

2. ACTIVATION OF THE NOCICEPTIVE MODULATORY SYSTEM

The functions of the motor and sensory systems were obvious long before their anatomy and physiology were described. In contrast, despite the wealth of information about the anatomy, pharmacology and physiology of nociceptive modulating systems, very little is known about their physiological function or the circumstances under which they are activated. In the awake cat, Casey & Morrow (1983) have shown that the thresholds for several behavioural responses to noxious heat are increased during feeding. In awake rats, a variety of intense and/or stressful stimuli can delay or abolish nocifensor reflexes such as the tail flick or hot plate response (Hayes *et al.* 1978; Maier *et al.* 1982). This phenomenon is referred to as stress-induced analgesia, and there is evidence that under certain circumstances the nociceptive modulatory system is required for its production. For example, analgesia induced by footshock can be consistently blocked by r.v.m. or d.l.f. lesions and by lumbar intrathecal naloxone (Watkins & Mayer 1982). These data clearly implicate descending pathways in the mechanisms of stress-induced analgesia.

The blockade of stress-induced analgesia by systemically administered naloxone (Lewis *et al.* 1980) provides an important parallel to the observation that naloxone reliably increases the severity of clinical pain in man (Lasagna 1965; Levine *et al.* 1982; Grevert *et al.* 1983) and supports the validity of stress-induced antinociception as a model for the physiological activation of the nociceptive modulatory system.

The definition of stress in this context is vague. Stimuli that are stressful are often, but not necessarily, noxious. It appears that the duration of the stimulus (Lewis *et al.* 1980), its location (Watkins & Mayer 1982) and its controllability by the animal (Maier *et al.* 1982) are all important features in determining which of the antinociceptive mechanisms are activated. It has also been shown that inherently neutral stimuli (such as a light or tone) can elicit analgesia after they have been contingently paired with a stressor such as inescapable shock (Bolles & Fanselow 1982).

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Although inhibition of nociceptive transmission is a common result of many manipulations intended to be stressful, the neural mechanisms that underlie the 'analgesia' may differ depending on how the 'stress' is produced. Thus, not all forms of stress-induced analgesia are naloxone-reversible (Lewis *et al.* 1980) and only certain types are blocked by d.l.f. lesions (Watkins & Mayer 1982). This raises the possibility that there are several nociceptive modulatory networks that can function independently (Hayes *et al.* 1978). Studies in this area are thus beginning to define the context in which nociceptive modulating systems can be reliably activated. Behavioural state, presence of a noxious stimulus and environmental stressors are all significant. Although these factors and the associated neural systems are only vaguely understood, it is clear that the nociceptive modulatory network can be activated by natural stimuli.

3. Some network properties of the nociceptive modulatory system

Withdrawal reflexes induced by noxious stimulation are polysynaptic and thus subject to some variability in latency and force when elicited at different times by apparently identical stimuli. Despite this, stimulation of r.v.m. or p.a.g. acts in an all-or-none fashion to either completely block or have no effect on tail flick and paw pinch withdrawal reflexes. We have used monopolar cathodal microstimulation (50 Hz continuous trains, 400 μs pulse duration) to map sites for suppression of the tail flick induced by noxious heat (46 °C or more). If the intensity of the noxious stimulus is raised while the brainstem stimulation parameters are held constant, the reflex may reappear at full force. If brainstem stimulation intensity is gradually raised, there is no antinociceptive effect in that individual until analgesia threshold is reached. At analgesia threshold, the reflex is completely blocked. Even at the lowest suprathreshold current employed (5 μA or less), tail flick is completely blocked during stimulation. If the heat is maintained throughout stimulation, the tail flick occurs within 0.5 s after the stimulation is turned off. There is a similar all-or-none component to the inhibition of the tail flick or paw-withdrawal reflex by systemically-administered morphine (Levine *et al.* 1980). Thus, the all-or-none component neither depends on the particular reflex nor on how the nociceptive modulatory system is activated.

By recording through the same metal electrodes used for microstimulation, we have been able to assess the number and kind of elements activated by stimuli at threshold for s.p.a. (Hentall *et al.* 1984*a*, *b*). First, chronaxies in r.v.m. for tail flick inhibition (162 μs) are closer to those for individual r.v.m. cell somata (170 μs), than for their axons in the d.l.f. (360 μs). This indicates that the inhibition is due to stimulation of r.v.m. somata rather than their axons or other fibres of passage. This conclusion is supported by the observation that glutamate microinjection into r.v.m. also produces analgesia (Satoh *et al.* 1983). By measuring the electrical threshold and spike amplitude of the nearest recordable r.v.m. neuron at several sites, it has been possible to determine the distance of the cell from the electrode (Hentall *et al.* 1984*a*). The results permit an estimate of the radius of effective current spread in r.v.m. at theshold for tail flick inhibition. By measuring cell densities in r.v.m., we were then able to calculate that approximately 30 cells are directly activated at threshold for tail flick inhibition. Since the rat nucleus raphe magnus alone has over 2000 cells, only a very small percentage of r.v.m. neurons need be directly activated to inhibit tail flick.

Thus synchronous activation of a small number of cells anywhere in r.v.m. can totally block

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the tail flick. These studies do not reveal whether tail flick inhibition requires only those few r.v.m. cells directly activated or whether the synchronous firing of that small number of cells activates a larger population of r.v.m. cells that in turn produces the behavioural effect.

