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Architecture and operation of a neuronal circuit that modulates pain

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SUMMARY

The present article is a narrative review that describes the functional characteristics of the neurons that constitute a system whose task is to facilitate or diminish the defensive reflexes and the sensation of pain elicited by real or potential damage to one of our tissues. These neurons are located in the medulla oblongata and are known as on-cells and off-cells. They have anatomical and functional links with the nociceptive circuits of the spinal cord and the sensory nuclei of cranial nerves and control the nocifensive reflexes as well as the activation of the thalamic neurons that in turn activate the cortical neurons responsible for the sensation of pain. It was shown that the on-cells facilitate nociception when they are activated by excitatory neurotransmitters and that the off-cells inhibit nociception when their activity increases due to the attenuation of the GABAergic synapses that keep them inhibited. The results obtained additionally indicate that on-cells and off-cells are a substrate for the action of endogenous and exogenous substances capable of augmenting or diminishing pain, such as opioids, analgesics like aspirin and metamizol, opioid receptor antagonists like naloxone, cannabinoids, antagonists to synaptic neurotransmitters, etc. Many of the findings described herein were obtained by doctoral students, young researchers or visiting faculty members under the present author's guidance in the Laboratorio de Neurofisiología of the Instituto Venezolano de Investigaciones Científicas (IVIC) or by the author during a one-year research stay as Visiting Full Professor in the University of California San Francisco.

Key words: *pain, analgesia, neuron, synapse, reflex, brainstem, spinal cord.*

RESUMEN

El presente artículo es una revisión narrativa que describe las características funcionales de las neuronas que constituyen un sistema cuya tarea es facilitar o mitigar los reflejos defensivos y la sensación de dolor generados por un daño real o potencial a alguno de nuestros tejidos. Estas neuronas están ubicadas en el bulbo raquídeo y se denominan células-on y células-off. Tienen vínculos anatómicos y funcionales con los circuitos nociceptivos de la médula espinal y de los núcleos sensoriales de los nervios craneales, y controlan tanto los reflejos nocidefensivos como la activación de las neuronas nociceptivas del tálamo que a su vez excitan a las neuronas corticales responsables de la sensación de dolor. Se demuestra que las células-on facilitan la nocicepción al ser activadas por neurotransmisores excitatorios, y que las células-off inhiben la nocicepción cuando su actividad aumenta debido a la atenuación de las sinapsis GABAérgicas que las mantienen inhibidas. Los resultados obtenidos además indican que las células-on y las células-off son substrato para la acción de substancias tanto endógenas como exógenas capaces de aumentar o disminuir el dolor, tales como los opioides, los analgésicos como la aspirina y la dipirona, antagonistas de los receptores a opioides

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como la naloxona, canabinoides, antagonistas de neurotransmisores sinápticos, etc. Muchos de los hallazgos descritos aquí fueron obtenidos por alumnos de doctorado, jóvenes investigadores o profesores universitarios, bajo la dirección de quien esto escribe, en el laboratorio de Neurofisiología del Instituto Venezolano de Investigaciones Científicas (IVIC), o por este autor durante su estadía de un año como Visiting Full Professor en la Universidad de California San Francisco.

Palabras clave: *Dolor, analgesia, neurona, sinapsis, reflejo, tallo cerebral, médula espinal.*

INTRODUCTION

According to the International Association for the Study of Pain, "pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage". Tissue damage triggers action potentials in distal axon terminals of neurons called primary afferent nociceptive neurons. These action potentials then lead to the excitation of nociceptive neurons located in the spinal cord dorsal horn and sensory nuclei of cranial nerves. These neurons in turn send their axons along two pathways. One pathway leads to the excitation of neighboring motoneurons of flexor muscles and thus to the reflex withdrawal of the damaged limb from the noxious agent. The other pathway runs rostral wards and leads to the excitation of neuronal groups in the brainstem and the ventrobasal thalamus (VB). The thalamic neurons then excite neurons in the somatosensory cortex and this excitation creates the *unpleasant sensory and emotional experience* that we call pain. But a noxious agent of a given intensity does not always elicit a withdrawal reflex of similar promptness and strength or a feeling of pain of similar intensity. Indeed, it has been discovered that neurons in the brainstem may increase or decrease the excitability of nociceptive neurons, and thus withdrawal reflexes and pain may become more or less intense or take a shorter or longer time ("latency") to occur. This is the subject of the present article.

