Chapter 48
Nociceptive On- and Off-Cells in the Mesopontine Tegmentum

Jonathan Dennis Carlson, M.D.., Ph..D., Nathan Richard Selden, M.D., Ph.D., Kim James Burchiel, M.D., and Mary Magdalen Heinricher, Ph.D.

INTRODUCTION

The brainstem modulates the spinal cord nociceptive system through important links between the periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM). This network can enhance or suppress nociceptive processing, and it plays a role in facilitated pain states as well as opioid analgesia (14, 37). Within the RVM the neurophysiological network producing antinociception has been studied extensively (14). This RVM noci-modulatory circuit is composed of two classes of neurons defined by changes in activity associated with nocifensive reflexes such as the tail flick or paw withdrawal evoked by noxious heat. ¡§On-cells¡¨ increase firing just before the occurrence of such reflexes, and these neurons play a pro-nociceptive role. By contrast, ¡§off-cells¡¨ pause just prior to a nocifensive reflex, and selective activation of off-cells produces antinociception (15, 25, 26, 36). Opioids produce analgesia by activating off-cells (25). Neurons without reflex-related changes in firing are termed ¡§neutral cells,¡¨ and whether they have any role in nociceptive modulation is not presently known.

Now that the functions of the on- and off-cells are well established a new question has arisen. Are there other brainstem regions and neurochemical systems that modulate the RVM in addition to the PAG and opioids? If there are other regions, then they likely will contain neurons with similar neurophysiologically characteristics as the RVM, specifically on- and off-cells. Thus the aim of the present study was to search for areas containing neurons with nociceptive properties similar to those in the RVM. Early data suggested that the mesopontine tegmentum, a region lateral to the periaqueductal gray, may be involved in nociceptive modulation (11, 19).

The mesopontine tegmentum encompasses several nuclei in the midbrain and dorsal pons. This includes the cuneiform nucleus, and the cholinergic neurons found in the pedunculopontine tegmental nucleus (PPTg) that extend caudo-medially into the PAG, where they comprise the lateral dorsal tegmental nucleus (LDTg) (38, 39). Electrical or chemical stimulation of the mesopontine tegmentum in rats increases the nociceptive threshold (2, 11, 13, 18, 19, 29-34, 47). This antinociception may be mediated via cholinergic connections from the PPTg to the RVM (38, 46), since focal administration of cholinergic agents into the RVM depresses nociceptive responding (3, 5, 6, 9, 41).

The current study searched for neurons in the mesopontine tegmentum using the same methods and equipment routinely used to identify on- and off-cells in the RVM. Briefly, neurons with neurophysiologically indistinguishable characteristics as those of the RVM were discovered in the mesopontine tegmentum.

METHODS

All procedures were approved by the Oregon Health Sciences University Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Sasco, 250¡V350 g) were anesthetized a continuous i.v. infusion of pentobarbital (3.5 to 6.5 mg/hr). Extracellular microelectrode recording was conducted in the mesopontine tegmentum using a stainless steel microelectrode. Electrolytic lesions were made at the deepest recorded neuron.

Data were collected from every spontaneously active neuron encountered along the electrode track. No search stimulus was used. Once a neuron was identified, the level of spontaneous activity was recorded for at least 3 minutes, or up to 30 minutes if the neuron displayed fluctuations in firing rate. Responses to noxious pinch (toothed forceps applied to the left hind paw or proximal tail, 2¡V5 s) or related to the tail flick reflex were then tested. The tail flick was evoked as follows. A controlled heat stimulus was applied to the blackened ventral surface of the tail. Feedback-controlled heat from a projector bulb was ramped from a resting temperature of 33 „aC at 2 „aC/s until tail flick movement was detected or until a maximum of 55 „aC.

At least three tail flick trials and three pinch trials were tested for each cell, typically in an alternating fashion, with at least 3 minutes separating the trials. If the noxious stimulus produced a prolonged neuronal response or induced a transition in cycling from inactive to active or vice versa, the next stimulus trial was delayed until the cell had returned to a baseline firing pattern. Peri-event time histograms were generated for each cell around the time of the heat-evoked tail movement and pinch onset. Cell cycling was defined when a neuron spontaneously transitioned between quiet and active periods (4, 21).