Data from morphine microinjection studies suggest that widespread activation of the nociceptive modulatory network can result from local activation in any subregion. Although analgesia is produced by microgram doses of morphine injected at p.a.g., r.v.m. or spinal cord, there is evidence that brainstem and spinal injections interact in a multiplicative fashion. Yeung & Rudy (1980) showed that when a single injection of morphine is made at either the brainstem or the spinal cord, a relatively large dose (10 μg) is required to produce significant analgesia. However, a concurrent injection of morphine at both sites produces analgesia at much smaller doses (1 μg or less at each site). This indicates that each site is potentially highly sensitive to morphine and that the sensitivity at each is influenced by activity at other opiate-sensitive loci. This concept is supported by the observation that injection of morphine at separate sites within the brainstem has an additive effect (Rosenfeld & Stocco 1981). These microinjection data indicate that a relatively large proportion of the cells in the modulatory network are involved in producing a behavioural effect, suggesting that the network acts as a unit.

Several endogenous opioid peptides (endorphins) are located at each of the opiate sensitive sites that compose the nociceptive modulatory system. β-Endorphin terminals as well as dynorphin and enkephalin terminals and somata are found in the ventral p.a.g. and nucleus cuneiformis. Dynorphin and enkephalin somata and terminals are also concentrated in the r.v.m. and in the spinal cord dorsal horn (see Basbaum & Fields (1984) for review). This widespread distribution of endorphins raises the possibility that systemically-administered opiates activate the nociceptive modulatory system by a multisite effect that mimics the local action of endorphins. There is also evidence that activation of the nociceptive modulatory system results in release of endorphins at multiple sites within the system. Intrathecal naloxone blocks the antinociceptive action of fourth ventricular morphine (15 μg) (Levine *et al.* 1983) and of r.v.m. stimulation (Zorman *et al.* 1982). The distribution of endorphins and of opiate-sensitive sites and the evidence for endorphin-mediated connections among these sites suggest that unitary action of the network depends upon multiple distributed endorphinergic links. The observation that activity in one part of the system increases the sensitivity to opiates throughout the system raises the possibility that the network as a whole is sensitive to circulating endorphins, such as may be released by stress or pain.

Our mapping studies have revealed that uniformly low intensity (10 μA or less) stimulation throughout r.v.m. and the midbrain region that projects to r.v.m. can inhibit the tail flick. Withdrawal to hindpaw pinch can also be blocked from identical r.v.m. sites at minimum currents that do not differ significantly from those required for tail flick suppression (Barbaro *et al.* 1983). This is in sharp contrast with the topographic arrangement of sensory and motor pathways. Consistent with this lack of topographic organization in r.v.m. are anatomical studies indicating that branches of individual spinally-projecting r.v.m. cells innervate the trigeminal nucleus caudalis (Lovick & Robinson 1983) and multiple levels of the cord (Huisman *et al.* 1981, 1982). On the other hand, Mayer & Liebeskind (1974) reported that p.a.g. stimulation often did not produce uniform whole body analgesia, and Soper & Melzack (1982) found a definite topography of the analgesic effects from p.a.g. Perhaps the p.a.g. differs from r.v.m. in this respect.

Studies of the anatomical relationships among brainstem regions implicated in nociceptive

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modulation also support the idea that this system is organized as a unit and is not a set of independently acting parallel subunits, each capable of inhibiting nocifensor reflexes. As mentioned above, the midbrain and r.v.m. regions are reciprocally connected, i.e. in addition to the midbrain to medulla projection there is a large rostral projection from r.v.m. to midbrain. At both midbrain and medullary levels, sites effective for s.p.a. are interconnected (Abols & Basbaum 1981; Mantyh 1982; Beitz 1982*a*, *b*). Furthermore, both regions receive spinal input.

Thus the weight of evidence supports the idea that the nociceptive modulatory system is organized as an interconnected network that acts as a unit to inhibit spinal nocifensor reflexes. Activation of only a small percentage of its component neurons by direct electrical stimulation or by opiate microinjection is sufficient to bring the whole network into play.

4. Physiology of r.v.m. neurons

The first studies of neurons in the rostral ventromedial medulla (r.v.m.) were inconclusive because the r.v.m. cell population responded in a heterogeneous fashion to most manipulations. Although noxious stimulation excited most r.v.m. cells, many were inhibited or unaffected by such stimuli (Fields & Anderson 1978; Guilbaud *et al.* 1980). Electrical stimulation of p.a.g., although predominantly excitatory, has no effect on many r.v.m. cells. Satoh *et al.* (1979) reported that a subgroup of r.v.m. cells was predominantly excited by iontophoresis of opiate agonists, but others have reported that opiates administered systemically, into p.a.g. or locally by iontophoresis produced excitation, inhibition or no effect in different r.v.m. cells (see Gebhart (1982) for review). Furthermore, division of r.v.m. cells into raphespinal and non-raphespinal groups did not result in classes of r.v.m. cells with more consistent physiological or pharmacological properties.