In 1969 Reynolds (1) found in laboratory rats that electrical stimulation of the gray matter that surrounds the Sylvian aqueduct in the midbrain (the periaqueductal gray, PAG) inhibits reflexes elicited by noxious manipulations. He called this effect "electrical analgesia". In 1977, Fields at al. (2) found that electrical stimulation of neuronal groups in the nearby rostral ventromedial medulla (RVM) inhibits neurons in the spinal dorsal horn and that this effect is markedly reduced by an ipsilateral lesion of the spinal dorsolateral funiculus (DLF) placed rostrally to the neurons under study. In 1981, Zorman et al. (3) found that stimulation in RVM inhibits withdrawal reflexes (so-called tail flick, TF) induced by noxious stimulation of the rat's tail and that this inhibition can be reversed by systemic administration of naloxone (an antagonist to opioid receptors). From these and other findings emerged the picture of a "descending pain control system" (DPCS) (Figure 1) whose top components were at the PAG and the RVM, whose target was the spinal dorsal horn sensory (Sens.) and motor (Mot.) neurons, whose neurochemical messengers were the endogenous opioids and whose function was the inhibition of nociception ("analgesia").

Fields et al. (4) then made in 1983 a seminal discovery. They recorded the activity of single neurons in RVM and applied noxious heat to the tail of lightly anesthetized rats; the TF occurred after a few seconds, as expected, and, shortly before the TF, a class of RVM neurons, then labeled off-cells, suddenly stopped ("pause") their ongoing firing, and another class of RVM neurons, then labeled on-cells, suddenly increased their firing rate ("burst"). Fields et al. proposed that these neurons were the ones in charge of regulating the activity of spinal nociceptive neurons so that the off-cell pause would disinhibit, and the on-cell burst would facilitate, spinal withdrawal reflexes elicited by tissue damage (Figure 2 on, off). More important: Fields et al. proposed that off-cells inhibit, and on-cells facilitate, the transmission of nociceptive messages up the spinal cord towards the brain and thus pain! Interestingly, in this model, exposure to a noxious agent like, e.g., heat, inhibits off-cells and activates on-cells, thus enhancing both the pain that is felt and the reflexes to avoid it.

Role of on- and off-cells

In 1983 Fields et al. (5) found that during

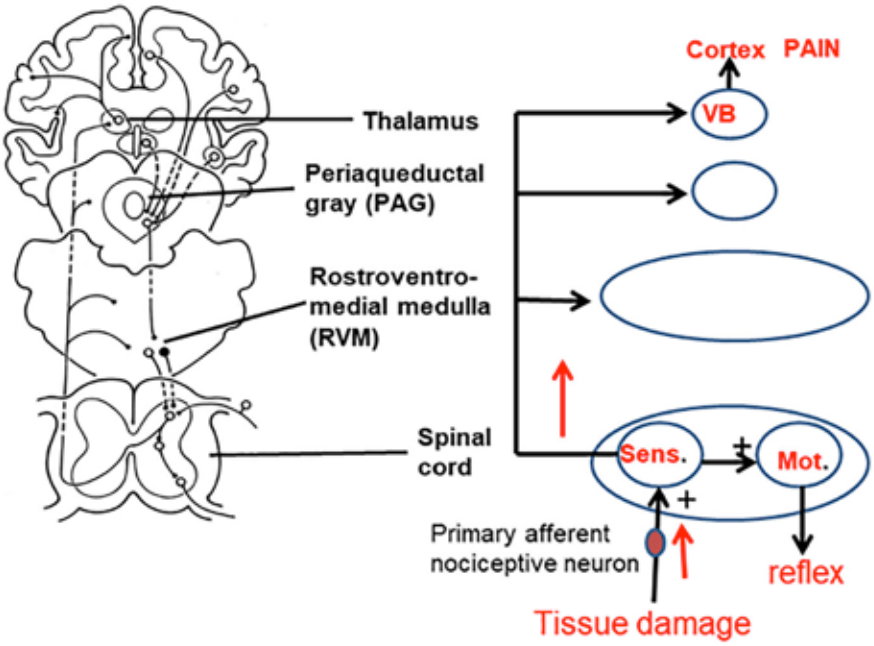

Figure 1. Simplified representation of the neuraxis.

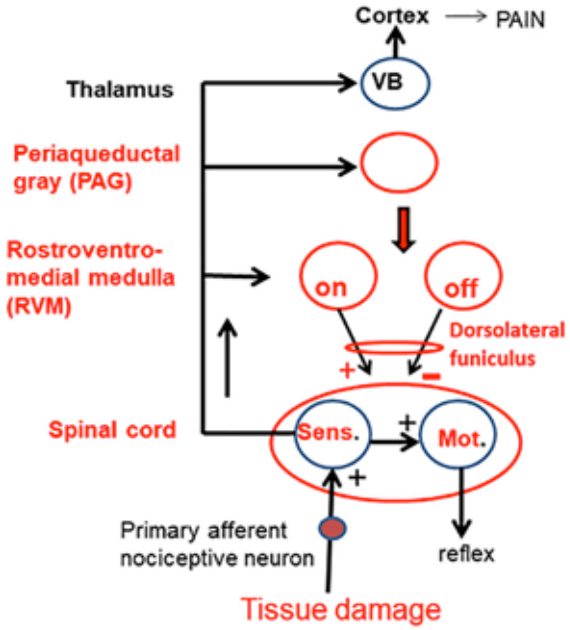

Figure 2. Components of the descending pain control system.

the inhibition of TF ("analgesia") induced by systemic injection of morphine the ongoing activity of on-cells is strongly depressed and their burst is delayed or eliminated whereas the ongoing activity of off-cells is markedly enhanced and their pause is delayed or even fails to occur. This finding was interpreted as showing that opioid analgesia was due to an inhibition of on-cells and thus diminished facilitation of spinal nociception, and activation of off-cells and thus an increased inhibition of nociception by these neurons (Figure 3). This interpretation was supported by the finding of Vanegas, Barbaro, and Fields (6) that excitation of off-cells was necessary for the induction of analgesia by electrical stimulation near the PAG. Vanegas, Barbaro, and Fields (7) simultaneously showed that the on- and off-cells indeed project to the spinal cord along the dorsolateral funiculus (Figure 2), a critical finding for the present model.

More important for the sensation of pain than the activation of a nocifensive reflex is the activation of the spino-thalamo-cortical projection, which leads to the activation of the cortical neurons responsible for this sensory

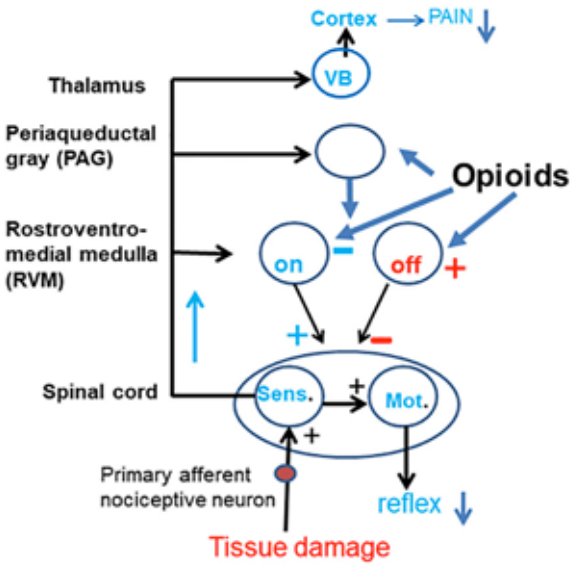

Figure 3. Effects of opioids.

and emotional experience. Hernandez Lopez and Vanegas (8) thus made in 1989, for the first time, recordings in rats from neuronal pairs consisting of a neuron in the ventrobasal (VB) thalamic nucleus and, simultaneously, an on- or an off-cell in RVM. When the tail was heated (45-55 ºC), within each neuronal pair the on-cell began to fire (burst) or the off-cell stopped firing (pause) 0.5-0.6s before the VB neuron responded to the tail stimulus, and also before the TF occurred. Obviously, then, on- and off-cells are related to, in fact, precede both reflexive and cognitive attributes of nociception. Furthermore, Hernandez and Vanegas (9) also found that administration of morphine i.v. concurrently inhibited the on-cell burst, the off-cell pause, and the VB neuron response, thus showing a tight relationship between the workings of on- and off-cells and the flow of pain signals that ascend towards the cortex and cause the sensation known as pain.

Regarding the question of whether on- and off-cells are also related to the action of non-opioid analgesics (e.g. "non-steroidal anti-inflammatory drugs" NSAIDs), Tortorici and Vanegas (10) in 1994, found that metamizol (dipyrone), either given i.v. or microinjected into PAG, caused, like morphine, a dose-related retardation of the off-cell pause, the on-cell burst, and the corresponding TF. Experiments by Tortorici and Vanegas (11) in 1995 with an injectable form of aspirin (lysine-acetylsalicylate) gave similar results: the noxious heat-elicited responses of on- and off-cells and TF retained their mutual time relationship but synchronously shifted toward longer latencies. Tortorici, Vasquez, and Vanegas (12) were then surprised to find in 1996 that the retardation of the off-cell pause, the on-cell burst, and the respective TF caused by metamizol microinjection into PAG was partly reverted by naloxone either i.v. or microinjected into PAG. This means that this NSAID has similar effects as morphine because it is acting by way of the endogenous opioids (Figure 4). These effects of opioids and NSAID sinvolve not only the spinal neurons responsible for the TF; in fact, more important for the sensation of pain are the spinal neurons that give rise to the spino-thalamo-cortical projection (*vide supra*), and Vanegas et al. (13) showed in 1997 that the responses of spinal nociceptive (wide dynamic range) neurons to noxious stimulation of their receptive fields were inhibited by a microinjection of metamizol into PAG. Very important for the experience of pain was the finding of Vasquez and Vanegas (14) in 2000 that the responses of spinal nociceptive neurons to noxious stimulation of their receptive fields were inhibited by a microinjection of metamizol into PAG (Figure 4) and that subsequent microinjection of naloxone into RVM reversed this inhibition.

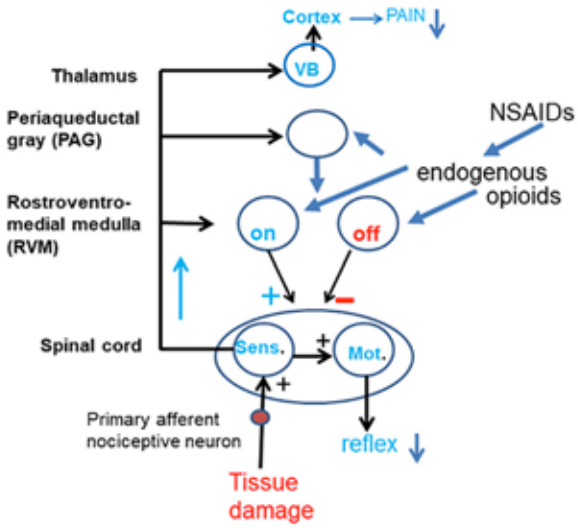

Figure 4. Effects of NSAIDS and opiods.

Marihuana (*Cannabis sativa*) has analgesic properties because some of its components ("cannabinoids") activate receptors (CB1 and CB2) located in the membrane of neurons of the nociceptive system. Meng et al. (15) found in1998 that i.v. injection of a CB1 agonist (WIN) affects on-cells, off-cells, and the TF like that of morphine but is specifically reversed by a CB1 antagonist.

Partial summary

The on- and off-cells are the output axis of a set of neural structures that modulate the transmission of pain signals from spinal nociceptive neurons towards nociceptive reflex circuits and towards the thalamus and which mediate the analgesia induced by opioids (both endogenous and exogenous), NSAIDs and cannabinoids.

The circuitry of on- and off-cells

It was originally assumed that opioids, known to inhibit neurons of various types, would directly inhibit on-cells, but a mechanism for the activation of off-cells by opioids was difficult to visualize. Then in 1992, Heinricher, Morgan, and Fields (16) directly applied morphine to single RVM neurons using an iontophoretic micropipette and found that morphine indeed depressed the activity of on-cells but, surprisingly, had no reliable effect on off-cells. Mason, Back, and Fields (17), in 1992, thus examined the RVM with laser confocal microscopy and found that enkephalin-immunoreactive appositions (i.e., opioid receptors) occur on somata and dendrites of on-cells, but, much less, off-cells. Thus the intense activation of off-cells by opioids remained a puzzle. There was the possibility that the off-cells were normally being inhibited by the on-cells and bounced to a high activity when the on-cells were depressed by opioids. However, in 2008, Cleary, Neubert, and Heinricher (18) carried out simultaneous recordings from one on-cell and one off-cell and found that upon heat application to the tail the off-cell pause always began before the on-cell burst; therefore the on-cells were not responsible for the inhibition of off-cells. Furthermore, infusion of DAMGO (an opioid receptor agonist) into RVM depressed on-cell firing irrespective of TF inhibition, but TF was inhibited only if off-cells were activated (Heinricher et al., 1994) (19). Thus, the disinhibition of off-cells, not inhibition of on-cells, is central to the antinociceptive action of opioids in RVM.

Heinricher, Haws, and Fields (20) found in 1991 that application of bicuculline (a GABA receptor antagonist) to individual RVM neurons by means of an iontophoretic micropipette blocked the TF-related pause of every off-cell they tested. This means that the off-cell pause is caused by a sudden increase in its GABAergic inhibition. On the other hand, bicuculline had no consistent effect on on-cells. Then in 1994, Heinricher and Tortorici (21) found that following a local infusion of bicuculline, off-cells enter a prolonged period of continuous firing that coincides with the period of TF inhibition. On the other hand, on-cells were inhibited by low dose and excited by high dose bicuculline but in both cases, there was TF inhibition. These findings indicate that off-cells are postsynaptic to GABAergic inhibitory neurons (not on-cells) and suggest that such neurons are inhibited by opioids and thereby are the off-cells disinhibited.

Regarding the cause of the on-cell burst, Heinricher and McGaraughty (22) showed in 1998 that an RVM infusion of kynurenate (a non-selective antagonist of excitatory neurotransmitters) produced a significant decrease in on-cell firing, with suppression of the on-cell burst. Off-cells nonetheless continued to display a TF-related pause. TF latency was unaffected by kynurenate infusions. These results demonstrate: 1) that there is a basal ongoing activation of the on-cell by an excitatory synapse and then the on-cell burst is caused by a sudden increase in postsynaptic excitation; 2) that a burst of on-cell firing is not required for the off-cell reflex-related pause and thus do not support the proposed role for on-cells as inhibitory interneurons within the RVM; and 3) that a decrease of on-cell firing does not lead to a depression of the TF. Additional evidence indicating that the role of on- and off-cells are separate, was provided by Neubert, Kincaid, and Heinricher (23). They showed in 2004, that RVM microinjection of low-dose neurotensin activates on-cells, does not affect off-cells and facilitates nocifensive paw withdrawal reflexes, whereas

high-dose neurotensin activates both on- and off-cells but inhibits paw withdrawal. Thus on-cells do facilitate nocifensive reflexes and plausibly pain but reflexes and plausibly pain are inhibited as long as off-cells are activated.

Cells in RVM that possess opioid receptors, including on-cells, can be selectively killed by microinjections of Derm-Sap, a conjugate of the opioid receptor agonist dermorphin and the neurotoxin saporin. Harasawa et al. (24) found in 2016 that, after RVM microinjection of Derm-Sap, microinjection of the opioid receptor agonist DAMGO (*vide supra*), which now has no on-cells to inhibit, does not induce analgesia, which also means, interestingly, that it is not disinhibiting off-cells. On the other hand, electrical stimulation of the PAG, or RVM microinjection of an agonist to synaptic receptors of the excitatory neurotransmitter glutamate, does produce analgesia. This means that off-cells, which have only a few opioid receptors (are not reliably affected by morphine, *vide supra*) indeed survive Derm-Sap treatment and can still be excited by glutamatergic axons from PAG neurons or by the direct action of glutamatergic agonists. Since in the normal animal, both endogenous and pharmacological opioids induce disinhibition of off-cells, it can be stated that opioids inhibit the neurons that are tonically inhibiting the off-cells. These inhibitory neurons must not express opioid receptors or must be located away from RVM because they are still functional after Derm-Sap microinjection in RVM. This is shown by the fact that, if the synaptic transmission in RVM after Derm-Sap is blocked by a microinjection of cobalt-chloride, analgesia ensues, which means that off-cells have become disinhibited (24).

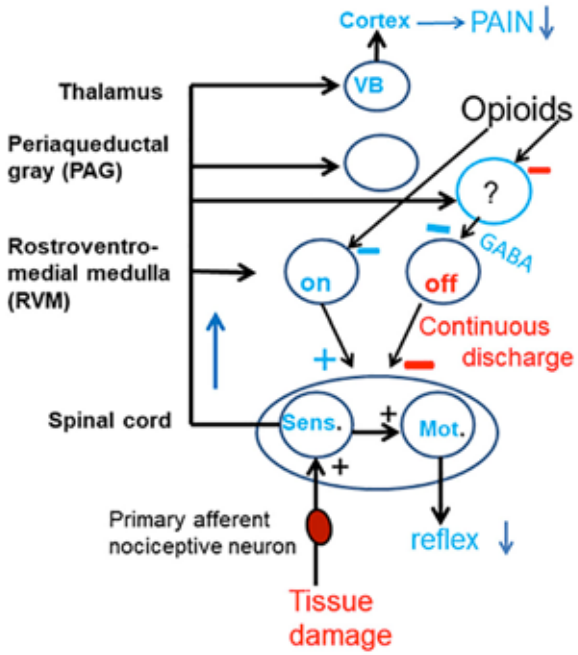

Figure 5. Opiods activate off-cells by inhibiting GABAergic neurons.

GABAergic neurons can in turn be inhibited (also through unknown pathways and mechanisms) by opioids, NSAIDs, and cannabinoids, thus giving rise to off-cell disinhibition and hence a delay or loss of the off-cell pause, a continuously high activity of off-cells and sustained analgesia. On-cells do not seem to be tonically inhibited by GABAergic synapses. On the other hand (Figure 6), there is a basal on going activation of on-cells by excitatory synapses, and the on-cell burst is caused by a surge in this postsynaptic excitation. On-cells are not inhibitory interneurons, and their burst is not the cause of the off-cell pause. A decrease of on-cell firing does not necessarily lead to analgesia, and an increase in on-cell firing facilitates nociception only if off-cells are not simultaneously activated.

Partial summary

Off-cells inhibit the transmission of nociceptive signals that spinally activate nocifensive reflexes and supraspinally excite VB thalamic neurons (Figure 5). Off-cells are activated by glutamatergic neurons from the PAG thus causing analgesia. Off-cells are inhibited by GABAergic neurons that can be activated (through unknown pathways and mechanisms) by noxious stimulation of widespread areas of the body, thus giving rise to the off-cell pause. These

Functional sequence triggered by damage

Based on the findings reviewed above, Figure 6 shows the sequence of events in the neuronal circuit of the Descending Pain Control System

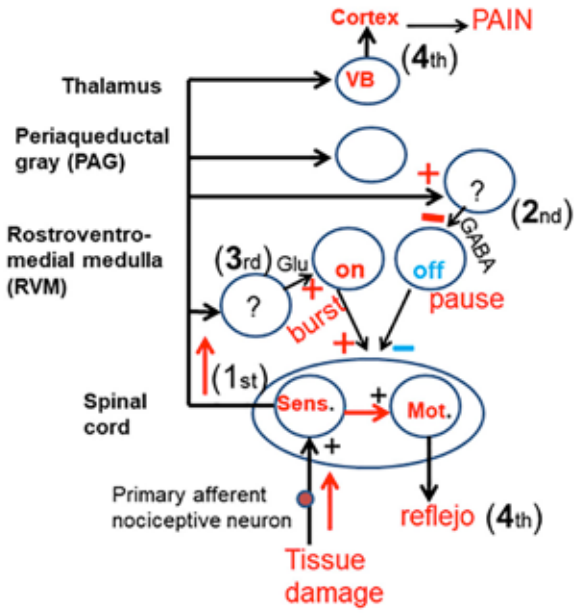

Figure 6. DPCS functional sequence triggered by damage to a tissue.

at the moment when a body tissue is subject to *an actual or potential damage*. In Figure 6 these events are labeled as first (1st), second (2nd), third (3rd), and fourth (4th). The sequence is triggered by the activation of the primary afferent nociceptive neurons in the peripheral nervous system, which then activates central nociceptive neurons (sens.) in the spinal cord or sensory nuclei of cranial nerves (1st). These neurons, in turn, fire action potentials that somehow excite (2nd) GABAergic neurons that inhibit the off-cells and cause their pause. Somehow these action potentials also reach excitatory neurons which cause (3rd) the burst of on-cells. Finally, the action potentials of central nociceptive neurons reach their neighboring flexor motoneurons (mot.) and cause a withdrawal reflex (4th), on one hand, or ascend to excite the VB thalamic neurons which in turn excite the cortical neurons (4th) that sustain the *unpleasant sensory and emotional experience* that we call pain.

Final comments

This circuit is useful not only when there is some tissue damage. Perhaps more important is its role as a substrate for endogenous mediators and pharmaceuticals which modulate the intensity of pain, like, e.g., opioids (whether endogenous or exogenous), NSAIDs, substance P, neurotensin, cholecystokinin, endogenous and exogenous transient receptor potential vanilloid 1 (TRPV1) receptor agonists (like, e.g. the capsaicin of hot peppers), etc. This circuit is also critically involved in clinically important disturbances like tolerance and withdrawal syndrome related to opioids or NSAIDs, and like neuropathic pain, inflammatory hyperalgesia, secondary pain, referred pain, and many others.

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