At the conclusion of the recording, the rat was euthanized and perfused. Brain sections were stained with NADPH diaphorase (2 mg/ml reduced ƒÒ-NADPH, 1 mg/ml nitroblue tetrazolium, Sigma), and counterstained with eosin (11, 17). Camera lucida drawings were made of each trajectory marking the lesion center and outline of major nuclear and white matter boundaries as well as the NADPH diaphorase labeled neurons in the PPTg and LDTg. The histological location of each recorded neuron was reconstructed from the microelectrode depth referenced to the lesion position on the specific camera lucida drawing for each trajectory.

RESULTS

Nocifensive Reflex Related Responses

A total of 188 neurons were studied in the mesopontine tegmentum in 23 rats. Seventy-seven cells displayed consistent activation associated with the tail flick (Fig. 48.1). The first spike in the reflex-related activation preceded the tail flick by 0.73 ¡Ó 0.72 s (mean „b SD) and in all but three neurons firing began prior to the tail movement. These 77 neurons were classified as mesopontine tegmental ¡§on cells.¡¨

Fourteen cells showed a consistent tail flick-related inhibition. In all cases, the pause in activity began before the tail flick (Fig. 48.1). These neurons were classified as mesopontine tegmental ¡§off cells.¡¨ A third group of cells displayed no consistent response to noxious thermal or mechanical stimuli. These neurons were categorized as ¡§neutral cells¡¨ (n = 97).

Seventy of 77 on cells were excited by noxious tail pinch, and 12 of 14 off-cells inhibited. In the remaining cells, the tail pinch had no effect. In no instance was a neuron excited by a tail flick and inhibited by a tail pinch or vice versa. However, 10 of the 97 neutral cells were excited by noxious tail pinch, but were still classified as neutral cells since there was no response to thermal noxious tail flick. One neutral cell was inhibited by a tail pinch. Four neutral neurons displayed no change in activity during the pinch, but with release of the pinch there was a transient burst in activity.

Fluctuations in Spontaneous Firing

The on- and off-cells exhibited spontaneous fluctuations in discharge with the absence of stimulation. This ¡§cycling¡¨ was displayed by 41 of the 77 on-cells and 4 of the 14 off-cells. Only two of the 97 neutral-cells demonstrated spontaneous fluctuations in firing. On some occasions, more than one neuron was isolated clearly. These simultaneous recordings from pairs of neurons allowed for an evaluation of whether fluctuations in spontaneous activity were in phase. In all cases in which the pair consisted of two on cells (11 pairs), alterations between active and inactive periods were in phase. By contrast, cycling was out of phase when the pair consisted of an on cell and off cell (4 pairs). These paired recordings suggest that there is a population of on cells that spontaneously cycle together and off cells that cycle together in the mesopontine tegmentum, and both groups cycle out of phase with each other, as has been reported in the RVM (4, 21).

Location of Neurons

The distribution of the recorded neurons is shown in Figure 48.2. On cells and off cells were distributed throughout the mesopontine tegmentum including the cuneiform nucleus, LDTg, and the PPTg. On- and off-cells were encountered frequently in the PPTg and, in particular, the LDTg in the ventral PAG. On- and off-cells were also found more dorsally in the PAG as in previous reports (20). However, active neurons were encountered relatively infrequently in the PAG. Few on cells and off cells were found ventral to the PPTg and PAG. However, this area was not carefully mapped.

DISCUSSION

This study found on- and off-cells in the mesopontine tegmentum using the same methodological approach that previously identified on-and off-cells in the RVM (15, 22-24, 26, 27). These findings extend previous reports of on- and off-cells in the periaqueductal gray and “dorsolateral pontomesencephalic reticular formation” (19, 20) to show that these neurons overlap the cholinergic boundaries of the PPTg and LDTg.