Because of this puzzling heterogeneity we took a different approach to physiological characterization using methods borrowed from motor systems physiology. We located cells of interest by recording only from sites in r.v.m. from which s.p.a. could be obtained at 10 μA or less. We then recorded single unit activity during controlled heating of the tail at temperatures sufficient to cause tail flick. Unit activity, tail temperature and tail movement were recorded. Unit data were analysed either with respect to the time of application of the heat stimulus or the occurrence of the tail flick.

This analysis identified three classes of neurons in r.v.m. (Fields *et al.* 1983*a*). One class (the on-cell) accelerates just prior to tail flick (figure 3). A second class (the off-cell) pauses in its discharge just prior to tail flick (figure 4). The third class shows no change in its firing pattern when the tail flicks. These cell types have been found throughout the r.v.m. In the lightly anaesthetized rat, all three classes of cell discharge spontaneously. The response properties of these cells mirror their flick-related activity. Thus the on-cells are excited by pinch or heat and the off-cells are inhibited by such stimuli. The neutral cells are unaffected by noxious stimuli. Inhibition of the off-cells and excitation of on-cells is produced by noxious stimulation anywhere on the body from face to tail. Occasionally cells are found with more restricted inputs or that respond to innocuous stimuli. When limbs are stimulated however, the most consistent neuronal responses are produced by stimuli that elicit withdrawal, suggesting that these 'responses' are not strictly sensory but are best correlated with withdrawal.

In fact, when quantitative observations are made it is clear that the changes in discharge are reflex-related. This is most clearly illustrated in figures 3 and 4 where spike probability

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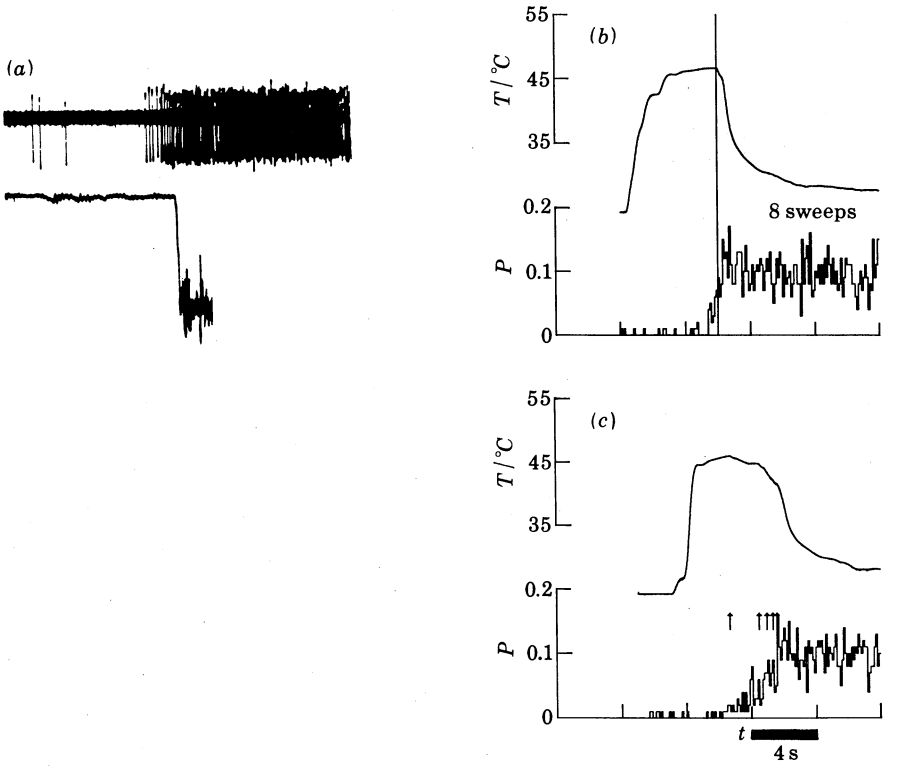

FIGURE 3. Representative on-cell in r.v.m. (*a*) Abrupt acceleration in unit activity (upper trace) before occurrence of tail flick (transducer output in lower trace). (Single 10 s sweep.) (*b*, *c*) Upper trace (*T*) of each figure indicates mean tail temperature at stimulation site, lower trace (*P*) is spike probability/number of sweeps. In (*b*) spike data are plotted with reference to time of the flick. An abrupt rise in spike probability is observed. In (*c*) temperature traces are aligned and the rise in spike probability is more gradual. Vertical arrows indicate times of occurrence of individual tail flicks. This illustrates a closer correlation of the acceleration in cell firing to the flick than to the stimulus that elicits it.

density is plotted against either tail temperature (stimulus) or tail flick. For both on- and off-cells, the changes in firing are more sharply correlated with flick than with temperature. In those cases where the same stimulus is applied and no flick occurs, there is neither on-cell acceleration nor off-cell pause. But the flick-related changes are not a result of the flick. The changes begin at least 250 ms before the flick. Furthermore, unpublished work in our laboratory (N. Barbaro) has shown that, in paralysed rats, on- and off-cell changes precede the sacral ventral root discharges that coincide with the tail flick in the unparalysed rat.

Thus the activity of on- and off-cells abruptly changes prior to nociceptor-induced reflexes (figure 5). What role does each cell type play in control of these responses? In view of available information, a potential role for the off-cell can be proposed. Since withdrawal reflexes are inhibited by electrical stimulation or the neuroexcitant glutamate, the r.v.m. to spinal cord output cell that inhibits tail flick should be activated by analgesia-producing manipulations. Such a cell should therefore be silent in order for the tail flick to occur. The off-cell clearly has the requisite firing characteristics. Its pause permits the tail flick. In contrast, the on-cell, which accelerates prior to the tail flick, is unlikely to have a direct inhibitory effect on the flick.

Consequently it may be concluded that electrical stimulation (or glutamate microinjection) prevents the tail flick by preventing the off-cell pause. Support for this conclusion comes from the finding that if r.v.m. stimulation is maintained during tail heating, flick does not occur until

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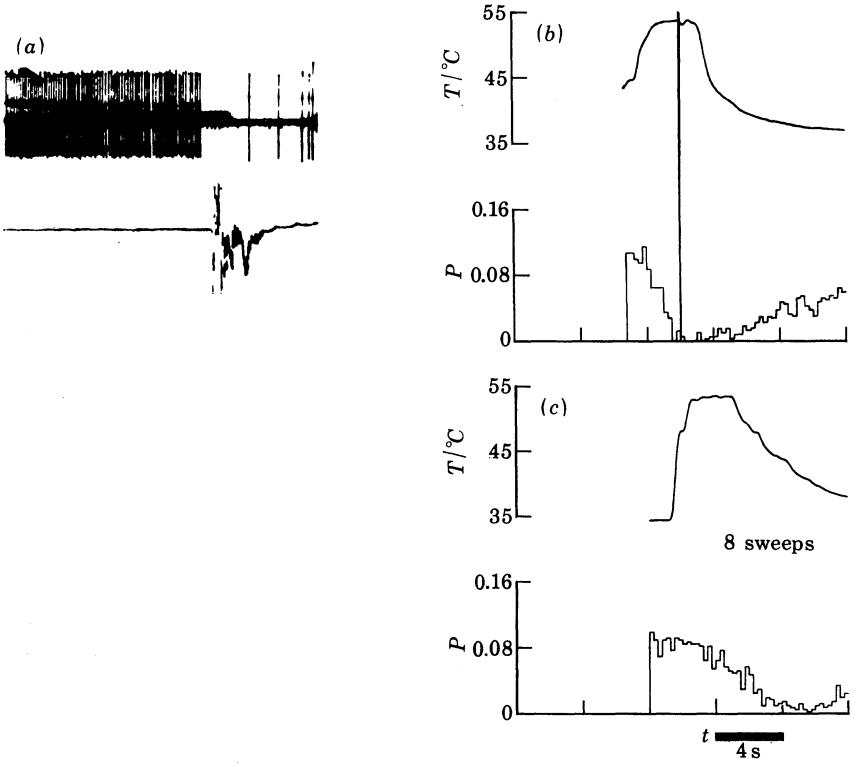

FIGURE 4. Representative off-cell in r.v.m. (*a*) Abrupt pause in unit activity (upper trace) before occurrence of tail flick (transducer output in lower trace). (Single 10 s sweep.) (*b*, *c*) Upper trace (*T*) of each figure indicates mean tail temperature at stimulation site, lower trace (*P*) is spike probability/number of sweeps. In (*b*) spike data are plotted with reference to time of the flick. An abrupt pause in spike probability is observed. In (*c*) temperature traces are aligned and the fall in spike probability is more gradual. This illustrates a closer correlation of the pause in cell firing to the flick than to the stimulus that elicits it.

r.v.m. stimulation is terminated. It then occurs with a latency similar to that observed from the onset of the off-cell pause in the absence of r.v.m. stimulation. As discussed in the previous section, the fact that very low currents are required to block the tail flick (or paw withdrawal) indicates either that sustained discharge in only a few (30 or less) off-cells is sufficient to block tail flick or that the effect of stimulation spreads by neural connections to maintain firing in a larger number of off-cells.

If the off-cells are the r.v.m. output cells that inhibit nociceptive transmission, it should be possible to demonstrate that off-cells project to the spinal cord. We have done this by antidromically activating physiologically characterized off-cells from the cervical d.l.f. (Vanegas *et al.* 1984*b*). However, significant percentages of on-cells and neutral cells in r.v.m. can also be antidromically activated from cervical spinal cord.

Electrical stimulation of p.a.g. similarly does not differentiate on- and off-cells. The fact that with p.a.g. stimulation off-cells are excited at or near the threshold for tail flick suppression is consistent with the idea that the off-cell is the r.v.m. output cell that mediates s.p.a. However, on-cells are also excited by p.a.g. stimulation (Vanegas *et al.* 1984*a*). On- and off-cells are, on the other hand, clearly distinguished by their responses to analgesic doses of morphine. Whether given systemically (Fields *et al.* 1983*b*; Barbaro *et al.* 1984) or by microinjection into the midbrain (Heinricher *et al.* 1984), opiates have opposite effects on these two cell types in r.v.m.