Furthermore, mesopontine on- and off-cells displayed other characteristics similar to RVM neurons. First, these cells cycled between silent and active phases in the absence of stimulation, similar to those observed in the RVM (4, 21, 35). Simultaneous recordings from more than one neuron demonstrated that the mesopontine on-cell population was active synchronously and out-of-phase with the off-cell population, as has been reported for the RVM (4, 21, 35). Thus, the presence of two populations of intermingled neurons displaying opposite response patterns associated with nociceptive reflex behaviors appears to be common to a number of areas within the brainstem reticular formation, including the mesopontine tegmentum. This neurophysiological property suggests that these regions compose a distributed noci-modulatory network.

It is important to note that the neurochemical identity of on- and off-cells in the mesopontine tegmentum was not definitively established in this study. On- and off-cells were found in both cholinergic and noncholinergic regions. Within the PPTg, and even more so in the LDTg, the density of cholinergic neurons is quite high. Thus it is likely that some of these neurons were cholinergic.

Other studies also suggest that cholinergic projections from the PPTg and LDTg nuclei modulate nociception in the RVM. The PPTg is the primary source of cholinergic projections to the RVM (38, 46). Microinjections of cholinergic

agents (muscarinic and nicotinic) into the RVM produce hypoalgesia (3, 9, 10, 29, 30, 34). Similarly, cholinergic agents iontophoresed onto single, but physiologically uncharacterized, RVM neuron are generally excitatory (5, 6, 12, 28, 45).

Some of the long-recognized antinociceptive effects of opioid injections in the PAG may in fact be mediated via cholinergic projections from the PPTg to the RVM. Selective cholinergic neurotoxins and nicotinic or muscarinic antagonists microinjected into the RVM attenuate or block the antinociceptive effect of morphine in the PAG (1, 41). Nevertheless, it remains to be determined specifically how acetylcholine modulates neurophysiologically identified on- and off-cells in the RVM.

Many functions have been attributed to the PPTg, including sleep-wake regulation and origination of sleep related ponto-geniculo-occipital waves (40, 42-44), cholinergic modulation of the thalamus (42), locomotion (16), and modulation of midbrain dopaminergic neurons (7, 8). Each of these diverse functions is related to the broader neuropsychological variable of arousal. An intriguing possibility is that reflex-related responses of mesopontine on- and off-cells contribute to behavioral and alerting responses to noxious stimuli. Such responses may directly influence descending nociceptive-modulatory systems or may alternatively be a part of a coordinated arousal response by the individual to challenging environmental contingencies.

CONCLUSIONS

In summary, the mesopontine tegmentum contains nocifensive reflex-related on- and off-cells with response characteristics indistinguishable from those found in the RVM. These neurons were distributed throughout the mesopontine tegmentum, including in the cholinergic PPTg and LDTg. Discovery of this neurophysiological network of on- and off-cells in the mesopontine tegmentum supports the theory that this area may modulate pain, in part through cholinergic projections to the RVM.

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Fig. 48.1. Representative nocifensive tail flick responses are shown for an on-cell, off-cell, and neutral cell. The top tracing is a rate meter (1 sec bins). The application of tail heat from a resting baseline (heat) is shown, as well as the movement of the tail (flick). *A*, the on-cell activity begins just prior to the tail flick, and in this case, tapered off to baseline. *B*, the off-cell decreased its activity during the heat application then paused just prior to the tail flick. *C*, the neutral cell had no change in activity associated with the heat application.

Fig.48.2. The distribution of neurons in this study is shown on representative outlines. On-cells (*filled circles*), off-cells (*filled squares*), and neutral cells (*open squares*) were distributed throughout the mesopontine tegmentum and periaqueductal gray (PAG). CN: cuneiform nucleus; DLL: dorsal nucleus lateral lemniscus; IC: inferior colliculus; LDTg: lateral dorsal tegmental nucleus; PnO: nucleus reticularis pontis oralis; PPTg: pedunculopontine tegmental nucleus; scp: superior cerebellar peduncle; VLL: ventral nucleus lateral lemniscus.