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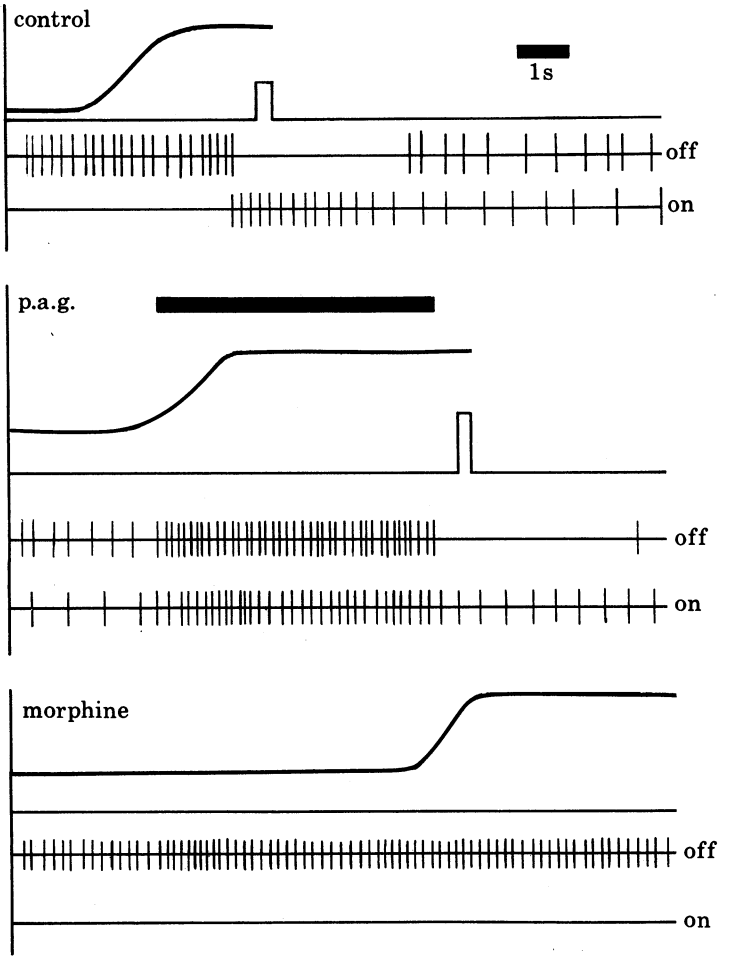

FIGURE 5. Summary diagram illustrating effect of p.a.g. stimulation and morphine on tail flick-related discharge of off- and on-cells in r.v.m. In each trace, the top line represents tail temperature, the second line the output of the tail movement transducer, and the third and fourth lines the discharge of an off- and on-cell respectively. Control: Off-cell pause and on-cell acceleration occur just prior to tail flick. P.a.g.: Heavy line indicates microstimulation in the p.a.g. (50 Hz continuous train, 400 μs pulse duration at threshold for tail flick suppression, 10 μA or less). Both off- and on-cell discharge increase. Tail flick occurs approximately 0.5 s after termination of p.a.g. stimulation. Effect of an analgesic dose of morphine sulphate administered systemically (2.5–5.0 mg kg^{-1}) or microinjected into the p.a.g. (5 μg). Tail flick is suppressed. Spontaneous discharge of the off-cell increases, and that of the on-cell ceases. Tail heating now has no effect on the firing of either cell. Naloxone (not shown) returns the pattern to that seen in the control trace.

Off-cells accelerate and on-cells stop firing when morphine sufficient to block the tail flick is administered. The characteristic on-cell acceleration and off-cell pause also disappear at this point. The effects on background activity and on flick-related activity are naloxone-reversible.

Thus the available evidence supports the hypothesis that the off-cells are the r.v.m. output elements that inhibit nociceptive transmission at the spinal cord. It also indicates that off-cells in the r.v.m. contribute to the analgesic effect of systemically administered morphine.

Off-cells are inhibited, rather than excited, by noxious input, meaning that they are not the feedback elements in a simple negative feedback loop that modulates nociceptive afferent input. In a negative feedback loop such as we (Basbaum & Fields 1978) and others (Melzack & Wall 1965; Mayer & Price 1976) have proposed, the inhibiting element should be excited by noxious

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input. That the off-cell is not excited by such stimuli requires an alternative organizing concept of the network that recognizes that factors other than noxious input, such as motivational state, stress and conditioning, are critical in determining the net activity of the nociceptive modulatory system. It will be important to study the neural mechanisms that produce the off-cell pause and those 'physiological' conditions under which noxious stimuli do *not* inhibit off-cells in order to identify the nature of the network in which the off-cell functions.

The role of the on-cell remains obscure. The fact that on-cell discharge is so tightly coupled to the nociceptor-induced tail flick, that it is excited by p.a.g. stimulation, projects to the spinal cord, and is profoundly inhibited by opiates, does suggest a role in nociceptive modulation. Although there is no direct evidence, the reciprocal relationship of its firing to that of the off-cell raises the possibility that one cell type inhibits the other. If so, the on-cell would have a facilitatory or permissive role in nociceptive control. This would also raise the possibility that the connections between on- and off-cells are crucial for the operation of the network. The nature of these connections, the transmitters involved and the contribution of the afferent systems that impinge upon r.v.m. and p.a.g. cells are important areas for future work.

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References

Abols, I. A. & Basbaum, A. I. 1981 Afferent connections of the rostral medulla of the cat: a neural substrate for midbrain-medullary interactions in the modulation of pain. *J. comp. Neurol.* **201**, 285–297.

Azami, J., Llewelyn, M. B. & Roberts, M. H. T. 1982 The contribution of nucleus reticularis paragigantocellularis and nucleus raphe magnus to the analgesia produced by systemically administered morphine, investigated with the microinjection technique. *Pain* **12**, 229–246.

Barbaro, N. M., Hammond, D. L. & Fields, H. L. 1983 Antagonism of microstimulation-produced analgesia by intrathecal injection of methysergide and yohimbine. *Soc. Neurosci. Abstr.* **9**, 791.

Barbaro, N. M., Heinricher, M. M. & Fields, H. L. 1984 Differential effect of morphine on three classes of medullary neurons. *Pain*, suppl. 2, p. 221.

Basbaum, A. I. & Fields, H. L. 1978 Endogenous pain control mechanisms: review and hypothesis. *Ann. Neurol.* **4**, 451–462.

Basbaum, A. I. & Fields, H. L. 1979 The origin of descending pathways in the dorsolateral funiculus of the cord of the cat and rat: further studies on the anatomy of pain modulation. *J. comp. Neurol.* **187**, 513–532.

Basbaum, A. I. & Fields, H. L. 1984 Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *A. Rev. Neurosci.* **7**, 309–338.

Basbaum, A. I., Clanton, C. H. & Fields, H. L. 1976 Opiate and stimulus-produced analgesia: functional anatomy of a medullo-spinal pathway. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4685–4688.

Basbaum, A. I., Clanton, C. H. & Fields, H. L. 1977 Three bulbospinal pathways from the rostral medulla of the cat. An autoradiographic study of pain modulating systems. *J. comp. Neurol.* **178**, 209–224.

Basbaum, A. I., Moss, M. S. & Glazer, E. J. 1983 Opiate and stimulation produced analgesia: the contribution of monoamines. *Adv. Pain Res. Ther.* **5**, 323–339.

Behbehani, M. M. & Fields, H. L. 1979 Evidence that an excitatory connection between the periaqueductal gray and nucleus raphe magnus mediates stimulation produced analgesia. *Brain Res.* **170**, 85–93.

Beitz, A. J. 1982*a* The sites of origin of brain stem neurotensin and serotonin projections to the rodent nucleus raphe magnus. *J. Neurosci.* **2**, 829–842.

Beitz, A. J. 1982*b* The organization of afferent projections to the midbrain periaqueductal grey of the rat. *Neuroscience* **7**, 133–159.

Bolles, R. C. & Fanselow, M. S. 1982 Endorphins and behavior. *A. Rev. Psychol.* **33**, 87–101.

Brodal, A. 1981 *Neurological Anatomy*, 3rd edn. Oxford University Press.

Carstens, E., Tulloch, I., Zieglgansberger, W. & Zimmerman, M. 1979 Presynaptic excitability changes induced by morphine in single cutaneous afferent C- and A-fibers. *Pflügers Arch. Eur. J. Physiol.* **379**, 143–147.

Casey, K. L. & Morrow, T. J. 1983 Nocifensive responses to cutaneous thermal stimuli in the cat: stimulus response profiles, latencies, and afferent activity. *J. Neurophysiol.* **50**, 1497–1515.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

D'Amour, F. E. & Smith, D. L. 1941 A method for determining loss of pain sensation. *J. Pharmac. exp. Ther.* **72**, 74–79.

Dickenson, A. H., Oliveras, J. L. & Besson, J. M. 1979 Role of the nucleus raphe magnus in opiate analgesia as studied by the microinjection technique in the rat. *Brain Res.* **170**, 95–111.

Dostrovsky, J. O., Hu, J. W., Sessle, B. J. & Sumino, R. 1982 Stimulation sites in periaqueductal gray, nucleus raphe magnus and adjacent regions effective in suppressing oral-facial reflexes. *Brain Res.* **252**, 287–297.

Dubner, R., Ruda, M. A., Miletic, V., Hoffert, M. J., Bennett, G. J. & Coffield, J. 1983 Neural circuitry mediating nociception in the medullary and spinal dorsal horns. In *Neural mechanisms of pain* (ed. L. Kruger & J. C. Liebeskind), vol. 6 (*Adv. Pain Res. Ther.*), pp. 151–166. New York: Raven Press.

Fields, H. L. & Anderson, S. D. 1978 Evidence that raphespinal neurons mediate opiate and midbrain stimulation produced analgesia. *Pain* **5**, 333–349.

Fields, H. L., Basbaum, A. I., Clanton, C. H. & Anderon, S. D. 1977 Nucleus raphe magnus inhibition of spinal cord dorsal horn neurons. *Brain Res.* **126**, 441–453.

Fields, H. L., Bry, J., Hentall, I. D. & Zorman, G. 1983*a* The activity of neurons in the rostral medulla of the rat during withdrawal from noxious heat. *J. Neurosci.* **3**, 2545–2552.

Fields, H. L., Emson, P. C., Leigh, B. K., Gilbert, R. F. T. & Iversen, L. L. 1980 Multiple opiate receptor sites on primary afferent fibres. *Nature, Lond.* **284**, 351–353.

Fields, H. L., Vanegas, H., Hentall, I. D. & Zorman, G. 1983*b* Evidence that disinhibition of brain stem neurones contributes to morphine analgesia. *Nature, Lond.* **306**, 684–686.

Gallager, D. W. & Pert, A. 1978 Afferents to brain stem nuclei (brain stem raphe, nucleus reticularis pontis caudalis and nucleus gigantocellularis) in the rat as demonstrated by microiontophoretically applied horseradish peroxidase. *Brain Res.* **144**, 257–275.

Gebhart, G. F. 1982 Opiate and opioid peptide effects on brain stem neurons: Relevance to nociception and antinociceptive mechanisms. *Pain* **12**, 93–140.

Gebhart, G. F., Sandkuhler, J., Thalhammer, J. G. & Zimmerman, M. 1983 Inhibition of spinal nociceptive information by stimulation in midbrain of the cat is blocked by lidocaine microinjected in nucleus raphe magnus and medullary reticular formation. *J. Neurophysiol.* **50**, 1446–1459.

Giesler, G. J., Gerhart, K. D., Yezierski, R. P., Wilcox, T. K. & Willis, W. D. 1981 Postsynaptic inhibition of primate spinothalamic neurons by stimulation in nucleus raphe magnus. *Brain Res.* **204**, 184–188.

Glazer, E. J. & Basbaum, A. I. 1983 Opioid neurons and pain modulation: an ultrastructural analysis of enkephalin in cat superficial dorsal horn. *Neuroscience* **310**, 357–376.

Glazer, E. J. & Basbaum, A. I. 1984 Axons which take up [^{3}H]serotonin are presynaptic to enkephalin immunoreactive neurons in cat dorsal horn. *Brain Res.* **298**, 389–391.

Grevert, P., Leonard, H. A. & Goldstein, A. 1983 Partial antagonism of placebo analgesia by naloxone. *Pain* **16**, 129–143.

Grumbach, L. & Chernov, H. I. 1965 The analgesic effect of opiate–opiate antagonist combinations in the rat. *J. Pharmac. exp. Ther.* **149**, 385–396.

Guilbaud, G., Peschanski, M., Gautron, M. & Binder, D. 1980 Responses of neurons of the nucleus raphe magnus to noxious stimuli. *Neurosci. Lett.* **17**, 149–154.

Hagbarth, K. E. & Kerr, D. I. B. 1954 Central influences on spinal afferent conduction. *J. Neurophysiol.* **17**, 295–307.

Hayes, R. L., Bennett, G. J., Newlon, P. G. & Mayer, D. J. 1978 Behavioral and physiological studies of non-narcotic analgesia in the rat elicited by certain environmental stimuli. *Brain Res.* **155**, 69–90.

Head, H. & Holmes, G. 1911 Sensory disturbances from cerebral lesions. *Brain* **34**, 102–254.

Heinricher, M. M., Cheng, Z. F. & Fields, H. L. 1984 Differential effect of microinjection of morphine into the periaqueductal gray on activity of two classes of neurons in the rostral ventromedial medulla. *Soc. Neurosci Abstr.* **10**, 100.

Hentall, I. D. & Fields, H. L. 1979 Segmental and descending influences on intraspinal thresholds of single C-fibers. *J. Neurophysiol.* **42**, 1527–1537.

Hentall, I. D., Zorman, G., Kansky, S. & Fields, H. L. 1984*a* The interrelations among threshold, spike height, electrode distance, and conduction velocity in electrical stimulation of certain medullospinal neurons. *J. Neurophysiol.* **51**, 968–977.

Hentall, I. D., Zorman, G., Kansky, S. & Fields, H. L. 1984*b* An estimate of the minimum number of brainstem neurons required for inhibition of a flexion reflex. *J. Neurophysiol.* **51**, 978–985.

Hosobuchi, Y., Adams, J. E. & Linchitz, R. 1977 Pain relief by electrical stimulation of the central gray matter in humans and its reversal by naloxone. *Science, Wash.* **197**, 183–186.

Huisman, A. M., Kuypers, H. G. J. M. & Verburgh, C. A. 1981 Quantitative differences in collateralization of the descending spinal pathways from red nucleus and other brainstem cell groups in rat as demonstrated with multiple fluorescent retrograde tracer technique. *Brain Res.* **209**, 271–286.

Huisman, A. M., Kuypers, H. G. J. M. & Verburgh, C. A. 1982 Differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in cat and monkey. In *Anatomy of descending pathways to the spinal cord* (ed. H. G. J. M. Kuypers & G. F. Martin), vol. 57 (*Progress in brain research*), pp. 185–217. Amsterdam: Elsevier Biomedical Press.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Lasagna, L. 1965 Drug interaction in the field of analgesic drugs. *Proc. R. Soc. Med.* **58**, 978–983.
Levine, J. D., Gordon, N. C. & Fields, H. L. 1979 Naloxone dose dependently produces analgesia and hyperalgesia in postoperative pain. *Nature, Lond.* **278**, 740–741.
Levine, J. D., Lane, S. R., Gordon, N. C. & Fields, H. L. 1982 A spinal opioid synapse mediates the interaction of spinal and brainstem sites in morphine analgesia. *Brain Res.* **236**, 85–91.
Levine, J. D., Murphy, D. T., Seidenwurm, D., Cortez, A. & Fields, H. L. 1980 A study of the quantal (all-or-none) change in reflex latency produced by opiate analgesics. *Brain Res.* **201**, 129–141.
Lewis, J. W., Cannon, J. T. & Liebeskind, J. C. 1980 Opioid and non-opioid mechanisms of stress analgesia. *Science, Wash.* **208**, 623–625.
Lovick, T. A. & Robinson, J. P. 1983 Bulbar raphe neurones with projections to the trigeminal nucleus caudalis and the lumbar cord in the rat: a fluorescence double-labelling study. *Expl Brain Res.* **50**, 299–308.
Maier, S. F., Drugan, R. C. & Grau, J. W. 1982 Controllability, coping behavior, and stress-induced analgesia in the rat. *Pain* **12**, 47–56.
Mantyh, P. W. 1982 The ascending input to the midbrain periaqueductal gray of the primate. *J. comp. Neurol.* **211**, 50–64.
Mantyh, P. W. & Peschanski, M. 1982 Spinal projections from the periaqueductal grey and dorsal raphe in the rat, cat and monkey. *Neuroscience* **7**, 2769–2776.
Mayer, D. J. & Liebeskind, J. C. 1974 Pain reduction by focal electrical stimulation of the brain: an anatomical and behavioral analysis. *Brain Res.* **68**, 73–93.
Mayer, D. J. & Price, D. D. 1976 Central nervous system mechanisms of analgesia. *Pain* **2**, 379–404.
Melzack, R. & Wall, P. D. 1965 Pain mechanisms: a new theory. *Science, Wash.* **150**, 971–979.
Pompeiano, O. 1973 Reticular formation. In *Handbook of sensory physiology* (ed. A. Iggo), vol. 2 (*Somatosensory system*), pp. 381–488. Berlin: Springer.
Prieto, G. J., Cannon, J. T. & Liebeskind, J. C. 1983 Nucleus raphe magnus lesions disrupt stimulation produced analgesia from ventral but not dorsal midbrain areas in the rat. *Brain Res.* **261**, 53–57.
Reynolds, D. V. 1969 Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science, Wash.* **164**, 444–445.
Richardson, D. E. & Akil, H. 1977 Pain reduction by electrical brain stimulation in man. *J. Neurosurg.* **47**, 178–183.
Rosenfeld, J. P. & Stocco, S. 1981 Effects of midbrain, bulbar and combined morphine microinjections and systemic injections on orofacial nociception and rostral trigeminal stimulation: Independent midbrain and bulbar opiate analgesia systems. *Brain Res.* **215**, 342–348.
Ruda, M. A. 1982 Opiates and pain pathways: demonstration of enkephalin synapses on dorsal horn projection neurons. *Science, Wash.* **215**, 1523–1525.
Satoh, M., Akaike, A. & Takagi, H. 1979 Excitation by morphine and enkephalin of single neurons of nucleus reticularis paragigantocellularis in the rat: a probable mechanism of analgesic action of opioids. *Brain Res.* **169**, 406–410.
Satoh, M., Oku, R. & Akaike, A. 1983 Analgesia produced by microinjection of l-glutamate into the rostral ventromedial bulbar nuclei of the rat and its inhibition by intrathecal α-adrenergic blocking agents. *Brain Res.* **261**, 361–364.
Satoh, M. & Takagi, H. 1971 Enhancement by morphine of the central descending inhibitory influence on spinal sensory transmission. *Eur. J. Pharmac.* **14**, 60–65.
Soper, W. Y. & Melzack, R. 1982 Stimulation-produced analgesia: evidence for somatotopic organization in the midbrain. *Brain Res.* **251**, 301–311.
Vanegas, H., Barbaro, N. M. & Fields, H. L. 1984*a* Midbrain stimulation inhibits tailflick only at currents sufficient to excite rostral medullary neurons. *Brain Res.* (In the press.)
Vanegas, H., Barbaro, N. M. & Fields, H. L. 1984*b* Tail flick related activity in medullospinal neurons. *Brain Res.* (In the press.)
Wall, P. D. 1967 The laminar organization of dorsal horn and effects of descending impulses. *J. Physiol., Lond.* **188**, 403–423.
Wall, P. D. & Sweet, W. H. 1967 Temporary abolition of pain in man. *Science, Wash.* **155**, 108–109.
Watkins, L. R. & Mayer, D. J. 1982 Organization of endogenous opiate and non-opiate pain control systems. *Science, Wash.* **216**, 1185–1192.
Willis, W. D., Haber, L. H. & Martin, R. F. 1977 Inhibition of spinothalamic tract cells and interneurons by brain stem stimulation in the monkey. *J. Neurophysiol.* **40**, 968–981.
Yaksh, T. L. 1978 Narcotic analgesics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. *Pain* **4**, 299–359.
Yeung, J. C. & Rudy, T. 1980 Sites of antinociceptive action of systemically injected morphine: involvement of supraspinal loci as revealed by intracerebroventricular injection of naloxone. *J. Pharmac. exp. Ther.* **215**, 626–632.
Zorman, G., Belcher, G., Adams, J. E. & Fields, H. L. 1982 Lumbar intrathecal naloxone blocks analgesia produced by microstimulation of the ventromedial medulla in the rat. *Brain Res.* **236**, 77–84.
Zorman, G., Hentall, I. D., Adams, J. E. & Fields, H. L. 1981 Naloxone-reversible analgesia produced by microstimulation in the rat medulla. *Brain Res.* **219**, 137–148.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES