

# Animal Models of Nociception

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**Abstract**—The study of pain in awake animals raises ethical, philosophical, and technical problems. We review the ethical standards for studying pain in animals and emphasize that there are scientific as well as moral reasons for keeping to them. Philosophically, there is the problem that pain cannot be monitored directly in animals but can only be estimated by examining their responses to nociceptive stimuli; however, such responses do not necessarily mean that there is a concomitant sensation. The types of nociceptive stimuli (electrical, thermal, mechanical, or chemical) that have been used in different pain models are reviewed with the conclusion that none is ideal, although chemical stimuli probably most closely mimic acute clinical pain. The monitored reactions are almost always motor responses ranging from spinal reflexes to complex

behaviors. Most have the weakness that they may be associated with, or modulated by, other physiological functions. The main tests are critically reviewed in terms of their sensitivity, specificity, and predictiveness. Weaknesses are highlighted, including 1) that in most tests responses are monitored around a nociceptive threshold, whereas clinical pain is almost always more severe; 2) differences in the fashion whereby responses are evoked from healthy and inflamed tissues; and 3) problems in assessing threshold responses to stimuli, which continue to increase in intensity. It is concluded that although the neural basis of the most used tests is poorly understood, their use will be more profitable if pain is considered within, rather than apart from, the body's homeostatic mechanisms.

## I. Introduction

Sensory systems have the role of informing the brain about the state of the external environment and the internal milieu of the organism. Pain is a perception, and as such, it is one of the outputs of a system in more highly evolved animals—the nociceptive system—which itself is a component of the overall set of controls responsible for homeostasis. In this context, pain constitutes an alarm that ultimately has the role of helping to protect the organism: it both triggers reactions and induces learned avoidance behaviors, which may decrease whatever is causing the pain and, as a result, may limit the (potentially) damaging consequences. At the beginning of the twentieth century, Sherrington (1910) developed

this concept and introduced the term *nociception* (from the Latin *nocere*, “to harm”). It seems appropriate to take the view of Dennis and Melzack (1983) that pain/nociception has at least three functions: 1) to warn the individual of the existence of real tissue damage; 2) to warn the individual of the probability that tissue damage is about to occur by realizing that a stimulus has the potential to cause such damage; and 3) to warn a social group of danger as soon as it exists for any one its members. Behaviors resulting from pain can facilitate other fundamental biological functions, such as the maintenance of tissue “trophicity” and regeneration (notably in the processes of inflammation and healing). The importance of these behaviors is well illustrated in humans through pathological cases of congenital insensitivity to painful stimuli, in which truly natural experiences can have catastrophic consequences.

The complexity of nociceptive systems, which ultimately produce pain, has increased during evolution as a result of the pressure to avoid organic lesions or their aggravation (Walters, 1994). One can easily see the evo-

<sup>2</sup> Abbreviations: IASP, International Association for the Study of Pain; NSAIDs, nonsteroidal anti-inflammatory drugs; R<sub>III</sub>, nociceptive flexion; FRA, flexor reflex afferent; R, reaction time; Lb, biological latency; Lp, physical latency; Tt, true threshold; %MPE, percentage of the maximum possible effect; Co, cutoff time; PAG, periaqueductal gray; RVM, rostral ventral medulla; Ta, apparent reaction threshold; Ta<sub>t</sub>, apparent reaction threshold after treatment.

lutionary advantage of cutaneous, muscular, and articular pains. However, the Sherringtonian concept of a nociceptor alarm system is more debatable in the context of visceral pain, given that serious lesions can develop painlessly in noninflammatory conditions and that viscera may contain only a few fibers that respond preferentially to nociceptive stimuli (Cervero, 1991, 1994; McMahon et al., 1995).

Therefore, like other body functions, the physiological system that generates pain can also be affected by pathological processes. In the context of chronic pain, which can last months or even years, the physiological protective effect gives way to a pathological state that is not only useless but also highly distressing. There are models of chronic pain in animals such as the rat with induced arthritis and rats that have had various lesions to the central or peripheral nervous systems (Colpaert, 1987; Butler, 1989; Dong, 1989; Rossitch, 1991; Zeltser and Selzer, 1994; Seltzer, 1995; Tjølsen and Hole, 1997; Kaupilla, 1998). However, these fall outside the purview of this review, which is restricted to models of acute pain, i.e., pain evoked by a brief noxious stimulus and generated by a nociceptive system functioning normally within its physiological limits.

The absence of verbal communication in animals is undoubtedly an obstacle to the evaluation of pain. There are circumstances during which there can be little doubt that an animal is feeling pain—notably when it is responding to stimuli through vocal responses such as squealing or groaning. On the other hand, it is far more difficult to certify that at a given moment, an animal feels no pain because it is presenting no typical physical signs or overt behaviors. This is particularly so given that we know that immobility and/or prostration are sometimes the only responses accompanying pain. The question of pain in animals can be approached only with anthropomorphic references, although differences probably do exist by comparison with humans, notably in respect of certain cerebral structures (Bateson, 1991). In this regard, the degree of cortical development has to be considered (Vierck, 1976), and it is reasonable to conclude that differences do exist between humans and animals, at least, but perhaps not only, with respect to the psychological repercussions. Neurological observations of human patients allow us to make some comparisons. Thus, like Lineberry (1981), one might question whether it is appropriate to consider any pain in patients who have undergone frontal lobotomies as being similar to the pains we feel: although the pain that they experience is unaltered at a sensory level, it has lost its emotional and motivational dimensions (Freemann and Watts, 1946; Foltz and White, 1962; Sweet, 1973). Furthermore, the International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage” (Merskey et al., 1979).

In contrast with the polymorphic nature of the pain that is described as a sensation in humans, pain in animals can be estimated only by examining their reactions. This is essentially the same difficulty that is faced by the pediatrician, the geriatrician, or the psychiatrist dealing with patients incapable of expressing themselves verbally. In those cases as well, the symptomatology is not unequivocal—it has to be taken in context and placed in an inventory, because its meaning will differ depending on the degree of maturation (or degradation) of the nervous system. In addition, one must never forget that the existence of a reaction is not necessarily evidence of a concomitant sensation (Hardy et al., 1943). Indeed, anesthetists see a dissociation between these phenomena every working day. Consequently, we must consider reactions within a more global context, which includes other considerations such as the homeostatic mechanisms of the animal (see *Section XII.E.*).

We will restrict this review to mammals, particularly rodents, since they are used in almost all animal models of pain. Reviews of nociception and/or pain in other species can be found in the articles by Kavaliers (1988), Bateson (1991), and Walters (1994). The essential mechanisms that make it possible for an organism to react to a stimulus, which might endanger its existence (including sensory perception), exist throughout the animal kingdom, except perhaps in arthropods and particularly in insects (Eisemann et al., 1984; Walters, 1994).

Generally speaking, the most reliable signs of pain are physical ones. Research in humans and in animals has focused on various biochemical indicators (catecholamines, corticoids, opioids, etc.), but these all seem to be without specificity. One can say the same for other methods such as electrophysiological parameters—electroencephalograms, evoked potentials, etc. (Molony, 1986; Ichinose et al., 1999). For the time being, the study of behavioral reactions provides the only indicator of the perceived disagreeable sensation resulting from a stimulus that would be algogenic in humans, but it must never be forgotten that these responses are often not very specific; for example, escape can result from any disagreeable stimulus whether or not it is noxious.

Descriptions of the “signs” of pain have been published on several occasions in a veterinary or an animal-welfare context (Gibson and Paterson, 1985; Morton and Griffith, 1985; Flecknell, 1986; Sanford et al., 1986; American Veterinary Medical Association, 1987; Sanford, 1992, 1994; Baumans et al., 1994). Above all else, it must be emphasized that these signs have no unequivocal value and that each species expresses pain in a manner related to its own behavioral repertoire. It is in that context that the description of each becomes interesting and allows the inventory of the principal clinical signs to be refined (Gibson and Paterson, 1985; Morton and Griffith, 1985).

For example, one can distinguish the following reactions produced by a (presumably) painful focus: 1) re-

sponses organized by centers that are relatively “low” within the hierarchy of the central nervous system; and 2) more complex responses organized by higher centers in the central nervous system. The former can be elicited in decerebrate animals and have been termed “pseudoaffective reflexes” (Woodworth and Sherrington, 1904; Sherrington, 1906b). They include basic motor responses (withdrawal, jumping, contractures, etc.), neurovegetative reactions—generally in the context of Selye’s “alarm reaction”, with an increase in sympathetic tone (tachycardia, arterial hypertension, hyperpnea, mydriasis, etc.), and vocalization.

The more complex reactions include conditioned motor responses, which result from a period of learning and sometimes can be very rapid, e.g., cattle avoiding an electrical enclosure. In general, the significance of these has as much to do with preventing new damage as with avoiding aggravating existing lesions. Behavioral reactions (escape, distrust of objects responsible for painful experiences, avoidance, aggressiveness, etc.) or modifications of behavior (social, food, sexual, sleep, etc.) are often observed. By comparison with the responses discussed above, these behaviors provide evidence of far more integrated reactions within the hierarchical organization of the central nervous system. If the stimulus is sufficiently intense, the reaction will be escape or attack. However, it must be noted that even if active motor reactions are frequent, passive motor responses are observed just as often in animals—immobility allowing the animal to preserve a painless posture. Whereas brief and sharp pains are associated with phasic motor responses (withdrawal, startle reactions), lasting pains may be associated with contractures, the consequence of which is to immobilize the painful region. This explains, for example, the frequent hunching of the back in dogs suffering from a slipped disc or abdominal pain. It reminds one of reflexes involving abdominopelvic muscles in humans—the classic “board-like” abdomen seen during peritonitis, hyperalgesic sciatica, etc. Furthermore, in animals, motor atonia is a general response to sickness, whether or not they are in pain. However, there can be spectacular exceptions, such as colic in horses or pancreatitis in dogs.

Zimmermann (1986) re-interpreted the IASP definition of pain so that it could be applied to animals: “an aversive sensory experience caused by actual or potential injury that elicits progressive motor and vegetative reactions, results in learned avoidance behavior, and may modify species specific behavior, including social behavior”.

The term *nociceptive* refers to the potential of a stimulus to produce a tissue lesion and a reaction from the organism. The “algogenic” character of a stimulus is defined by its capacity to produce pain—in an affective and motivational as well as a sensory context. None of these can be observed directly in animals. Cervero and Merskey (1996) recently discussed these terms and gave

some examples related to acute pain. In the same way that menthol excites cold receptors without being a thermal stimulus, capsaicin evokes a sensation of burning without producing tissue damage. Thus, it is a nociceptive stimulus (it activates nociceptors) and an algogenic stimulus (it produces pain), but it is not harmful (and as such cannot be called a “noxious” stimulus). Similarly, a thermal stimulus of 45°C may or may not be harmful depending on the duration of its application (Stoll and Greene, 1959).

In this review, we describe and critically analyze the most commonly used behavioral tests of nociception in animals (Fig. 1). However, we do not claim that the review will be exhaustive. For example, some often complex tests that depend on a period of learning by the animals have been omitted deliberately (Hill et al., 1957; Weiss and Laties, 1958; Weitzman et al., 1961; Evans, 1964; Lineberry, 1981; Chapman et al., 1985; Hammond, 1989; Vierck et al., 1989). We will simply illustrate this type of method with an example (Fig. 2). In addition, complementary information can be found in other reviews (Jacob, 1966; Domer, 1971; Vyklicky, 1979; Vierck and Cooper, 1984; Wood, 1984; Chau, 1989; Watkins, 1989; Dubner, 1994; Tjølsen and Hole, 1997; Dubner and Ren, 1999).

Before describing the most commonly used tests, we will consider successively the ethical problems posed by the study of acute pain in animals, the choice of stimulus, and which type of response can or should be monitored. However, the main part of this review will be devoted to a critical analysis of these tests. Most notably, we will comment on relationships between tests of acute pain and motor responses and then consider successively the sensitivity, specificity, and predictiveness of the tests. The analysis of their sensitivities will lead us to pose the question of which fibers underlie the observed responses and what meaning can be ascribed to measurements of reaction time when a stimulus is grad-

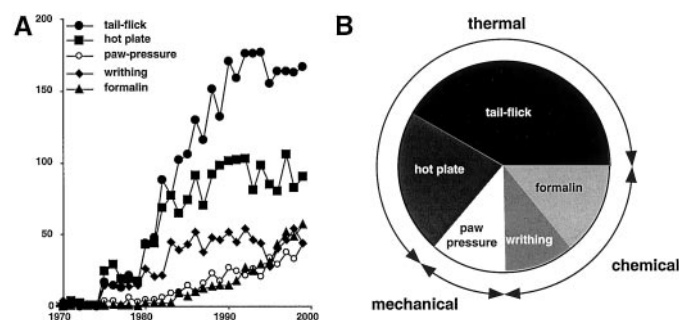


FIG. 1. A, evolution of the number of original articles published during each of the years between 1970 and 1999 (ordinate) in which the authors used one of the five most common tests of nociception (based on Medline). B, relative proportions of these categories of articles appearing during 1999. Of all of these tests, the tail-flick and the hot plate tests remain the most commonly used. Note that the rate of publications regarding the tail-flick, hot plate, and writhing tests stabilized in the 1990s, whereas there was a progressive increase in the number of articles describing the use of the formalin test and the various different tests involving withdrawal of the paws from mechanical stimuli.

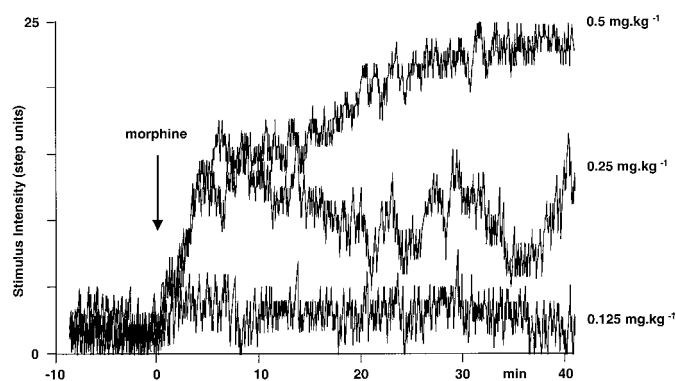


FIG. 2. Results from a test involving a substantial amount of learning by the experimental animal (rhesus monkey). Short bursts of electrical stimuli were applied through electrodes implanted in the trigeminal ganglion of the animal. The stimulus intensity (ordinate) was automatically increased in preset "steps" (approximately 0.75 V) every 5 s. However, the animals had been trained over a period of several weeks to press a lever to reduce this stimulus intensity and the intensity at which they did this was taken as the pain threshold. Thus, upward deflections on the records were produced by subthreshold, automatic increases in the stimulus intensity, whereas downward deflections indicate that the pain threshold had been reached (and that the animal had pressed the lever). The graphs show the effects with time (abscissa) on this pain threshold of three doses of morphine administered intravenously. Records traced from original chart recordings in Weitzman et al. (1961) and modified for clarity of presentation with permission.

ually increasing in intensity. We will also consider some methods of analyzing the results of the tests. Finally, we will examine factors that can disturb the measurement of behavioral responses in animals; in this respect, we pay particular attention to intercurrent physiological functions.

Because the goal of these animal models is the understanding of acute pain in humans, we make reference throughout this review to analogous experimental situations in humans. Notwithstanding the normal caution one must have with any anthropomorphic approach, we believe this is a necessary counterbalance to the reductionism of the so-called basic sciences.

## II. Ethical Problems

As with all biomedical research involving animals, pain research presents ethical problems at two levels (Morton and Griffith, 1985). From a general point of view, investigators have to follow the recommendations of ethics committees and, notably, those of international scientific review boards so as to ensure a given level of physiological well being in the animal. Indeed, if the animal is miserable or in a state of stress in which neurovegetative reactions are exacerbated, it is clear that scientific observations will not be valid from a physiological point of view. Thus, it is not only for moral reasons but also for scientific reasons that some rules have to be observed.

The second point is more specific to studies on pain (Wall, 1975; Sternbach, 1976; Zimmermann, 1983; Casey and Handwerker, 1989; Roberts, 1989). The ethics committee of the IASP has formulated a certain number

of recommendations on this subject. The practical consequences of these are summarized below (Covino et al., 1980; Zimmermann, 1983). In a preamble, the committee stated that experiments are indispensable if we are to gain a better understanding of the mechanisms of pain. As in other areas of biomedical research, the attitudes of scientists is conditioned by how they regard their subject of study: they have to consider the animal not as an object but as a living being gifted with sensations. The committee, while acknowledging that some experiments have the aim of trying to reproduce chronic syndromes in animals, stated clearly that experimental protocols have to minimize or avoid pain (this notion could, a priori, seem paradoxical; however, as we shall see, one can study nociception without producing pain).

The committee's other recommendations with respect to studies of acute pain may be summarized as stating that 1) experiments involving the study of pain on conscious animals must be reviewed beforehand by scientists and lay persons, and the potential benefit of these experiments must be shown; 2) as far as possible, the scientist must test the painful stimuli on himself or herself, and this should apply to most noninvasive stimuli; 3) the scientist should carefully assess all behavioral and physiological changes in the animal and report them in resulting manuscripts; 4) as in other areas of neuroscience, there must be no question of using animals paralyzed with a neuromuscular blocking agent without a general anesthetic or an appropriate surgical procedure that eliminates sensory awareness; and 5) the duration of experiments must be as short as possible, and the number of animals involved must be kept to the minimum.

Studies in conscious animals most commonly involve monitoring the threshold for obtaining a response to a stimulus that would produce pain if applied to humans. Such responses include flexion reflexes and vocalization. In such experimental scenarios, the stimulus is stopped once the response has been obtained. Sometimes algogenic substances are applied briefly—generally under conditions similar to when they are used in studies of experimental pain in humans.

## III. Input and Output: the Stimulus and the Response

There are numerous tests of nociception, and in this review, we do not give an exhaustive list. In his magnificent review published in 1957, Beecher cited 60 original publications related to the description, development, and application of experimental tests of pain in animals. Twenty-seven of these publications were based on the use of thermal stimuli, whereas 10 involved electrical and 23 mechanical stimulation. None was based on the use of chemical stimulation. By comparison, at that time the corresponding numbers of studies in humans were

167 publications: 63 based on thermal, 49 on electrical, 46 on mechanical, and 9 on chemical stimulation.

Experimental studies on conscious animals are often designated “behavioral studies”. Sometimes, this may seem to be stretching the meaning of the word “behavioral”, but what it means is simply and implicitly that all responses—including simple withdrawal reflexes—are part of an animal’s behavioral repertoire. The behavioral tests that are used to study nociception—nociceptive tests—constitute “input-output” systems that function via “black boxes”, which the neurobiologist wishes to decode. As a result, when describing these tests, one must specify the characteristics of the input (the stimulus applied by the scientist) and the output (the reaction of the animal).

Thus, describing these tests should be a simple matter of first accounting for the nature of the stimulus (electrical, thermal, mechanical, or chemical) and then describing the behavioral parameters that are measured. This latter task may involve defining the responses as a function of their increasing complexity (e.g., one might distinguish reflexes from more integrated reactions). In fact, the inputs and outputs of these systems are very intimately linked by the physical characteristics (notably the temporal nature) of the stimulus.

#### A. *The Stimulus*

In humans and in animals, experimental studies of the mechanisms underlying acute pain necessitate the use of appropriate stimuli to provoke the sensation. To be adequate, these stimuli have to be quantifiable, reproducible, and noninvasive (Beecher, 1957; Lineberry, 1981). Although thermal and electrical stimuli can meet these requirements, they also have serious drawbacks.

*1. Electrical Stimulation.* The application of electrical stimuli has the advantages of being quantifiable, reproducible, and noninvasive and of producing synchronized afferent signals. However, it also has serious disadvantages. First, electrical stimuli are not a natural type of stimulus like those encountered by an animal in its normal environment. More importantly, intense electrical stimuli excite in a nondifferential fashion all peripheral fibers, including large diameter fibers, which are not directly implicated in nociception, as well as fine A $\delta$  and C fibers, which mediate sensations of cold and hot as well as nociceptive information. Furthermore, this type of stimulation completely short-circuits peripheral receptors, thus preventing any study of peripheral transduction mechanisms with these methods. On the other hand, this last disadvantage becomes an advantage when one wants to study the actions of a systemically administered substance in the central nervous system: as long as the substance has no action on peripheral fibers, any effect will be of central origin because the transduction processes have been bypassed. Finally, there are difficulties introduced by variations in the impedances of the tissues being stimulated, although

these can be minimized by the use of a constant current stimulator and the monitoring of the voltage as well as the current of the applied stimulus. However, this precaution is not always respected, which may explain the difficulties that are sometimes encountered, notably, the variability in evoked vocal responses (Fennessy and Lee, 1975). It must be emphasized that the use of a constant current stimulator does not solve all the problems because it allows one only to verify that a given current has been delivered, not that it has gone in a consistent fashion to the intended target (e.g., the paws of an animal). The possibility always exists that a variable amount of current may be shunted through other conducting media (e.g., the urine of the animal). To assess whether this is happening, it is essential to monitor the voltage required to generate the current, because this will vary with the impedance of the tissues and thus will indicate whether this impedance is changing during an experiment.

Electricity can be applied in a very brief and sudden fashion. This results in the signals in the afferent nerve fibers being synchronized. Thus, electrical stimuli can release a vast repertoire of behavioral responses that are graded as a function of intensity—from spinal reflexes, through complex vocalizations, and up to very organized types of behavior (escape, aggression, etc.). The electrical thresholds of individual fibers are related to their diameters; thus, when the applied intensity of an electrical stimulus to a cutaneous nerve is increased progressively, it is first the A $\beta$ , then the A $\delta$ , and finally the C fibers that are activated. This can be an advantage, but it also means that one cannot usually excite small-diameter nerves without additionally exciting the others. Thus, when electrical stimuli are applied to a sensory nerve in humans, they evoke a variety of sensations, including pain, which result from the nonselective activation of all types of peripheral fibers, be they of large or small diameter. It is probably this nonselectivity of activation and the aforementioned synchronization of the resulting afferent inputs that can make these sensations rather unusual or even bizarre. Thus, electricity does not constitute a specific stimulus of the type that can be produced under physiological conditions, when one can even selectively excite those fine-diameter afferent fibers connected to nociceptors (notably to polymodal nociceptors) without exciting other small fibers such as those that are connected to thermoreceptors and are activated by non-nociceptive thermal stimulation. Finally, it should be added that because of the differences in conduction velocities, there is a small (but possibly significant, depending on peripheral conduction distance) time gap in the arrival at the spinal cord of the afferent volleys evoked by electrical stimuli in fibers having different diameters. This gap can be useful in some carefully planned neurophysiological protocols, but it can also produce other problems such as activating the inhibitory mechanisms produced by large diameter,

fast-conducting fibers before the arrival of the signal in the finer diameter, slow-conducting fibers.

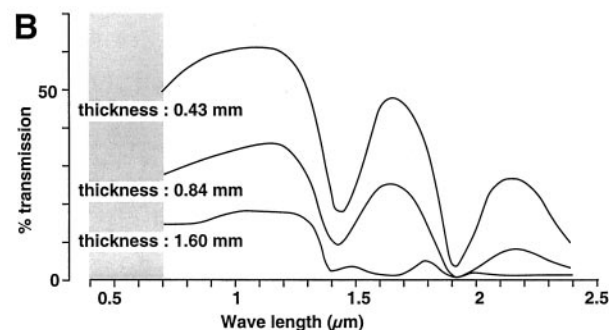
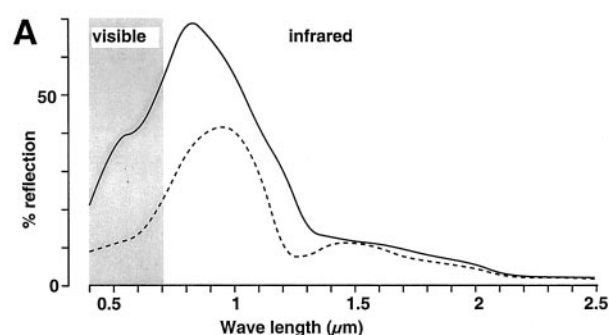
Because conduction velocities in different peripheral fibers are of approximately the same order in all mammals, it is obvious that these problems will be influenced by both the size of the studied species and the chosen site of stimulation. This also makes one think that if these conduction velocities varied in accordance with the size of the species (which they do not), it would undoubtedly confer an advantage, and yet evolution has not produced such a situation.

Mention must be made of the “double pain” phenomenon observed in humans following a brief nociceptive stimulus (Handwerker and Kopal, 1993): the first or “fast” pain is typically stinging and well localized, and it results from the activation of A $\delta$  fibers; the second or “slow” pain is slower in onset, typically burning in nature, more intense, and more difficult to localize, and it results from activation of C fibers. In this article, we return on several occasions to the consequences of these phenomena for animal tests of nociception.

2. *Thermal Stimulation.* Heat is more selective in the way it stimulates cutaneous receptors. Consequently, specific categories of peripheral axons, including thermosensitive and nociceptive fibers, can be excited. However, the weak caloric power of the stimulators that are generally used (radiant lamps or contact thermodes) has always been a limitation of this method. Indeed, the speed of cutaneous heating induced in this way is slow (<10°C/s), which results in an asynchronous activation of peripheral and central neurons. Thus, it does not allow for an appropriate study of neural phenomena classically seen in other sensory systems (e.g., reflexes, evoked potentials, and reaction times) for which a synchronous excitation of fibers is required.

Conventional radiant heat sources have the additional disadvantage of emitting radiation within the visible and adjacent infrared spectra for which the skin is a poor absorber and a good reflector. In humans, Hardy and his coworkers (1940, 1943, 1947, 1951, 1952, 1953) thoroughly studied the “stinging” type of pain evoked by heat produced by the beam emitted from a powerful lamp, which passed through a lens and was controlled by an obturator. This device made it possible to apply a constant amount of radiant heat energy for a given period of time. The beam was directed toward the skin of the subject, which had been blackened beforehand by the application of India Ink. The stimulation site was often the forehead because the baseline cutaneous temperature ( $34.0 \pm 0.5^\circ\text{C}$ ) of the forehead is less prone to interindividual variations. Blackening had two objectives: 1) to limit reflection, which is particularly high for the visible and adjacent infrared parts of the spectrum of electromagnetic waves and varies with the pigmentation of the skin (Fig. 3A); and 2) to limit the penetration of the rays beneath the skin surface. This factor is not negligible, as shown by the observations of Winder et al.

### Radiation properties of the skin



### Emission properties of the bulb

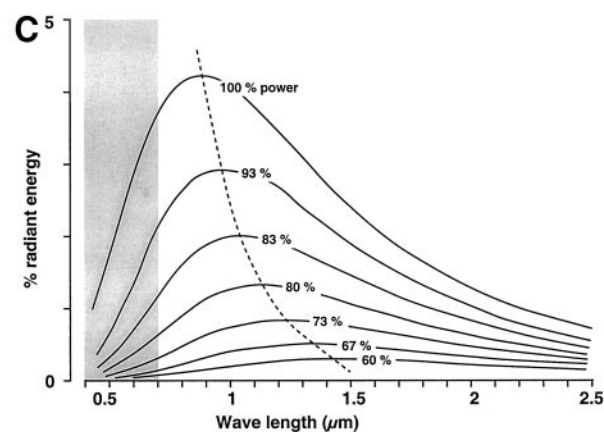


FIG. 3. A, when radiant heat is used, the percentage of the energy reflected by the skin (ordinate) depends on the wavelength emitted by the source of radiation (generally an incandescent lamp). In the visible and adjacent infrared spectra, it also depends on the degree of pigmentation of the skin (white skin: solid line; black skin: broken line). From data from Hardy (1980). B, the percentage of the energy transmitted through the skin (ordinate) also depends on the wavelength emitted by the source of radiation and decreases within depth. Modified from Hardy et al. (1956) with permission. C, the electromagnetic emission spectrum of a lamp varies with the intensity of the electrical current that is being applied to it. In this example, using a lamp in a commercially available apparatus that is marketed for the tail-flick test, it can be seen that at full power, the spectrum is centered in the infrared range (around  $0.9 \mu\text{m}$ ). When the power is reduced, the center shifts gradually to the right. (Graph courtesy of Michel Morel, Philips, 54700 Pont à Mousson, France.)

(1946): the nociceptive threshold with radiant heat was 8% lower in black guinea pigs than in white ones, and blackening the skin in the former group with India Ink actually reduced the threshold by a further 31%. In fact, the thermal radiation needed to increase the tempera-

ture to the pain threshold depends on several parameters: 1) the radiation properties of the skin, namely reflectance (Fig. 3A), transmittance (Fig. 3B), and absorbance, which depend on the electromagnetic spectrum emitted by the source of radiation, which itself varies with the intensity of the electrical current through incandescent bulbs (Fig. 3C); 2) the conduction properties of the skin (diffusivity); 3) the initial temperature of the skin (Fig. 4); and 4) the amount of caloric energy delivered to a given surface area of skin, which in turn depends on both the power spectral density of the bulb and the duration of exposure (Fig. 5).

Under normal conditions, skin temperature results from an equilibrium between heating via the arterio-venous capillary bed and heat loss from the skin surface (Fig. 6A). Radiant heat produces a local and transient disruption of such an equilibrium (Fig. 6B). However, it should be emphasized that a constant power from a source of radiant heat will change the skin surface temperature in relation to the square root of time (Buettner, 1951; Hendler et al., 1965; Stolwijk and Hardy, 1965; Fig. 7A).

Thermodes, such as those based on the Peltier principle (Kenshalo and Bergen, 1975; Fruhstorfer et al., 1976), heat the thermosensitive receptors by means of the conduction properties of the skin (Fig. 6C). However, these generate a different set of problems since by necessity they are in contact with the skin (heat transfer by conduction). As a result, when they activate nociceptors, they can concomitantly activate low-threshold non-nociceptive nerves that exert an inhibitory influence on pain mechanisms (Nathan et al., 1986; Svensson et al.,

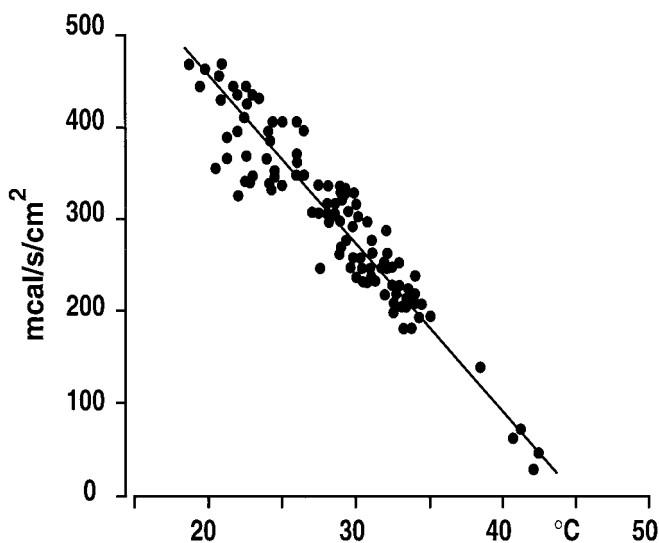


FIG. 4. Relationship between the thermal energy necessary to increase the temperature to the threshold for "stinging" pain and the initial temperature of the skin. In four subjects, radiant heat from a preheated lamp controlled by an obturator was applied for 3 s to a 3.5-cm<sup>2</sup> area of skin on the face that had been blackened by India Ink. The threshold fell in a linear fashion with the increase in temperature and tended toward a value of approximately 45°C. Adapted with permission from Hardy et al. (1951). Copyright 1951 American Association for the Advancement of Science.

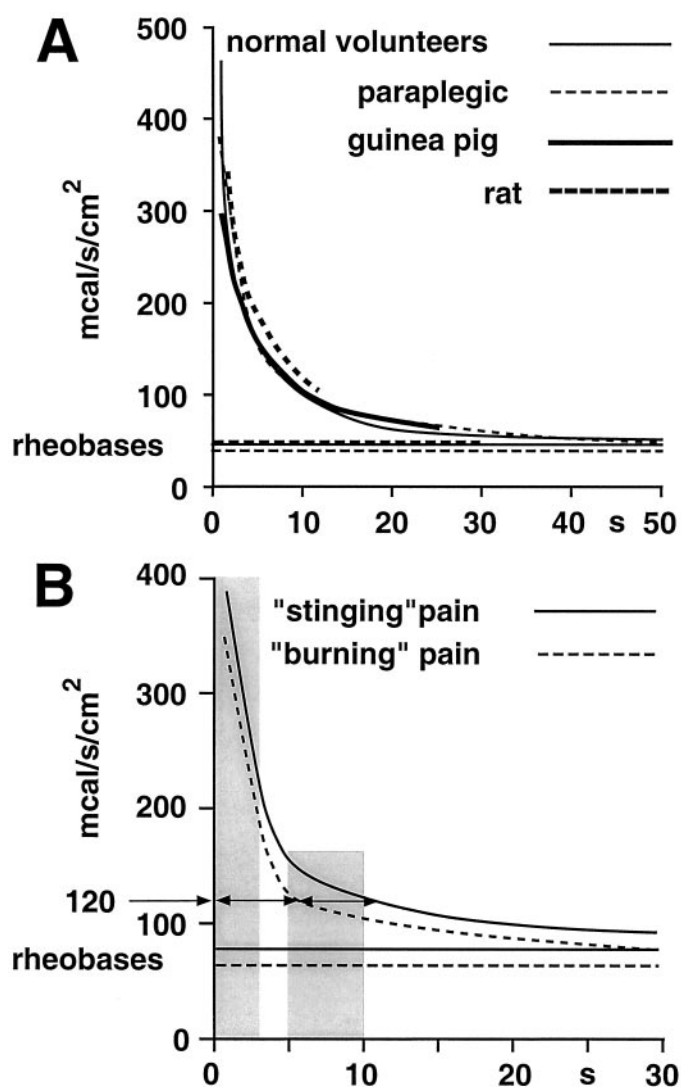


FIG. 5. Relationships between the thermal energy necessary to increase the temperature to a pain or nociceptive threshold and the stimulus duration. A, variations in the threshold for "stinging" pain in six healthy volunteers (thin solid curve). This graph also shows a curve obtained in a paraplegic patient by measuring the threshold for producing a reflex withdrawal movement of the foot when its dorsal surface was stimulated (broken curve). Very similar curves were obtained with similar protocols in guinea pigs by measuring the threshold for producing a reflex in back muscles by stimulation of the previously shaven back (thick solid curve) and in rats by measuring the threshold for producing a reflex movement of the tail (tail-flick, thick dotted curve). Adapted from Hardy et al. (1953) with permission. Each of these curves tends toward a limit (the rheobase) below which one can never evoke a sensation or a response. The four rheobases are very close together. B, in this experiment, 11 healthy volunteer subjects were asked to differentiate stinging from burning pain. With stimulus durations of less than 3 s (left gray zone), the subjects were unable to distinguish the two pains. It was with durations between 5 and 10 s (right gray zone) that they could most easily determine the threshold for "burning" pain. Moreover, this pain was produced by weaker stimulus intensities than was stinging pain. For example, at an intensity of 120 mcal/s/cm<sup>2</sup>, it was necessary to double the duration to pass from the threshold for burning pain to that for stinging pain (arrows). Adapted from Bigelow et al. (1945) with permission.

1997). Furthermore, the surface of the thermodes is fixed and rigid, which limits their use because most skin surfaces are not flat. Finally, the rate of thermal transfer is dependent on the quality of the thermode-skin contact and thus on the pressure of application of the



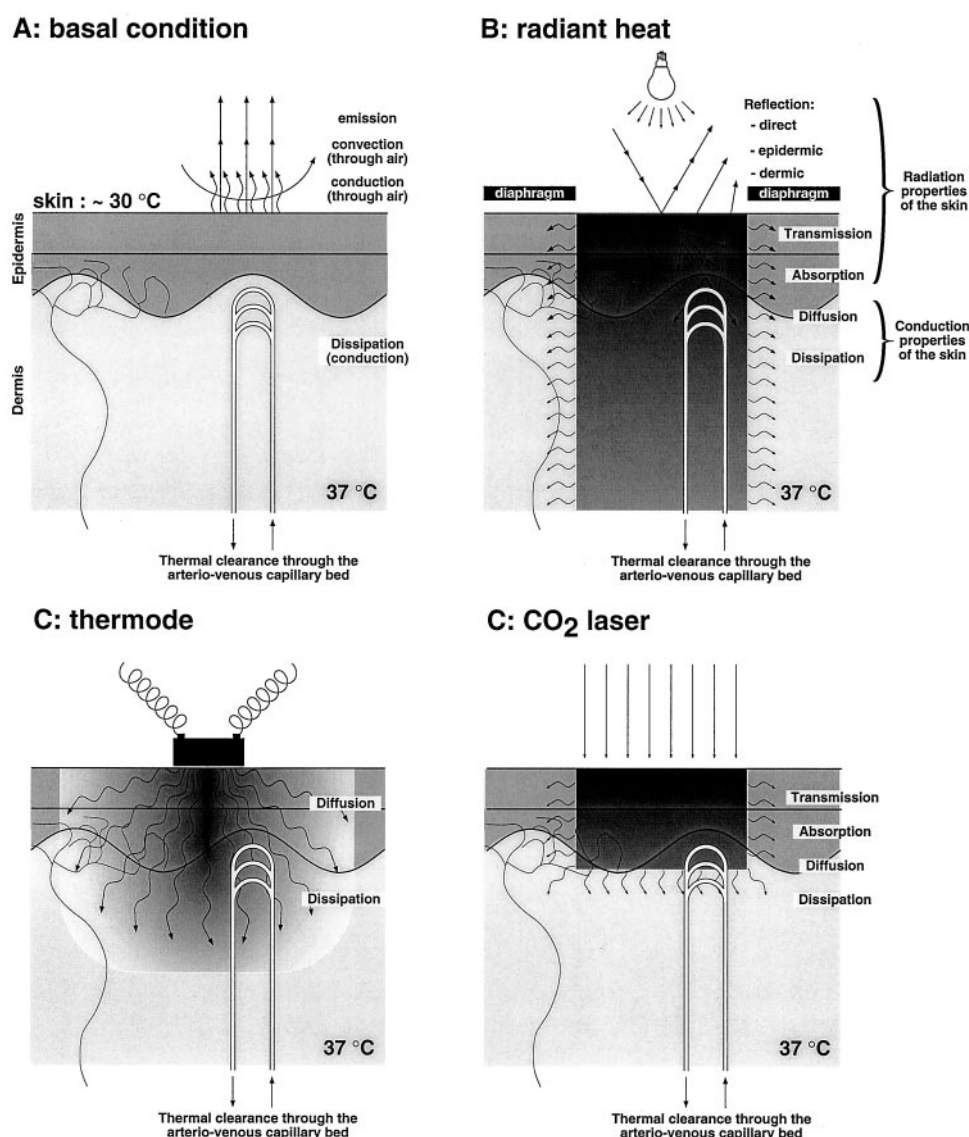


FIG. 6. Schematic representation of heat transfer through the skin at rest (A) and when subjected to heat by a radiant lamp (B), a contact thermode (C), or a CO<sub>2</sub> laser (D). For clarity of presentation, we have indicated a diaphragm close to the cutaneous surface during the exposure to radiant heat (B). It should be noted 1) that radiation from an incandescent lamp easily passes through the thickness of the skin (B) in a fashion dependent on the wavelength emitted by the source of radiation (Fig. 3B); 2) that there is a finite speed of conduction of heat within the skin (C); and 3) that radiation from a CO<sub>2</sub> laser penetrates little more than 100  $\mu$ m (D).

thermode—a parameter that is not easy to control (Yarnitsky and Ochoa, 1990), particularly in animals. In fact, thermodes have been used in animals only rarely (Rosenfeld et al., 1978; Morris et al., 1982; Casey and Morrow, 1983; Carstens and Ansley, 1993; Hämäläinen et al., 1996), although they do have one major advantage, viz., that they can deliver a slope of heating that grows linearly with time (Fig. 7B), albeit with a maximum rate of heating of only 4°C/s (Wilcox and Giesler, 1984).

On the other hand, the immersion of an animal's limb or tail in a thermostatic bath allows a more rapid, although not instantaneous, increase in skin temperature (Fig. 7C; Hardy et al., 1965). In general, even though one might know the surface temperature achieved with one of these methods, that is not the same as the tempera-

ture reached by the various layers within the skin (dotted white lines in Fig. 7). The latter can be estimated only by modeling or simulation (Hardy et al., 1965; Stolwijk and Hardy, 1965; Meyer et al., 1976; Bromm and Treede, 1983; Tillman et al., 1995b) because the types of probe that might be used to make direct measurements (e.g., thermocouples) have not yet been miniaturized to the extent that they would not disturb heat exchange.

To a large extent, these disadvantages can be overcome by using a CO<sub>2</sub> laser thermal stimulator (Fig. 6D). Such stimulators have many advantages from a physiological point of view (Plaghki et al., 1989, 1994): 1) a monochromatic, long wavelength (10.6  $\mu$ m) infrared source of radiation that results in near-total absorption no matter what the degree of pigmentation of the skin or the incidence of the radiation; 2) penetration which is so

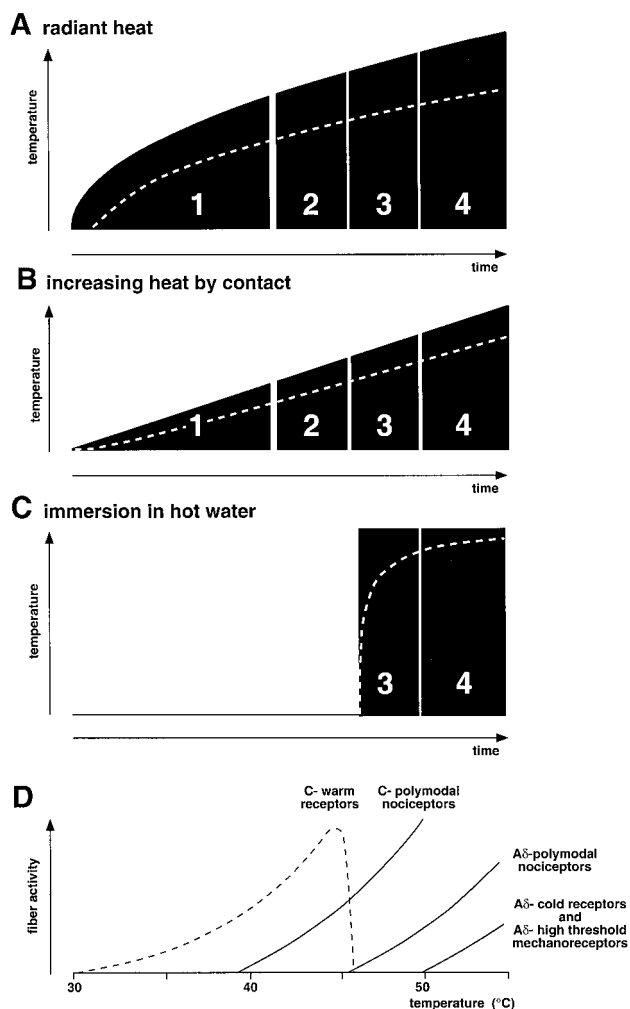


FIG. 7. A, when subjected to a constant-power heat source, the temperature of the skin surface increases in proportion to the square root of time. This increase is more gradual deep within the skin (broken white line). Moreover, an additional inaccuracy is introduced at the beginning of stimulation by the thermal inertia of the lamp when it is not preheated. The combination of these factors can give an illusion of linearity in the increase in temperature. B, increase in temperature can be proportional to time when contact thermodes are used. The more gradual increase in temperature within the skin (broken white line) is highly dependent on the rate of heating of the thermode. C, when part of the body is immersed in a hot, soaking fluid such as water, the temperature of the skin interface almost immediately achieves the temperature of the bath if the bath has an adequate thermostatic control and the water is agitated. In this case, one must bear in mind that the increase in temperature within the skin (broken white line) will be smoother and more gradual. D, diagrammatic representation of the activity evoked in cutaneous receptors by different temperatures applied to the skin (Hensel, 1973; Duclaux and Kenshalo, 1980; Meyer et al., 1994; Treede et al., 1995). When the temperature is gradually increased from the normal value for the skin (around 30°C with an ambient temperature of 20°C) to within the noxious range, there is a successive activation of thermoreceptors and then C- and A $\delta$ -polymodal nociceptors. At the highest temperatures, high-threshold mechanoreceptors and cold receptors are also activated. On the basis of the recruitment of these different receptors, four successive periods shown in A, B, and C can be defined: 1) thermoreceptors; 2) thermoreceptors and C-polymodal nociceptors; 3) C- and A $\delta$ -polymodal nociceptors; and 4) polymodal nociceptors, high-threshold mechanoreceptors, and cold receptors.

weak (around 100  $\mu$ m) that the thermal energy absorbed at the skin surface is concentrated in the region in which the thermosensitive nerve terminals are located (around

the dermoepidermal junction interface, 60–120  $\mu$ m below the skin surface); 3) a beam with highly controllable temporal and spatial energy profiles; and 4) a heating slope that, when measured at the skin surface, is extremely steep (achieving the target temperature within milliseconds)—this, together with the lack of cutaneous contact and the fact that the beam is outside the visible spectrum, ensures a quasisynchronous and selective activation of free endings of small nerve fibers (Treede et al., 1984). Perhaps as a result of these properties, such stimulators have been used in the rat to evoke motor responses and vocalization (Carmon and Frostig, 1981; Schouenborg et al., 1992; Danneman et al., 1994; Fan et al., 1995; Bragard et al., 1998), the latter being very sensitive to morphine (Bragard et al., 1998). Primary afferent and spinal sensory neurons have also been reported to respond to brief pulses of intense infrared laser radiation (Devor et al., 1982). However, for financial and technical reasons, the use of the CO<sub>2</sub> laser to study pain is still in the domain of only a few research groups.

**3. Mechanical Stimulation.** The application of a noxious mechanical stimulus can be progressive or coarse. Responses produced by noxious mechanical stimuli are graded in relation to the intensity and/or duration of the stimulus, from reflexes up through vocalizations ultimately to complex motor behaviors. The stimulus is stopped as soon as a response is obtained. The type of mechanical stimulus used by von Frey in the last century (Handwerker and Brune, 1987) is often almost revered by neurologists, but it has the disadvantage of activating low-threshold mechanoreceptors as well as nociceptors. Consequently, the stimulus is not specific. There are also technical difficulties in applying mechanical stimuli, especially in freely moving animals. In addition, when mechanical stimuli are truly nociceptive, they are likely to produce changes in the tissues (sensitization or actual lesions). Furthermore, conventional techniques do not allow noxious mechanical stimuli to be delivered rapidly and briefly enough to produce synchronous excitation of the nerve fibers—with disadvantages identical to those discussed above for thermal stimuli. Finally, especially in small animals such as rodents, the parts of the body that are stimulated are themselves small, which can produce problems for the scientist in separating cause (stimulus) and effect (reaction). This problem is so great that the most common mechanical stimuli (pinches) are really double stimuli. There are also animal models of visceral pain triggered by mechanical stimuli, in this case involving the dilatation of hollow organs (see *Section VI.C.*).

**4. Chemical Stimulation.** Chemical stimulation involving the administration of algogenic agents represents a slow, or even very slow, form of stimulation. In this respect, chemical stimuli are clearly different from other forms of stimulation; they are also progressive, are of longer duration, and have an inescapable character once they have been applied. As a result, typical re-

flexes, which necessitate a minimum level of synchronization of activity in primary afferent nerves, are not produced by these stimuli (although reflexes can be facilitated by algogenic agents such as capsaicin; Gilchrist et al., 1996; Yeomans et al., 1996b).

The behaviors that are produced vary but are relatively stereotyped in rodents. Tests using chemical stimuli can be distinguished very clearly from those mentioned above, not only by their physical nature and duration, but also and equally importantly by the fact that it is never the threshold that is measured but a behavioral score, in units of time, in response to an inescapable suprathreshold stimulus. Without doubt, these experimental models are the closest in nature to clinical pain. Models of visceral or peritoneal pain in animals also involve the administration of algogenic agents (see *Sections VI.B.* and *VI.C.*).

5. *The Choice of Stimulus Parameters.* All nociceptive stimuli can be defined by a number of different parameters that can be placed into three categories: physical nature, site of application, and past history of the site of application.

The first parameter is the physical nature of the stimuli and those parameters that we can control with some precision. From a physiological point of view, it seems essential that three such parameters be controlled: the intensity, the duration, and the surface area of stimulation. These three parameters determine the “global quantity of nociceptive information” that will be carried to the central nervous system by the peripheral nervous system. However, the choice of these parameters is not as simple as one might expect because one has to consider the consequences of temporal and/or spatial summation phenomena at the spinal level when the global quantity of nociceptive information exceeds a given value (Bouhassira et al., 1995; Gozariu et al., 1997).

The second parameter is the site on the body at which the stimulus is applied. Obviously, it is important to distinguish the principal tissue types from which clinical pains originate: somatic, visceral, articular, and musculotendinous. In nociceptive tests, stimuli are usually applied to cutaneous and, to a lesser extent, visceral structures. Furthermore, it is known that in both humans and animals, there are differences in the sensitivity of cutaneous tissues that have to be taken into account. We also know that in some species, some areas of skin can have a specific particular function. For example, the rodent tail, which is a structure used in many nociceptive tests, is an essential organ for thermoregulation and balance. Finally, in this regard, the simultaneous application of stimuli to several topographically distinct areas of the body—as happens in some classic tests—can introduce bias to a study by triggering inhibitory controls involving supraspinal structures (see *Section XII.B.*).

The third parameter is the previous history of the stimulated site. Tests for acute pain involve healthy

tissues and, occasionally, acutely inflamed tissues (of a few days standing at most). Tests for chronic pain—which are beyond the scope of this review—relate to rheumatic or neuropathic pain that lasts for a long time (from weeks up to several months).

Since the application of the stimulus must not produce lesions, one often defines a limit for how long the animal should be exposed to the stimulus (the “cutoff time”). This limit is absolutely necessary when the intensity of the stimulus is increasing; a compromise has to be found between the dynamics of the effect being studied (for which one would wish the longest time limit possible) and the prevention of tissue damage (for which one would wish the shortest time limit possible). It has been suggested that the time limit should be set at 3 times the reaction time of the controls (Carroll, 1959).

Furthermore, the repeated application of a stimulus up to the time limit during an antinociceptive effect can sensitize peripheral receptors and/or produce a central sensitization (e.g., by accumulation of mediators at the level of the spinal cord). These phenomena in turn can badly affect the findings during the final phase of the antinociceptive effect and give the appearance of a rebound “facilitation” (Kallina and Grau, 1995; Baldwin and Cannon, 1996).

#### B. *The Response*

It also seems reasonable to classify tests in terms of the biological function being recorded. The observed reactions cover a very wide spectrum ranging from the most elementary reflexes to far more integrated behaviors (escape, avoidance). In almost every case, it is a motor response that is monitored; vegetative responses are considered only occasionally (Ness and Gebhart, 1988; Gebhart and Ness, 1991; Holzer-Petsche, 1992; Sherman and Loomis, 1994; Holzer-Petsche and Rordorf-Nikolic, 1995; Reina and Yeziarski, 1995; Roza and Laird, 1995; Culman et al., 1997; Taylor et al., 1998). It is important to bear this in mind when considering all results obtained from such tests. Indeed the analysis of the results should take account of the possibility that nociceptive processes interact with other linked phenomena, particularly motility itself (Chapman et al., 1985; Schomburg, 1997; see *Section VIII.*). It is equally necessary to consider nociception along with the other phenomena responsible for the homeostatic balance of the organism (see *Section XII.*). When you consider that even today there are no major analgesics without secondary side effects, you realize how difficult it is to analyze the results of tests of nociception.

### IV. Behavioral Models of Nociception

Ideally, a behavioral model for nociception in an animal should possess the characteristics detailed below (Goetzl et al., 1943; Taber, 1974; Lineberry, 1981; Vierck and Cooper, 1984; Ramabadran and Bansinath, 1986;

Hammond, 1989; Watkins, 1989; Dubner, 1994; Tjølsen and Hole, 1997).

**Specificity.** 1) The stimulus must be nociceptive (“input specificity”). Although this is common sense, it is not always easy to confirm that it is being achieved. For example, the appearance of a flexion reflex does not inevitably mean that the stimulus is nociceptive or that it is a nociceptive flexion reflex. Indeed, flexion reflexes are not triggered exclusively by nociceptive stimuli (see *Section VIII.*). This can lead to misinterpretations.

2) It must be possible in the behavioral model to differentiate responses to nociceptive stimuli from responses to non-nociceptive stimuli. In other words, the quantified response has to be exclusively or preferentially triggered by nociceptive stimuli (“output specificity”). In this respect, one has to bear in mind that some innate or acquired behaviors can be triggered by aversive stimuli that are not nociceptive/painful.

**Sensitivity.** 3) It must be possible to quantify the response and to correlate this variable with the stimulus intensity within a reasonable range (from the pain threshold to the pain tolerance threshold). In other words, the quantified response must be appropriate for a given type of stimulus and monotonically related to its intensity.

4) The model must be sensitive to manipulations and notably pharmacological ones, which would reduce the nociceptive behavior in a specific fashion. A sensitive test must be able to show effects for the different classes of antinociceptive agents at doses comparable to those used for analgesics in humans.

**Validity.** 5) The model must allow the differentiation of nonspecific behavioral changes (e.g., in motility and attention) from those triggered by the nociceptive stimulus itself. In other words, the response being monitored must not be contaminated by simultaneous perturbations related to other functions, notably if they have been introduced by a pharmacological agent. The test validity, i.e., the degree to which the test actually measures what it purports to measure, is undoubtedly one of the most difficult problems to resolve (see *Section XII.*).

**Reliability.** 6) Consistency of scores must be obtained when animals are retested with an identical test or equivalent form of the test. In this context, the repeated application of the stimulus must not produce lesions.

**Reproducibility.** 7) Results obtained with a test must be reproducible not only within the same laboratory but also between different laboratories.

Because no test of nociception meets all these criteria, the choice of which test to use has to be a compromise. Before describing these tests, it is worth noting that in general, they can be divided into two overall categories depending on whether it is a threshold or a supraliminal response to a given stimulus that is being measured. Note that both these categories permit one to investigate

only one point on the stimulus-response curve, be it the threshold or an arbitrary point further up the curve. As a result, they allow only a rough appreciation of the gain of the process (Tjølsen and Hole, 1997). For the main part, the models involve rodents, most often the rat. In this review, when the species is not explicitly mentioned, we are referring to models that are based on the rat.

If we restrict ourselves to acute cutaneous and acute visceral pain, it is useful to classify the animal models used on the basis of the physical characteristics of the stimuli. We therefore successively consider tests based on the use of short-duration stimuli (in the order of seconds) and then those based on the use of longer-duration stimuli (in the order of minutes). The former relate to pains of cutaneous origin, with physical stimuli (thermal, mechanical, electrical) applied to small areas, often at increasing intensities. The latter relate to pains of cutaneous or visceral origin, with chemical stimuli (algogenic substances) being applied usually subcutaneously or intraperitoneally. In addition, one can add to the latter category tests based on the distension of hollow organs (visceral mechanical stimulation); such stimuli last for intermediate periods of time.

## V. Use of Short-Duration Stimuli (“Phasic Pain”)

These tests are the most commonly used. In general they 1) involve a short period of stimulation; 2) have somatic rather than visceral sites of stimulation; 3) involve measuring thresholds with the result that they generate no information whatsoever regarding responses to frankly nociceptive stimuli; 4) usually involve measuring the response time to a stimulus of increasing intensity with the explicit or implicit assumption that this reaction time is related to the threshold; 5) involve stimulation of minimal surface areas, with two important exceptions: the hot plate and the electrified grid, where the four paws and tail of the animal are stimulated simultaneously; and 6) can be classified by the nature of the stimulus, be it thermal, mechanical, or electrical.

### A. Tests Based on the Use of Thermal Stimuli

In tests involving thermal stimuli, it is always the skin that is stimulated. These tests do not involve visceral or musculoskeletal tissues. However, it is important not to forget that radiant heat also stimulates thermoreceptors and that, consequently, the application of a ramped thermal stimulus will result in an organized and unalterable sequence of activation, namely thermoreceptors, then thermoreceptors plus nociceptors, then nociceptors alone, and finally (possibly) nociceptors plus “paradoxical cold” receptors (Fig. 7D). In practice, the animal withdraws itself quickly from the stimulus, and therefore only the first part of this scenario takes place.

The source of nociceptive stimulation can be distant from its target (e.g., radiant heat from a lamp) or can be

in direct contact with the skin. Radiant heat constitutes a relatively selective stimulus for nociceptors and has an advantage over the other modes of thermal stimulation in that it produces no tactile stimulus.

We first mention the work of Ercoli and Lewis (1945), who applied the method used in humans by Hardy et al. (1940) to 2200 rats. The beam was directed at the animal's back, which had been shaven the previous day. By simultaneously opening the obturator and starting timers, it was possible to measure two successive response times: that of a local response, which consisted of a "twitch", and then that of a general response involving an "escape" reaction by the animal. The most interesting observation by these authors related to morphine: although it affected both responses, it was more powerful against the second, to such an extent that increasing the dose resulted primarily in an increase in the difference between the two response times, with the second becoming increasingly longer. Andrews and Workman (1941) and Winder et al. (1946) studied similar muscle responses in the dog and the guinea pig.

1. *The Tail-Flick Test.* There are two variants of the tail-flick test. One consists of applying radiant heat to a small surface of the tail. The other involves immersing the tail in water at a predetermined temperature. Although apparently similar, these two alternatives are actually quite different at a physical level: the cutaneous temperature varies with the square root of time in the first case and more rapidly in the second (Fig. 7, A and C). In addition, the stimulated surface areas can be very different. Indeed it is surprising that authors generally consider these two tests as though they were equivalent.

a. *The Tail-Flick Test Using Radiant Heat.* The tail-flick test with radiant heat is an extremely simplified version of the method used on human subjects by Hardy et al. (1940). Indeed, Hardy and his colleagues eventually used the technique in the rat (Hardy, 1953; Hardy et al., 1957). The application of thermal radiation to the tail of an animal provokes the withdrawal of the tail by a brief vigorous movement (D'Amour and Smith, 1941; Smith et al., 1943). It is the reaction time of this movement that is recorded (often referred to as "tail-flick latency"). This is achieved by starting a timer at the same time as the application of the heat source. By using a rheostat, the intensity of current through the filament and therefore of radiant heat emission can be controlled in such a way that one can empirically predetermine the time until the withdrawal of the tail. This is usually between 2 and 10 s (most commonly between 2 and 4 s), although it can be much longer (e.g., Raffa et al., 1992). A photoelectric cell stops the timer and switches off the lamp at the moment the tail is withdrawn (Bass and Vanderbrook, 1952). A lengthening of the reaction time is interpreted as an analgesic action. It is advisable not to prolong the exposure to radiant heat beyond 10 to 20 s, otherwise the skin may be burned. The advantages of this method are its simplicity and the small interani-

mal variability in reaction time measurements under a given set of controlled conditions. Some authors have recorded these motor responses electrophysiologically (e.g., Cargill et al., 1985; Peets and Pomeranz, 1987), but this approach has not been adopted by most investigators.

The reaction time of the tail movement varies with the intensity (power) of the source of radiant heat: when it is more intense, the temperature slope is steeper and, consequently, the reaction time is shorter (Carroll, 1959; Granat and Saelens, 1973; Ren and Han, 1979; Levine et al., 1980; Ness and Gebhart, 1986; Carstens and Wilson, 1993) and the movement is greater (Hamann and Martin, 1992; Carstens and Wilson, 1993). This is discussed in detail under *Section IX*. Equally, the reaction time varies with the surface area stimulated: when the area increases, the reaction time decreases (Kawakita and Funakoshi, 1987). Similar findings were obtained when electromyographic responses were recorded in the tail muscles (Tsuruoka et al., 1988). However, this reaction time also varies with the site stimulated; paradoxically, it decreases when the stimulus is applied to increasingly distal parts of the tail (Ness et al., 1987) even though the pathway for the afferent signals is longer. Also paradoxically, and perhaps as a result of this, pharmacological data can depend on the part of the tail being stimulated. Thus, it can be shown that the test is more sensitive to morphine when the distal part of the tail is stimulated than when a more proximal part is stimulated, with the middle part giving an intermediate effect (Yoburn et al., 1984; Martinez-Gomez et al., 1994; Prentice et al., 1996). To this day, no one has found a satisfactory explanation for these observations. All that can be said is that the tail of the rat is a complex structure, the movement of which is effected by between 8 and 14 muscles (Brink and Pfaff, 1980), and the conical form of which could influence how much of it [and what type(s) of receptors] are affected by thermal stimulation. It is also possible that heat reaches the nociceptors more rapidly at the tip of the tail where the skin is thinner.

One can demonstrate that the tail-flick is a spinal reflex in that, at least in its shorter latency form, it persists after section or cold block of upper parts of the spinal cord (Irwin et al., 1951; Bonnycastle et al., 1953; Sinclair et al., 1988). As with all reflexes, it is subject to control by supraspinal structures (Mitchell and Hellon, 1977). Details of the spinal pathways implicated in this reflex can be found elsewhere (Grossman et al., 1982; Carstens and Wilson, 1993; Douglas and Carstens, 1997). It is triggered by C fibers when it is elicited by heat delivered by a CO<sub>2</sub> laser (Danneman et al., 1994). However, this does not mean that the same applies when a conventional source of energy is used to provide radiant heat (see *Section XI.B*).

The tail-flick reflex may not always be purely spinal, notably when the heating slope is slower and there is an increase in the reaction time. Under these conditions,

the tail-flick can disappear in the spinal animal. In this regard, Jensen and Yaksh (1986) compared intact animals and chronic spinal animals (48 h after spinalization and thus free from spinal shock) and submitted them to two intensities of stimulation. When the intensity of stimulation was powerful enough to produce a movement within 2 s in the intact animal, it occurred with a similar reaction time in the spinal animal; when the intensity took 4 to 5 s to produce a movement in the intact animal, it was incapable of producing one within the imposed limit of 10 s in the spinal animal. Thus, under these conditions, it is possible that the tail-flick is not a purely spinal reflex but is a more complicated one involving higher neural structures. It might, for example, be mediated by a spino-bulbo-spinal circuit. A much more likely explanation would be that the light emitted by the incandescent lamp used to stimulate the tail might cause a learning process (King et al., 1997; see *Section XII.D.*).

The tail-flick is prone to habituation, viz., a reduction in the response with repetitive stimulation (Groves and Thompson, 1970). This habituation increases with a shortening of the interstimulus interval and with the intensity of stimulation (Carstens and Wilson, 1993). It may be noted that the phenomenon of habituation is generally reported for reflexes evoked by stimulation of myelinated fibers and recorded electrophysiologically (Spencer et al., 1966a,b,c; Wickelgren, 1967a,b; Groves et al., 1969; Egger, 1978; Mendell, 1984).

From a pharmacological point of view, there is a consensus that this test is truly efficient only for revealing the activity of opioid analgesics (but not of opioid partial agonists). In this context, it is adequate for predicting their analgesic effects in humans (Archer and Harris, 1965; Grumbach, 1966). For morphine itself, it is not difficult to construct dose-response curves for intravenous doses between 1 and 10 mg/kg. However, although this statistical way of presenting results is legitimate, it hides a curious observation made in 1941 by D'Amour and Smith. These authors found that the blocking of the tail-flick by morphine was "quantal" or absolute in the sense that, for a given dose, animals either responded or did not respond (before the cutoff time), but that although the proportion of nonresponding animals increased with the dose of the analgesic, those that continued to respond always showed a reaction time close to that of the controls. It is the proportion of animals whose tail-flick reaction time reaches the cutoff time that increases when one the dose of morphine increases (Levine et al., 1980; Carstens and Wilson, 1993). However, in a number of individual cases, a graduated effect of morphine has been observed (Yoburn et al., 1984; Carstens and Wilson, 1993). As Miller emphasized in 1948, these observations make it tricky to interpret results obtained with this model since no equivalent observations (i.e., all-or-none analgesic effects) have been seen in human patients in clinical practice.

As far as opioid partial agonists are concerned, some have been shown to increase the tail-flick reaction time when slow rates of heating are applied (Gray et al., 1970). It is probable that this pharmacological observation resulted from the aforementioned fact that supraspinal structures are involved when the test is carried out in this fashion.

*b. The Tail-Flick Test Using Immersion of the Tail.* The use of immersion of the tail is apparently a variant of the test described above. The most obvious difference is that the area of stimulation is far greater. Immersion of an animal's tail in hot water provokes an abrupt movement of the tail and sometimes the recoiling of the whole body. Again, it is the reaction time that is monitored (Ben-Bassat et al., 1959; Janssen et al., 1963; Grotto and Sulman, 1967). This test can be used on monkeys (Dykstra and Woods, 1986), and some investigators have used cold instead of hot stimuli (Pizziketti et al., 1985; Wang et al., 1995).

This test is actually quite different from the previous one insofar as immersion of the tail in a hot liquid increases its temperature very quickly and in a more or less linear fashion, which, as we have discussed, is not the case with radiant heat. The main interest in this response—which arguably has not been exploited sufficiently—lies in the possibility of applying different temperatures. Thus, lower temperatures can be used to seek evidence for the effects of minor analgesics (Sewell and Spencer, 1976; Luttinger, 1985). This also applies to using a bath in which the temperature increases slowly (Farré et al., 1989).

*2. The Paw Withdrawal Test.* In principle, this test is entirely comparable to the test of D'Amour and Smith (1941) but offers the advantage that it does not involve the preeminent organ of thermoregulation in rats and mice, i.e., the tail (Hargreaves et al., 1988; Yeomans and Proudfit, 1994). One can improve the test by minimizing variations in the baseline temperature of the skin (Galbraith et al., 1993; Dirig et al., 1997). With the aim of studying hyperalgesic phenomena resulting from inflammation, Hargreaves et al. (1988) had an inspired idea for supplementing the model of Randall and Selitto (1957; see *Section V.C.*): radiant heat was applied to a paw that had already been inflamed by a subcutaneous injection of carrageenin. For this purpose, inflammation can also be produced by exposure to ultraviolet rays (Perkins et al., 1993). One advantage in these tests is that heat is applied (to the plantar surface of the foot) of a freely moving animal. However, there is a disadvantage in that the position of the leg becomes a factor since the background level of activity in the flexors varies with the position of the animal (see *Section VIII.*).

Yeomans and Proudfit (1994, 1996) and Yeomans et al. (1996b) studied the withdrawal of the hind paw in the anesthetized rat and came to the following conclusions: when the heating slope is steep (6.5°C/s), the paw withdrawal reaction time is short and the skin surface tem-

perature reaches a high level, suggesting A $\delta$  fibers are activated; when the heating is slow (1°C/s), the reaction time is longer and skin temperature increases less, activating only C fibers (see *Section IX.B.*). Morphine is far more active in the second than in the first of these tests (Lu et al., 1997).

**3. The Hot Plate Test.** This test consists of introducing a rat or mouse into an open-ended cylindrical space with a floor consisting of a metallic plate that is heated by a thermode or a boiling liquid (Woolfe and MacDonald, 1944; Eddy and Leimbach, 1953; O'Callaghan and Holzman, 1975). A plate heated to a constant temperature produces two behavioral components that can be measured in terms of their reaction times, namely paw licking and jumping. Both are considered to be supraspinally integrated responses.

As far as analgesic substances are concerned, the paw-licking behavior is affected only by opioids. On the other hand, the jumping reaction time is increased equally by less powerful analgesics such as acetylsalicylic acid or paracetamol, especially when the temperature of the plate is 50°C or less (Ankier, 1974) or if the temperature is increased in a progressive and linear fashion, e.g., from 43 to 52°C at 2.5°C/min (Hunskar et al., 1985). The specificity and sensitivity of the test can be increased by measuring the reaction time of the first evoked behavior regardless of whether it is paw-licking or jumping (Carter, 1991), or by lowering the temperature (Plone et al., 1996).

The behavior is relatively stereotyped in the mouse but is more complex in the rat, which sniffs, licks its forepaws, licks its hind paws, straightens up, stamps its feet, starts and stops washing itself, among other things. These behaviors have been labeled "chaotic defensive movements" (Knoll et al., 1955). Espejo and Mir (1993) identified and described 12 different behaviors. Because so many of these behaviors exist, observation of them is difficult. Furthermore, this test is very susceptible to learning phenomena, which result in a progressive shortening of the jumping reaction time accompanied by the disappearance of the licking behavior (Knoll et al., 1955). Thus, the animal may lick the paws and then jump during the first test but will jump almost immediately—certainly with a much shorter reaction time—during subsequent tests (Takagi and Iwamoto, 1952; Jacob, 1963; Kayan et al., 1969; Van Ree and Leys, 1985; Espejo and Mir, 1993). Similarly, even putting the animals on an unheated plate just once to watch the test leads in subsequent tests to a diminution in the reaction time under standard conditions with a constant noxious temperature (Bardo and Hughes, 1979; Hunskar et al., 1986). Finally, reiteration of the test once a day or once a week inevitably leads to a progressive decrease in the reaction time (Fig. 8; Lai and Chan, 1982; Gamble and Milne, 1989; Plone et al., 1996; Sandkühler et al., 1996). In general, these measures are very variable, even within a single laboratory (Miller, 1948; Tjølsen and

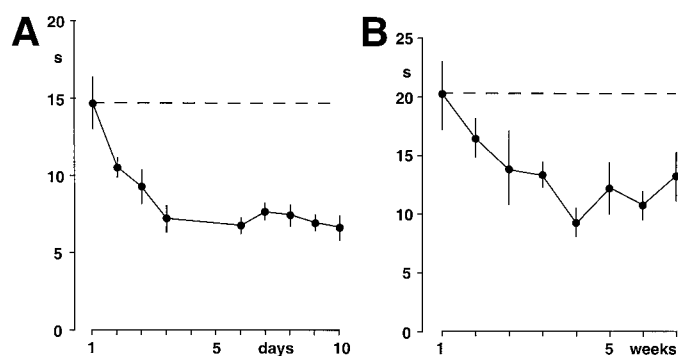


FIG. 8. Learning phenomena can make it difficult to use the hot plate test. In these experiments, measurements were made of the delay after the animal had been put on the hot plate, before they began to lick their paws. In A, Sandkühler et al. (1996) repeated the test daily in Sprague-Dawley rats; in B, Lai and Chan (1982) repeated the test each week on a Wistar strain. In both cases, it can be seen that four to five tests were sufficient to almost halve the reaction time. Modified from Sandkühler et al. (1996) and Lai et al. (1982), copyright 1996 and 1982, respectively, with permission from Elsevier Science.

Hole, 1997). All these factors make this test a very delicate one to use.

In the final analysis, it should be noted that this test consists of stimulating the four limbs and even the tail of the animal simultaneously (Knoll et al., 1955). Such heterotopic stimuli involving large body areas undoubtedly trigger diffuse inhibitory controls that are likely to disturb the observed responses (see *Section XII.B.*).

**4. Tests Using Cold Stimuli.** Cold is very rarely used to test acute pain. On the other hand, it is more common to test cold allodynia in animal models of neuropathies. The techniques are directly inspired by those that use heat by contact: immersion of the tail or a limb (Piz-ziketti et al., 1985; Attal et al., 1990; Briggs et al., 1998), or placing the animal on a cold surface (Bennett and Xie, 1988; Jasmin et al., 1998).

#### B. Tests Based on the Use of Mechanical Stimuli

The preferred sites for applying nociceptive mechanical stimuli are the hind paw and the tail. Tests using constant pressure (Haffner, 1929; Brodie et al., 1952; Bianchi and Francheschini, 1954; Collier et al., 1961; Vinegar et al., 1990) have been abandoned progressively for those applying gradually increasing pressures.

In the course of such a test, a pressure of increasing intensity is applied to a punctiform area on the hind paw or, far less commonly, on the tail. In practice, the paw or tail is jammed between a plane surface and a blunt point mounted on top of a system of cogwheels with a cursor that can be displaced along the length of a graduated beam (Green et al., 1951). These devices permit the application of increasing measurable pressures and the interruption of the test when the threshold is reached. The measured parameter is the threshold (weight in grams) for the appearance of a given behavior. When the pressure increases, one can see successively the reflex withdrawal of the paw, a more complex movement

whereby the animal tries to release its trapped limb, then a sort of struggle, and finally a vocal reaction.

If the first of these reactions is undoubtedly a proper spinal reflex, the last two clearly involve supraspinal structures. This type of mechanical stimulation has a certain number of disadvantages (Fennessy and Lee, 1975): 1) it is sometimes difficult to measure the intensity of the stimulus with precision; 2) repetition of the mechanical stimulus can produce a diminution or conversely an increase in the sensitivity of the stimulated part of the body—in the latter case, this carries the risk that the tissues may be altered by inflammatory reactions that could call into question the validity of repeated tests; 3) the necessity of applying relatively high pressures—which explains the weak sensitivity of the method and the relatively small number of substances that have been shown to be active by this test; and 4) a non-negligible level of variability of the responses.

With the aim of improving the sensitivity of the test, Randall and Selitto (1957) proposed comparing thresholds observed with a healthy paw and with an inflamed paw. The inflammation was induced beforehand by a subcutaneous injection into the area to be stimulated of substances such as croton oil, beer yeast, or carrageenin, the last of these being the most commonly used today (Gilfoil et al., 1963; Winter and Flataker, 1965b; Vinegar et al., 1976; Chipkin et al., 1983; Kayser and Guilbaud, 1987; Ardid et al., 1991). Even though it was found that the sensitivity of the method was improved, it was to the detriment of its specificity because, a priori, two different pharmacological effects—analgesic and anti-inflammatory—could be confused. It is therefore quite difficult to state that there has been analgesic or even “antalgic” activity. However, a comparison in the same animal of responses triggered from a healthy and an inflamed paw allows this problem to be overcome: nonsteroidal anti-inflammatory drugs (NSAIDs) are inactive on the former but do increase the (lowered) vocalization threshold when pressure is applied to the latter (Winter and Flataker, 1965b). One can increase the discrimination between different antalgic substances with this test by reducing the rate at which the pressure applied to the paw is increased and by increasing the time limit for subjecting the animal to the stimulus—the cutoff time (Chipkin et al., 1983).

### C. Tests Based on the Use of Electrical Stimuli

#### 1. Use of Long-Lasting Trains of Electrical Stimuli.

*a. Electrical Stimulation of the Tail.* Electrical stimuli of gradually increasing intensities can be delivered in the form of trains (lasting some hundreds of milliseconds) through subcutaneous electrodes in the tail of the rat or the mouse (Carroll and Lim, 1960; Paalzow, 1969; Paalzow and Paalzow, 1975; Levine et al., 1984; Borszcz et al., 1994). When such gradually increasing intensities of electrical stimuli are applied, one can observe successively a reflex movement of the tail, vocalization at the

time of stimulation, and then vocalization continuing beyond the period of stimulation (“vocalization after-discharge”). These responses are organized on a hierarchical basis; they depend on the different levels of integration of the nociceptive signal in the central nervous system: the spinal cord, the brainstem, and the thalamus/rhinencephalon. The last of these can reflect affective and motivational aspects of pain behavior (Borszcz, 1995a). The sensitivity to morphine of the thresholds for these three responses increases with the levels themselves: reflex < vocalization during stimulation < prolonged vocalization (Paalzow and Paalzow, 1975; Fig. 9). This differential effect on the different behavioral responses suggests different sites of action that are organized hierarchically.

*b. Electrical Stimulation of the Paw (and Tail).* In these tests, electrical stimuli of increasing intensities are delivered in the form of trains through the floor of a cage in which the animal is free to move (Evans, 1961;

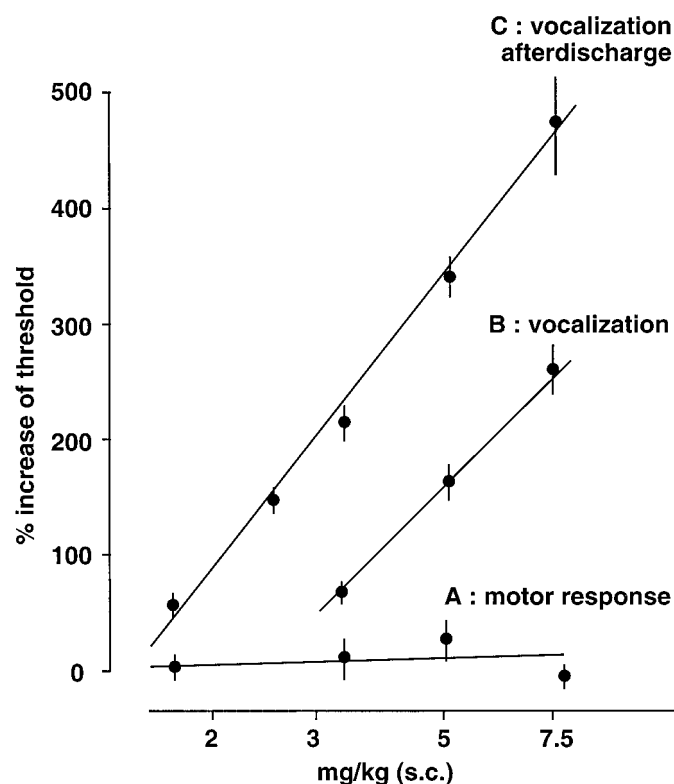


FIG. 9. Effects of morphine on various behavioral responses produced by electrical stimulation. In the experiments of Paalzow and Paalzow (1975), a train of 125 electrical shocks (each of 1.6 ms duration) were applied to the rat tail over a 1-s period. These trains were delivered at intensities that increased logarithmically. The following responses were seen successively: A, movement of the tail; B, vocalization during stimulation; C, vocalization outlasting the period of stimulation (“vocalization after-discharge”). The thresholds for these responses were determined before (relative ratio: 1, 1.5, and 3, approximately) and 45 min after the subcutaneous administration of morphine. The results are expressed as percentages of these thresholds on a semilogarithmic chart. Over the range of doses used, morphine did not modify the threshold for the reflex movement of the tail but did increase, in a dose-dependent fashion, the thresholds for vocalization and the vocalization after-discharge—the latter being the most sensitive. Modified from Paalzow and Paalzow (1975), copyright 1975 Springer-Verlag.



Blake et al., 1963; Bonnet and Peterson, 1975; Crocker and Russell, 1984). Measurements are made of the thresholds for various behaviors: the animal twitching, squeaking or attempting to escape by jumping (the “flinch-jump” test). The vocal response can be recorded, measured and analyzed objectively (Eschaliier et al., 1988). One method inspired by tests using heat or pressure consists of applying continually increasing currents and determining the reaction time for obtaining a squeak with a given amplitude (Swedberg, 1994). As in the hot plate test, the concomitant stimulation of the four legs and the tail of the animal is undoubtedly the source of diffuse inhibitory controls which are likely to modify the response that is being monitored (see Section XII.B.). This test has now fallen into disuse.

## 2. Use of Single Shocks or Very Short Trains of Electrical Stimuli.

*a. Stimulation of the Tail.* This test differs from those described above in that 1) the electrical stimuli applied to the tail are single and of short duration (10 or 20 ms), which allows latencies to be measured, and 2) the observed behaviors are different, albeit related (Charpentier, 1961, 1964, 1965, 1968). When the intensity of stimulation increases, the following responses are observed successively: twitching, escape behavior, vocalization, and biting the electrodes. Again, these responses are hierarchically organized, with the last one being the most coordinated; they depend on different levels of integration of the nociceptive signal within the central nervous system. The sensitivity of this test to morphine increases with the level of integration of the nociceptive signal (twitching = escape < vocalization < biting the electrodes). This method has been adapted to the mouse (Nilsen, 1961; McKensie and Beechey, 1962; Perrine et al., 1972) and seems adequately predictive for analgesics, including opioid partial agonists (Taber, 1974).

One can trigger complex vocal behaviors with electrical stimuli of very short duration—either single shocks or very short trains (Ardid et al., 1993; Jourdan et al., 1995). Such stimuli can be applied at regular intervals to the tail or paw of a rat placed in a restraint box inside an echo-free chamber. The vocal response of the animal is picked up by a microphone situated at a fixed distance from the animal’s head. Thanks to progress in the computer-assisted processing of such signals, it is now possible to study the characteristics (spectrum, reaction time, threshold, envelope) of the sounds emitted in response to a large range of stimulation intensities. Three types of emissions can be identified: 1) two distinct “peeps” (Fig. 10A), the energies of which are distributed across a large range of audible frequencies without a defined structure (Fig. 10B), and these amount to noise because they are simply emissions from the vocal cords; the first peep results from activation of relatively rapidly conducting A $\delta$  fibers, whereas the second results from activation of slowly conducting C fibers; 2) “chatters”, characterized by formants composed of a funda-

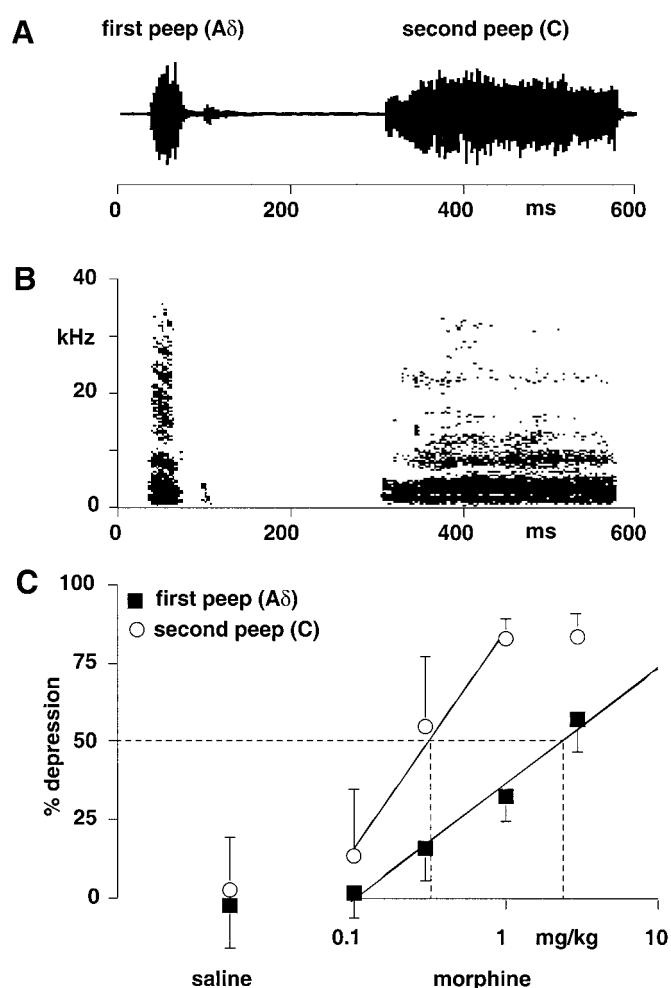


FIG. 10. Example of vocal responses evoked by an electric shock (duration, 2 ms; intensity, 20 mA) applied to the tail of a rat. A, recording during the first 600 ms after the stimulus. This was made with a microphone placed 5 cm from the muzzle of the animal and visualized on an oscilloscope. Two “peeps” produced by activation of A $\delta$  and C fibers can be seen. B, corresponding spectrogram. The two peeps correspond to strong emissions from the vocal cords over a broad frequency band in both the audible and the ultrasonic (>20 kHz) ranges. C, effect of morphine (0.1–10 mg/kg i.v.) on the successive peeps. Doses that reduced the peeps by 50% (ED<sub>50</sub>) are represented by the broken line. The response produced by C fibers is 6 times more sensitive to morphine (ED<sub>50</sub> = 0.3 mg/kg) than that produced by activation of A $\delta$  fibers (ED<sub>50</sub> = 1.9 mg/kg). From Jourdan et al., 1995, 1998 with permission.

mental frequency and its corresponding harmonics; this constitutes a very elaborate response, the physical characteristics of which are similar to human words; and 3) ultrasonic emissions inaudible to humans and made up of a fundamental frequency, without harmonics, between 20 and 35 kHz with mild modulations (Dinh et al., 1999). Each component of the response undoubtedly reflects a certain level of organization that has to be related to a particular physiological function. The characteristics of the first two peeps emitted by the rat are reminiscent of the phenomenon of double pain observed in humans following a brief, sharp nociceptive stimulus. The physiological meanings of the other components of the response are more difficult to understand. In line with Pavlovian conditioning, the chatters can be trig-

gered by a light signal (Borszcz, 1995b). The ultrasonic emissions may reflect the affective state and the degree of anxiety of the animal because they can also be recorded in other experimental situations that generate fear or stress (Sales and Pye, 1974; Haney and Miczek, 1994). In addition, they are sensitive to anxiolytic drugs (Tonoue et al., 1987; Cuomo et al., 1992).

The second peep is particularly sensitive to morphine with an  $ED_{50}$  value (0.3 mg/kg; i.v.) 5 times less than that for the first peep (Fig. 10C). Morphine equally decreases the chatters but has no significant effect on the ultrasonic emissions. Cooper and Vierck (1986b) recorded vocalizations produced by electrical stimulation of the lower leg in two monkeys and also observed that 0.25 mg/kg morphine reduced this response.

*b. Stimulation of the Dental Pulp.* As discussed above (see Section III.A.1.a.), one important weakness of using electrical stimulation to study pain lies in the nonselective fashion in which it excites different types of primary afferent nerve fibers. This can be overcome to some extent by using selective stimulation of small nerve fibers with triangular (Accornero et al., 1977) or trapezoidal (Fang and Mortimer, 1991) electrical pulses (as opposed to conventional rectangular waves) or by differential nerve blocks. The latter techniques involve preventing the conduction of impulses in larger diameter nerves by applying pressure, cold, or an anodal electrical field to the nerve proximal to the point of stimulation (e.g., see Mendell and Wall, 1964; Franz and Iggo, 1968a,b; Torebjörk and Hallin, 1973; Hopp et al., 1980). However, such techniques are imperfect, do not block activity in small non-nociceptive (e.g., thermoreceptive) nerves, and are not easy to apply in awake, unrestrained animals. As an alternative, many investigators have sought to find a tissue in which all the afferent nerve fibers are nociceptive. Most commonly, the dental pulp has been used for this purpose. Indeed, it was identified for use in the study of pain many decades ago (Goetzl et al., 1943).

Before discussing the various models of pain that have used stimulation of the dental pulp, it is important to consider the contention that all its afferent nerve fibers are nociceptive. This widely held belief is based on three lines of evidence (Anderson et al., 1970, Matthews, 1985; Carter and Matthews, 1989): 1) that the afferent nerves in the pulp can be activated only by stimuli which produce pain when similarly applied in humans; 2) that activation of these nerves in humans never produces any sensation other than pain (Naylor 1964; Edwall and Olgart, 1977; Jyväskylä and Kniffki, 1987); and 3) that all these fibers have small diameters (Beasley and Holland, 1978) and slow conduction velocities (Brookhart et al., 1953; Wagers and Smith, 1960) like those which are associated with nociception elsewhere in the body. However, in each case, there exists contrary evidence. One research group (Dong et al., 1985, 1993) has claimed to show that some pulpal nerves can be excited by gentle

mechanical stimulation through the dental enamel—a stimulus which would not produce pain in humans (but see Matthews, 1986; Carter and Matthews, 1989). Another group has provided evidence that it is possible to produce sensations of cold as well as of pain by applying a thermode at  $0.5^{\circ}\text{C}$  to the outside of the tooth (Grüsser et al., 1982, 1987). Furthermore, it has long been known that weak electrical stimulation of teeth can produce sharp but not overtly painful sensations (Vargas, 1956; see also Section X.). Finally, although it is undoubtedly true that the nerve fibers inside tooth pulp have small diameters, many of these have parent axons in the alveolar nerves that are large and rapidly conducting (Cadden et al., 1982, 1983; Holland and Robinson, 1983) and therefore have to be classified as  $A\beta$  fibers (Fig. 11). There is little evidence that  $A\beta$  fibers from other parts of the body are involved in signaling pain. In view of these conflicting lines of evidence and of the marked species differences between dental tissues (see below), one should be cautious before regarding all responses to stimulation of the pulp as being nociceptive. However, it is probably safe to conclude that at worst, electrical stimulation of pulpal nerves is closer to being a selective nociceptive stimulus than any similar stimulation of nerves elsewhere in the body.

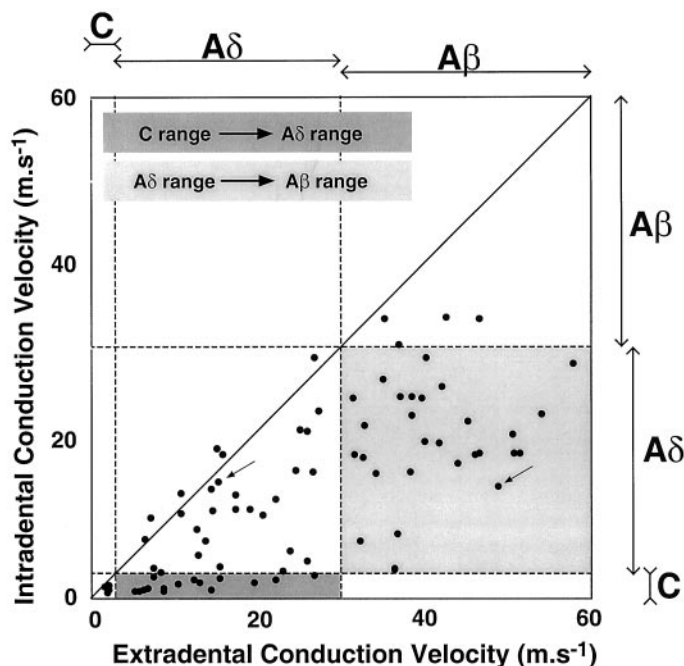


FIG. 11. The relationship between the conduction velocities of the terminal portions (ordinate, intradental conduction velocity) and parent axons (abscissa, extradental conduction velocity) of 80 nerve fibers supplying the pulp of cat canine teeth. Note that if these fibers were classified on the basis of their intradental velocities, they would almost all be in the  $A\delta$ - or C-fiber categories (labeling to the right). However, more conventionally, nerve fibers are classified by the velocities of their main axons (labeling at the top)—thus a large proportion of these “ $A\delta$ ” fibers have to be classed as  $A\beta$  (light shading) and many of the “C” fibers as  $A\delta$  (dark shading). The arrows indicate two fibers with similar intradental but very different extradental velocities—one an  $A\delta$  and one an  $A\beta$  fiber. Modified from Cadden et al. (1983), copyright 1983, with permission from Elsevier Science.

Regardless of whether all pulpal afferent nerves are nociceptive, it is undoubtedly true that, under controlled conditions, it is usually possible to excite pulpal nerves selectively with electrical stimuli, i.e., without exciting any other nerves. This is true at least for teeth of limited growth such as those in the dog and the cat (e.g., Matthews, 1977; Cadden et al., 1983). However, there is some doubt as to whether this is possible for continuously erupting teeth such as rat incisors, which have been used in a number of models of pain. These teeth are anatomically very different, and several studies have shown that the application of electrical stimuli through the dentine or directly to the pulp of such teeth at intensities sufficient to elicit nociceptive behavior is likely to excite nerves outside the tooth (periodontal nerves) as well as pulpal fibers (Hayashi, 1980; Jiffry, 1981; Engstrand et al., 1983). Other groups have suggested that the exclusive activation of pulpal nerves is possible, provided adequate care is taken (Toda et al., 1981; Rajaona et al., 1986; Myslinski and Matthews, 1987). However, one has to conclude that there are several biological and technical reasons for caution before ascribing all the responses to electrical stimulation of rodent incisors as being nociceptive.

Despite these problems, models of pain using tooth-pulp stimulation have been created using continuously erupting teeth in a number of species. The rat (Steinfels and Cook, 1985), the rabbit (Cheymol et al., 1959; Gouret, 1975; Piercey and Schroeder, 1980), and the guinea pig (Radouco-Thomas et al., 1957) have all been used in addition to species with teeth of limited growth, such as dogs, cats (Mitchell, 1964; Anderson and Mahan, 1971; Skingle and Tyers, 1979), and monkeys (Ha et al., 1978).

Two types of response have been monitored in such models: either the appearance of coordinated reactions involving licking, chewing, changes of facial expression, and head movements in the awake animal (Radouco-Thomas et al., 1957; Cheymol et al., 1959; Gouret, 1975; Ha et al., 1978; Skingle and Tyers, 1979; Piercey and Schroeder, 1980; Steinfels and Cook, 1985), or the disynaptic jaw opening reflex, which can be recorded electromyographically from the digastric muscle in awake or anesthetized preparations (Mitchell, 1964; Anderson and Mahan, 1971). Some studies have monitored both types of response (Rajaona et al., 1986). Although monitoring the reflex has the advantage of providing an easily quantifiable response, it has the same drawback as discussed elsewhere in this review in regard to flexion reflexes in the limbs (see *Sections IV. and VIII.A.*)—namely, that such reflexes have been produced by stimulation of mechanoreceptors (Hannam and Matthews, 1969; Cadden, 1985; but see Dessem et al., 1988) as well as by stimulation of nociceptors (Fig. 12), i.e., there is doubt about the output specificity of such a model. Indeed, given the near-total absence of myelinated axons within the rat pulp (Bishop, 1981), the relatively short

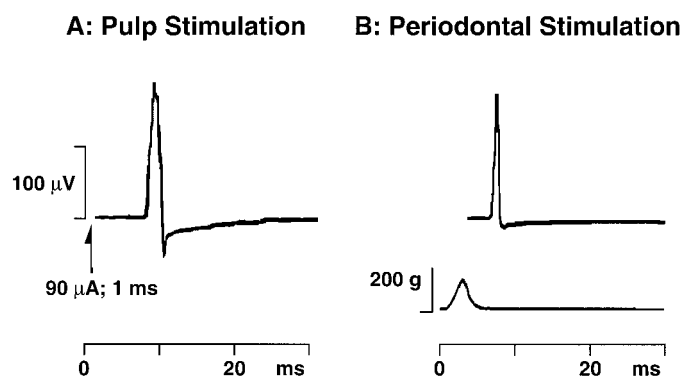


FIG. 12. Recordings from the nerve to the digastric (jaw opening) muscle in an anesthetized cat showing reflex responses to (A) electrical stimulation of the pulp of an upper canine tooth (at time indicated by arrow) and (B) gentle mechanical stimulation of the same tooth (which would excite periodontal nerves). The timing and force profile of the mechanical stimulus is shown beneath the neurogram in B. All traces have been retouched for clarity of presentation. Note the qualitative similarity in these digastric reflex responses to nociceptive and non-nociceptive stimuli. Modified from Cadden (1985), copyright 1985, with permission from Elsevier Science.

latencies of many reflexes recorded in that species are likely to have resulted from activation of myelinated fibers in the adjacent periodontal tissues (Jiffry, 1981), at least some of which are likely to be mechanoreceptors.

Notwithstanding their limitations, models using stimulation of the dental pulp discriminate well for opioid analgesics (Collier, 1964; Fennessy and Lee, 1975; Chau, 1989). They are more selective than the abdominal contortion test (see *Section IV.B.*) and can reveal the activity of nonopioid analgesics that cannot be revealed by the hot plate test. However these models are not very sensitive to the analgesic effects of nonsteroidal anti-inflammatory agents, which show effects only at high (sometimes close to toxic) doses. They are little used nowadays.

*c. Stimulation of the Limbs.* Electromyographic recordings of nociceptive limb reflexes have been used for pharmacological studies of nociception, but they are far less common than behavioral tests. Nevertheless, a wide variety of preparations have been used [e.g., the intact or spinalized, anesthetized, or decerebrate cat (McClane and Martin, 1967; Bell and Martin, 1977; Duggan et al., 1984; Bell et al., 1985), the chronically spinalized dog (Martin et al., 1964), the spinalized decerebrate rabbit (Clarke and Ford, 1987), the anesthetized (Parsons et al., 1989; Parsons and Headley, 1989) or decerebrate (Woolf and Wiesenfeld-Hallin, 1986) spinalized rat]. These electromyographic studies have allowed the quantification of responses regardless of whether there is any movement; they have also allowed the evolution of responses to suprathreshold stimuli to be studied. In a series of studies from 1944 onward, Wikler noted among other things that in chronically spinalized cats or dogs, morphine depressed spinal reflexes when they were characterized by long-lasting after-discharges, but that other spinal reflexes were either not affected or facilitated (Wikler, 1950).

Nociceptive flexion reflexes have also been recorded in humans using electrophysiological methods to study the spinal and supraspinal controls that exert an influence on motor activity under normal or pathological conditions (Kugelberg, 1948; Hagbarth, 1960; Kugelberg et al., 1960; Dimitrijevic and Nathan, 1968; Hugon, 1973). From 1977 onward, Willer developed a dual electrophysiological and neuropharmacological approach for studying the nociceptive flexion reflex of the lower limb. His work strongly suggested that the nociceptive flexion reflex can be used as an objective index for the study of nociception (Willer, 1977, 1985). Indeed, there is a close relationship between the pain threshold and the threshold of the nociceptive flexion ( $R_{III}$ ) reflex which can be evoked by electrical stimulation of the sural (or external saphenous) nerve at the ankle. Thus, the  $R_{III}$  reflex constitutes a useful tool for the study of pain in humans. Campbell et al. (1991) arrived at a similar conclusion with respect to a withdrawal reflex produced by nociceptive thermal stimulation.

By using the methods of the Electromyography Clinic, it is possible to record a reflex to activation of C fibers in anesthetized rats (Fig. 13). Electrical stimulation within the cutaneous distribution of the sural nerve provokes a two-component reflex in the biceps femoris muscle: the

first component has a short latency and is a response to stimulation of rapidly conducting fibers; the second occurs between 150 and 450 ms after the stimulus and corresponds to the activation of C fibers (Duysens and Gybels, 1988; Strimbu-Gozariu et al., 1993; Falinower et al., 1994). The main advantage of this model lies in that it is easy to quantify responses to various intensities of stimuli; the reflex magnitude increases with stimulus intensity from threshold until it reaches a plateau (Guirimand et al., 1994). One can see that the curve in Fig. 13C closely follows a logarithmic function or, even more so, a power function. Thus, it is possible to analyze responses to a wide range of stimuli and get closer to the variety of nociceptive situations found clinically, both with respect to chronic pain and to pain in the perioperative period (Guirimand et al., 1994). Using this model, one can show that the  $ED_{50}$  value for the depressive effects of morphine increases 3-fold when the stimulus intensity goes from threshold to 7 times the threshold (Fig. 14).

The validity of using flexor reflexes as measurements of nociception in animals is perhaps more ambiguous than it is in humans. It is not difficult to imagine that an acute noxious stimulus simultaneously causes pain and a withdrawal reflex. However these two phenomena be-

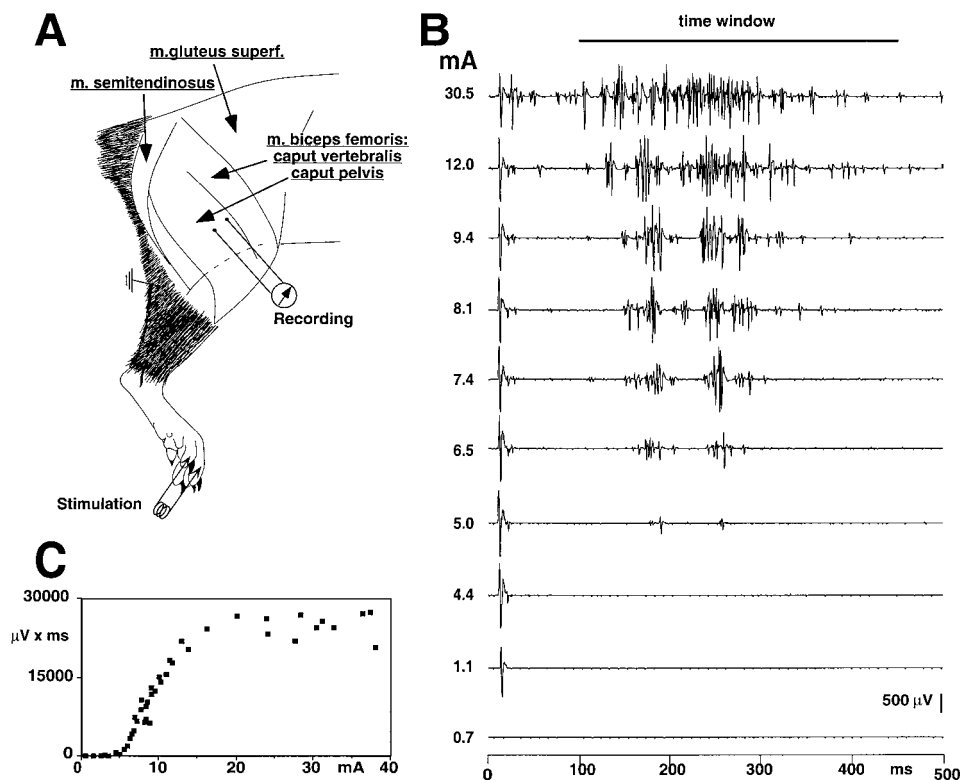


FIG. 13. Example of C fiber-mediated reflex evoked by electrical stimulation. A, experimental set-up for stimulation and recording. B, electromyographic recording from the biceps femoris muscle. The response was evoked by electrical stimulation within the territory of the sural nerve (2 ms square-wave stimulus applied at time 0). The stimulus intensity is indicated to the left of each record. At the lower stimulus intensities, there was an early response caused by the activation of myelinated fibers. As the intensity was increased to 5 mA and higher, there was a second response produced by the activation of C fibers. The signals were rectified and integrated within the time window 100 to 450 ms (horizontal line at top) to build the curve presented in C. C, corresponding recruitment curve. Abscissa: stimulus intensity (in mA). Ordinate: responses in terms of integrated electromyogram within a window 100 to 450 ms after the stimulus (in  $\mu V \cdot ms$ ). The magnitude of the C fiber-evoked response increases from its threshold until it reaches a plateau at around 3 times threshold. After Falinower et al., 1994 with permission.

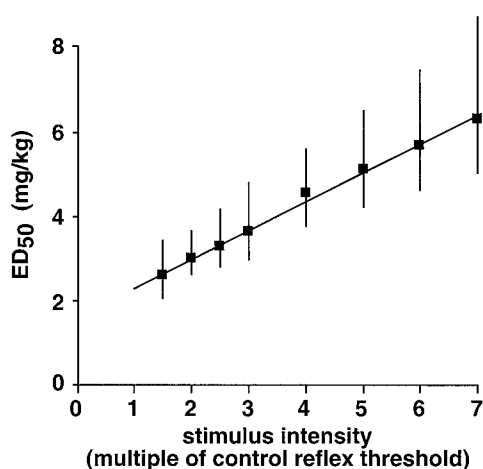


FIG. 14. Influence of stimulus intensity on dose-response relationships. The results obtained from the recruitment curves for the C-fiber reflex (see Fig. 13C) make it possible to determine dose-response curves for each intensity of stimulation and to deduce the corresponding  $ED_{50}$  value. The  $ED_{50}$  value for intravenous morphine varies with stimulus intensity. On the abscissa, the stimulus intensity is expressed as a multiple of the reflex threshold determined during the control period. On the ordinate, the  $ED_{50}$  values are shown ( $\pm$  a 95% confidence interval), as obtained from the dose-response curves for each stimulus intensity. A linear relationship can be seen between the intensity of stimulation and the  $ED_{50}$  values for morphine. Modified from Guirimand et al., 1995 with permission.

come dissociated under certain conditions, particularly when the animal is anesthetized. The paradox of dissociation between suppression of pain and the presence of a reflex response could be explained by the particular sensitivity of the brain to the general anesthetic. However, it could also possibly be explained by reflexes and ascending pathways using different circuits at a spinal level (Jurna and Zetler, 1981; Doi and Jurna, 1982; Schouenborg et al., 1995; Jasmin et al., 1997).

## VI. Tests Based on the Use of Long Duration Stimuli ("Tonic Pain")

Basically, these tests involve using an irritant, algogenic chemical agent as the nociceptive stimulus. They differ from the vast majority of other tests in that they abandon the principle of determining the nociceptive threshold and involve a quantitative approach to the behavior observed after the application of a stimulus with a potency that is going to vary with time. They can be thought of as a kind of model for tonic pain. However, they are not models for chronic pain because their duration is only in the order of some tens of minutes.

The main types of behavioral test based on such stimuli use intradermal or intraperitoneal injections. The use of intra-arterial or intradental bradykinin is less common (Guzman et al., 1964; Deffenu et al., 1966; Lim and Guzman, 1968; Foong et al., 1982), although intracapsular (jaw) injections of algogenic substances have also been used recently in pharmacological studies of pain in nonbehavioral models in which the animals are anesthetized (Broton and Sessle, 1988; Yu et al., 1994,

1995, 1996). In addition, there are behavioral tests that use the intracapsular administration of urate crystals, Freund's adjuvant, or carrageenin, but these are related to models of chronic inflammatory pain (Okuda et al., 1984; Otsuki et al., 1986; Coderre and Wall, 1987; Butler et al., 1992; Tonussi and Ferreira, 1992).

In this section, we also consider tests based on the stimulation of hollow organs. These animal models of visceral pain can be split into two categories on the basis of stimulus type: those involving the administration of algogenic agents, and those involving distension of hollow organs. In the latter case, one can add a subcategory of distension following induced inflammation of the hollow organ.

### A. Intradermal Injections

The most commonly used substance for intradermal injections is formalin (the "formalin test"). The term *formalin* usually means a 37% solution of formaldehyde. Less commonly used are hypertonic saline (Lewis and Kellgren, 1939; Hwang and Wilcox, 1986), ethylene diamine tetra-acetic acid (Teiger, 1976), Freund's adjuvant (Iadarola et al., 1988), capsaicin (Sakurada et al., 1992), and bee sting (Larivière and Melzack, 1996). Other substances have been tested but with less success (Wheeler-Aceto et al., 1990).

A 0.5 to 15% solution of formalin injected into the dorsal surface of the rat forepaw provokes a painful behavior that can be assessed on a four-level scale related to posture: 0, normal posture; 1, with the injected paw remaining on the ground but not supporting the animal; 2, with the injected paw clearly raised; and 3, with the injected paw being licked, nibbled, or shaken (Dubuisson and Dennis, 1977). The response is given a mark, and the results are expressed either continuously per unit of time or at regular time intervals when several animals are observed sequentially (Abbott et al., 1999). Each level on this scale can be weighted to optimize the test (Coderre et al., 1993; Abbott et al., 1995; Watson et al., 1997). This method has also been used in the mouse, cat, and monkey (Dubuisson and Dennis, 1977; Alreja et al., 1984; Hunskaar et al., 1985; Murray et al., 1988; Tjølsen et al., 1992). The measured parameter can also be the number of licks or twitches of the paw per unit of time (Wheeler-Aceto and Cowan, 1991), the cumulative time spent biting/licking the paw (Sufka et al., 1998), or even a measure of the overall agitation of the animal obtained by a strain gauge coupled to the cage (Jett and Michelson, 1996). Such specific behaviors resulting from an injection of formalin can be captured automatically by a camera attached to a computer; in this way, the effects of a pharmacological substance on such motor activity can be identified, analyzed, and uncoupled from antinociceptive effects (Jourdan et al., 1997). This test has been adapted for use in the trigeminal region (Clavelou et al., 1989, 1995).

In the rat and the mouse, intraplantar injections of formalin produce a biphasic behavioral reaction. This behavior consists of an initial phase, occurring about 3 min after the injection, and then after a quiescent period, a second phase between the 20th and 30th minutes. The intensities of these behaviors are dependent on the concentration of formalin that is administered (Rosland et al., 1990; Aloisi et al., 1995; Clavelou et al., 1995). The first phase results essentially from the direct stimulation of nociceptors, whereas the second involves a period of sensitization during which inflammatory phenomena occur. The central or peripheral origin of this second phase has been the subject of debate (Tjølsen et al., 1992). For some, the second phase results from central processes triggered by the neuronal activation during the first phase (Coderre et al., 1993). However, this hypothesis seems unlikely not only because formalin provokes biphasic activity in afferent fibers (McCall et al., 1996; Puig and Sorokin, 1996), but even more so because the blocking of the first phase by substances with rapid actions (e.g., subcutaneous lidocaine or intravenous remifentanyl) does not suppress the second phase (Dallel et al., 1995; Taylor et al., 1995, 1997). Thus, the second phase cannot be interpreted as a consequence of the first; it clearly also originates from peripheral mechanisms.

Opioid analgesics seem to be antinociceptive for both phases, although the second is more sensitive to these substances. In contrast, NSAIDs such as indomethacin seem to suppress only the second phase (Hunskar and Hole, 1987; Shibata et al., 1989; Malmberg and Yaksh, 1992; Jourdan et al., 1997), especially when the formalin is injected in high concentrations (Yashpal and Coderre, 1998).

Another model of tonic cutaneous pain has been proposed recently. This test involves mimicking postoperative pain triggered by a cutaneous incision (Brennan et al., 1996; Zahn et al., 1997).

### *B. Intraperitoneal Injections of Irritant Agents (the "Writhing Test")*

The intraperitoneal administration of agents that irritate serous membranes provokes a very stereotyped behavior in the mouse and the rat which is characterized by abdominal contractions, movements of the body as a whole (particularly of the hind paws), twisting of dorso-abdominal muscles, and a reduction in motor activity and motor incoordination. The test is sometimes called the abdominal contortion test, the abdominal constriction response, or the stretching test, but more commonly it is known as the "writhing test". Generally the measurements are of the occurrence per unit of time of abdominal cramps resulting from the injection of the algogenic agent. These behaviors are considered to be reflexes (Hammond, 1989) and to be evidence of visceral pain (Vycklicky, 1979); however, it would probably be wiser to call it peritoneovisceral pain. Indeed, given the

well established fact that the parietal peritoneum receives a somatic innervation (Williams et al., 1995), it is possible that the pain may not be visceral at all. However, the pain is probably similar to that resulting from peritonitis. Unfortunately, the frequency of cramps decreases spontaneously with time (Michael-Titus and Costentin, 1988) to such an extent that it is impossible to evaluate the duration of action of an analgesic on a single animal. Furthermore, the number of cramps is subject to a great deal of variability (Hendershot and Forsaith, 1959).

Many modifications have been made to the original test using phenylbenzoquinone, which was described in 1957 by Siegmund et al. after analogous observations had been made following the intraperitoneal injection of radio-opaque elements (Van der Wende et al., 1956). These modifications mainly concern the chemical agent that, in turn, determines the duration of the effect: acetylcholine, dilute hydrochloric or acetic acid (Eckhardt et al., 1958; Koster et al., 1959; Niemegeers et al., 1975), bradykinin (Emele and Shanaman, 1963), adrenaline (Matsumoto and Nickander, 1967), adenosine triphosphate, potassium chloride, tryptamine (Collier et al., 1968), and oxytocin (Murray and Miller, 1960) have all been used. Modifications have also been made to the concentration, temperature, and volume of the injected solution, the experimental conditions, and ways of monitoring behavioral changes so as to simplify the test and increase its sensitivity (Linée and Gouret, 1972; Harada et al., 1979). The test has also been used in monkeys (Pearl et al., 1969a).

These methods have the advantage of allowing evidence to be obtained for effects produced by weak analgesics. On the other hand, they lack specificity. Indeed, these tests work not only for all major and minor analgesics, but equally for numerous other substances, including some that have no analgesic action, e.g., adrenergic blockers, antihistamines, muscle relaxants, monoamine oxidase inhibitors, and neuroleptics. (Hendershot and Forsaith, 1959; Chernov et al., 1967; Pearl et al., 1968; Loux et al., 1978). Thus, a positive result with this test does not necessarily mean there is analgesic activity. Nevertheless, because all analgesics inhibit abdominal cramps, this method is useful for sifting molecules whose pharmacodynamic properties are unknown (Hendershot and Forsaith, 1959; Chernov et al., 1967; Loux et al., 1978). The specificity can be improved by undertaking a preliminary Rotorod test to detect and eliminate molecules that alter the motor performance of the animal (Pearl et al., 1969b). Although the writhing test has a poor specificity, it is sensitive and, after a fashion, predictive, as shown by the correlation between ED<sub>50</sub> values obtained in rats using this test and analgesic doses in humans (Collier et al., 1968; Dubinsky et al., 1987).

Intraperitoneal injections of algogenic substances have also been used in nonbehavioral models of nocicep-

tion, i.e., models in which the animal is anesthetized. For example, changes in mean arterial blood pressure and intragastric pressure have been used as indicators of nociceptive responses to intraperitoneal bradykinin in anesthetized rats (Holzer-Petsche, 1992; Holzer-Petsche and Rordorf-Nikolic, 1995; Griesbacher et al., 1998).

### C. Stimulation of Hollow Organs

In addition to such tests of peritoneal or visceral nociception, other tests involve injecting algogenic substances directly into hollow organs and, as such, may be regarded as models for true visceral pain. For example, administration of formalin into the rat colon can produce a complex biphasic type of "pain behavior" involving an initial phase of body stretching and contraction of either the flanks or the whole body and a second phase that predominantly involves abdominal licking and nibbling (Miampamba et al., 1994). Intracolonic infusions of glycerol also produce abdominal constrictions (Botella et al., 1998). Similarly, a number of models have been developed for bladder pain, whereby reflexes and/or more complex behaviors have been observed following intravesical administration of capsaicin, capsaicin-like substances (Craft et al., 1993, 1995; Pandita et al., 1997), or turpentine (McMahon and Abel, 1987; Jaggar et al., 1998). More recently, a model for inflammatory uterine pain was developed, whereby intrauterine injections of mustard oil produced complex behavior patterns in rats (Wesselmann et al., 1998).

Arguably, a more natural noxious visceral stimulus is that produced by distension of hollow organs. Although distension of viscera has been used in electrophysiological studies for many years (e.g., Talaat, 1937; Paintal, 1954; Iggo, 1955; Cervero, 1994), the use of such stimuli at noxious intensities in behavioral studies is a more recent development. In this context, colorectal distension by means of an inflatable balloon in the rat is the most commonly used stimulus. Ness and Gebhart (1988) used such a stimulus and found that it produced avoidance behavior as well as reflex activities that could be recorded electromyographically from the abdominal muscles. It also evoked quantifiable vegetative responses that, in the awake animal, involved increased arterial pressure and tachycardia, although these were attenuated or even reversed by certain anesthetic agents (Ness and Gebhart; 1988). This group (Danzebrink and Gebhart, 1991; Ness et al., 1991; Maves and Gebhart, 1992; Kolhekar and Gebhart, 1994; Maves et al., 1994; Danzebrink et al., 1995; Traub et al., 1995) and others (e.g., Omote et al., 1994; Harada et al., 1995a,b; Saito et al., 1995; Yamamori et al., 1996; Hara et al., 1998, 1999) have subsequently used this or similar behavioral models to test a wide range of pharmacological agents. In some of these models (Harada et al., 1995a,b; Saito et al., 1995; Yamamori et al., 1996), the abdominal reflexes were monitored as increases in intra-abdominal pressure rather than electromyographically. A development

of these models involves firstly inflaming the colon by the administration of acetic acid. As was shown originally in the anesthetized rat, this procedure sensitizes responses to colonic distension (Langlois et al., 1994). The application of acetic acid followed by distension of the colon has subsequently been used in the conscious rat; under such conditions, the abdominal reflexes produced by distension are enhanced (e.g., Burton and Gebhart, 1995, 1998; Langlois et al., 1996, 1997), although the threshold for these responses is unaltered as are the reflex alterations in arterial blood pressure (Burton and Gebhart, 1995). In other studies, colonic distension has been applied after the colon has been inflamed by other chemical agents, e.g., turpentine (Ness et al., 1991), trinitrobenzene sulfonic acid (Morteau et al., 1994; Goldhill et al., 1998), or zymosan (Coutinho et al., 1996).

Other models of nociception involving the gastrointestinal tract of conscious rats have used distension of the stomach (Rouzade et al., 1998) or duodenum (Colburn et al., 1989; DeLeo et al., 1989; Feng et al., 1998) as the test stimulus. In addition, colonic distension has been used in models involving other species, including the rabbit (Jensen et al., 1992; Crawford et al., 1993; Borgbjerg et al., 1996a,b) and the dog (Houghton et al., 1991).

Models of visceral nociception have also used mechanical stimulation of parts of the genitourinary system in conscious animals, although such stimuli are more common in tests including anesthetized animals (see below). However, Giamberardino et al. (1995) studied the behavior produced by the surgical introduction of dental cement—to mimic a calculus—into the ureter and found something akin to episodes of writhing behavior over a 4-day period. In addition, these authors observed a concomitant hyperalgesia in the abdominal muscles (Giamberardino et al., 1990, 1995), which taken together with their own electrophysiological data (Giamberardino et al., 1996) provided clear evidence for visceromuscular convergence at a spinal level.

It is possible to record a number of responses to intense mechanical stimulation of hollow viscera in anesthetized animals, and these have formed the basis of a number of tests. For example cardiovascular responses can be produced in anesthetized rats by colorectal distension with (Langlois et al., 1994) or without (Banner et al., 1995) inflammation, distension of the duodenum (Moss and Sanger, 1990; Diop et al., 1994), distension of the jejunum with (McLean et al., 1997) or without (Lembeck and Skofitsch, 1982; McLean et al., 1998) sensitization by an experimental nematode infection, distension of the ileum (Clark and Smith, 1985), distension of the renal pelvis (Brasch and Zetler, 1982), distension of the ureter (Roza and Laird, 1995), or distension of the uterus (Robbins and Sato, 1991). In all but one (Roza and Laird, 1995) of these studies, the cardiovascular response involved a decrease in systemic arterial blood pressure, which is in contrast to the increases in blood pressure evoked by visceral distension in awake animals

(see above). Clearly, the cardiovascular responses to visceral distension are preparation-dependent (Ness and Gebhart, 1990), and as a result, it may be even more important than in other models of pain to establish a good normal baseline response before the administration of drugs being tested. Finally, other responses to visceral distension have been monitored in models involving anesthetized preparations, notably changes in intragastric pressure during duodenal distension (Moss and Sanger, 1990) and desynchronization of the electrocorticogram during urinary bladder distension (Conte et al., 1996).

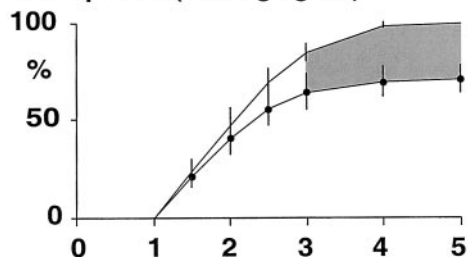
## VII. Nociceptive Tests and Stimulus-Response Relationships

It cannot be stated often enough that in most animal models of pain, the only measurement is of a nociceptive threshold. However, clinical pain is rarely limited to threshold intensities (0–1 on a visual analog scale of 1–10). Benedetti et al. (1984) summarized data from many previous reports and concluded that the occurrence, severity, and duration of postsurgical pain varied *inter alia* with the site, nature, and duration of an operation. It is usually worse after intrathoracic or intra-abdominal surgery or surgery to the joints and bones, but less after most superficial operations.

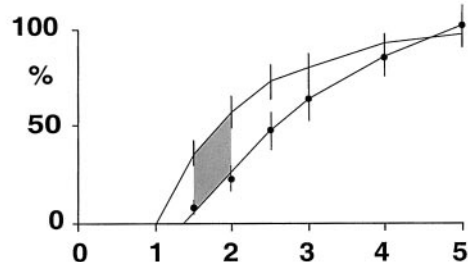
This restriction to measurements of threshold in classic tests is very limiting. From a theoretical point of view, imagine if you could construct a stimulus-response curve for each test. Imagine also that you then administer to the animal an analgesic substance with an unknown action. If the stimulus-response curve is displaced to the right in a more or less parallel fashion, then measuring the threshold will provide evidence of the action of the substance. If, on the other hand, the slope of the stimulus-response curve is reduced without any overall shift, then the measurement of the threshold alone will not permit any conclusion to be drawn about the substance (or may lead to the wrong conclusion that the substance has no effect). In other words, measuring a threshold does not permit an evaluation of changes in the gain of a system, no matter what that is. It is well known that the nociceptive systems that generate pain can show changes in gain. One can illustrate this by considering the effects of three different analgesics on the recruitment curves for an electromyographic response evoked in the biceps femoris muscle of the anesthetized rat by stimulation of C fibers: ketoprofen depresses only the responses to the strongest stimulus intensities and does not modify the threshold; buprenorphine acts only against the responses to the lowest stimulus intensities; and morphine acts against all the responses (Fig. 15).

One further comment is necessary with respect to the measurement of nociceptive thresholds. In general, there are several psychophysical methods for measuring

### A : ketoprofen (100 mg/kg i.v.)



### B : buprenorphine (300 µg/kg i.v.)



### C : morphine (6 mg/kg i.v.)

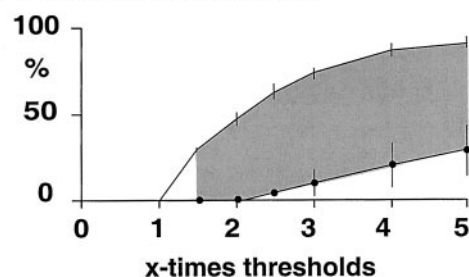


FIG. 15. The effects of an analgesic can depend on the intensity of stimulation: an example of reflexes recorded electromyographically from the biceps femoris in response to stimulation of C fibers in the sural nerve of the anesthetized rat. The individual responses are normalized in terms of the percentage of the maximum control reflex (ordinate in %). The reflex response increases with the intensity of the stimulus and then reaches a plateau at the highest values of stimulation. For each animal, the threshold of the reflex is defined as the intersection of the curve with the abscissa, then the intensities of stimulation are expressed as multiples of this threshold. The gray zone corresponds to the points that were significantly different after treatment (black circles) from the corresponding control points (without symbols). A, ketoprofen depressed only the responses to the strongest intensities of stimulation and did not modify the threshold. B, in contrast, buprenorphine was active only on the responses produced by the lowest intensities. C, morphine acted against all the responses. Modified from Guirimand et al., 1995, and Bustamante et al., 1996 with permission.

a sensory threshold. It is beyond the scope of this review to consider all of them. However, one of them—the method of limits—merits some discussion. This method consists of gradually increasing the intensity of a stimulus until it is detected by the subject. To reduce bias, series with decreasing intensities of stimuli are also presented to the subject to determine the point at which the stimulus is no longer perceived. It is the combination of results obtained with the ascending and descending series that ultimately gives the threshold. For various reasons (the risks of tissue damage or of stressing the animal, etc.), in many tests of nociception, the stimulus



is presented only at increasing intensities. This approach undoubtedly introduces a systematic error in the measurement of nociceptive thresholds (Lineberry, 1981). Thus, in the guinea pig, the threshold for evoking a muscular contraction using noxious radiant heat was 10% lower when determined during a series of increasing stimulus intensities than when it was determined while the stimulus was being reduced (Winder et al., 1946). As far as animal models of nociception are concerned, to the best of our knowledge, the method of limits has been used only with the flinch-jump test (Evans, 1961).

### VIII. Nociceptive Tests and Motor Activity

Most tests that are used to study pain in animals involve motor responses to nociceptive stimuli. These depend on an implicit hypothesis that there is a strong relationship between nociception and motor activity. No one can deny the existence of such a relationship. However, it has to be put in context, and above all else, it must not be considered as being unequivocal. It should always be remembered, for example, that the modulation of reflex motor responses can be different from that of dorsal horn neurons (Carstens and Campbell, 1992).

Furthermore, the activity of motoneurons is controlled by structures in the brain, which can influence motor activity regardless of whether it is initiated reflexly or from a supraspinal level. Electromyographic recordings provide a complementary approach to many of the behavioral tests described above and, more particularly, allow them to be placed in a physiological context.

Sherrington (1906a,b, 1910) suggested that pain is “the psychical adjunct of a protective reflex”, and it was in characterizing protective flexion reflexes that he introduced the concept of nociception. These reflexes result from activation of polysynaptic spinal neuronal circuits, which themselves are under the control of spinal and/or supraspinal influences. The amplitudes and durations of these reflexes are functions of stimulus intensity (Creed et al., 1932; Lloyd, 1943a,b). Nowadays, the term *reflex* is often applied to two phenomena that, although they are intimately linked, are distinct from one another: the reflex activation of the muscle(s), which is quantifiable using electromyography, and the reflex movement, which is characterized by its latency, force, and direction. Considering these two phenomena as the same thing can cause great confusion. One must remember, for example, that flexion reflex movements result from the contraction of flexor muscles and the relaxation of extensors (Sherrington, 1906b; Hagbarth, 1952).

In the vertical posture, the extensors (the “antigravity” muscles) are tonically active while activity in the flexors is inhibited. In healthy human subjects, the transfer of weight from one leg to the other results in a gradual inhibition of flexion reflexes in the limb taking the weight with an accompanying and symmetrical fa-

cilitation of their counterparts in the opposite limb (Rossi and Decchi, 1994). Some nociceptive tests necessitate postural adjustments of the animal. For example, in the orthostatic position, the motoneurons of the flexors are inhibited (and those of the extensors facilitated), and a flexion reflex is more difficult to evoke. Thus, it is not surprising that different results can be obtained depending on whether an animal is upright or not (e.g., Kauppila et al., 1998). On the other hand, when an animal takes up an “antalgic position”, the injured limb is flexed, the flexor motoneurons are facilitated (and those of the extensors inhibited), and a flexion reflex is easier to evoke. However, the position of the resting animal involves a motor equilibrium between one limb and its contralateral counterpart. Thus, the antalgic position will also result in increased tonic activity in the extensors of the contralateral limb as more of the animal’s weight is transferred there. These mechanisms, which strictly speaking fall into the field of motor control, are likely to affect results from some animal models of nociception. This underlines the fact that such results should not be interpreted only in terms of nociception. Thus, if one were to compare in a single animal a response in a control limb with that of its contralateral counterpart, which was in the antalgic position, one would introduce a systematic imbalance related to motor-control mechanisms. This could result in an overestimation of hyperalgesia or allodynia. In this context, it is interesting to note that the extent of allodynia estimated after administration of Freund’s adjuvant into a paw is an order of magnitude greater than that within the orofacial region in which there is no equivalent postural adjustment (Ren, 1999).

#### A. Not All Flexion Reflexes Are Nociceptive

Electromyographic techniques allow the recording and analysis of one or more reflex responses in flexor muscles. These responses follow each other, separated by periods of silence, in a fashion that reflects the activation of afferent fibers with different diameters and, hence, different conduction velocities. According to the studies of Lloyd (1943a,b) in the spinal cat, flexion reflexes are made up of two components: the first has a short latency and low threshold and can be produced by weak-intensity stimuli; the second has a longer latency and higher threshold and results only from intense stimulation—it corresponds to the activation of high-threshold afferent fibers with slow conduction velocities. In humans, C-fiber afferents are responsible for the delayed component of the flexion reflex produced by intense stimulation of the sural or plantar nerves (Kugelberg, 1948). The activation of A $\beta$  fibers can also produce a flexion reflex in the anesthetized rat—proof that mechanoreceptive impulses can also modify the excitability of flexor motoneurons (Schouenborg and Sjölund, 1983). Moreover, non-noxious heat, which on its own cannot evoke flexion reflexes, can nevertheless influence them.

In this context, the R<sub>III</sub> reflex in humans is facilitated by non-noxious heat applied by a CO<sub>2</sub> laser to the territory of the sural nerve (Plaghki et al., 1998). Thus, an increase in cutaneous temperature can, by itself and independently of other factors, contribute to hyperalgesic phenomena (e.g., during inflammation).

Lundberg's group investigated the nature of afferent impulses that converge onto interneurons in polysynaptic flexion reflex arcs. The concept of "flexor reflex afferents" (FRAs) includes all fibers (groups II, III, and IV) of muscular, cutaneous, and articular origin, which when activated provoke flexion reflexes. The reflex circuits are tightly controlled by descending pathways originating from supraspinal centers (Eccles and Lundberg, 1959a; Holmqvist and Lundberg, 1959, 1961; references in Lundberg, 1982; Schomburg, 1990). Some afferents have the function of controlling active movements, whereas others (the nociceptive A $\delta$  and C fibers) have roles entirely related to nociception (references in Schomburg, 1990). Schomburg (1997) recently retraced the historical relationship between FRAs and nociception. In fact, the early studies leading up to the definition of FRAs were concerned mainly with which types of afferents (particularly muscle afferents) could activate flexor motoneurons, inhibit extensor motoneurons, and thus produce a flexion reflex; the concept of nociception was hardly mentioned (Eccles and Lundberg, 1959b; Lundberg, 1959). Indeed, these authors even stated that there was no evidence to support the assumption that group III (A $\delta$ ) muscle afferents mediating flexion reflexes were nociceptive (Eccles and Lundberg, 1959b). The notion of FRAs came to be associated with reflex pathways that exhibited a certain amount of convergence. The inclusion of nociceptors in this larger group of afferent nerve fibers that constitute FRAs was suggested by the finding of a spatial facilitation resulting from the convergence of signals from cutaneous nociceptors and non-nociceptive mechanoreceptors (Behrends et al., 1983), muscle spindles (Kirkwood et al., 1987), or articular and muscle group I to III afferents (Steffens and Schomburg, 1993).

It is perfectly clear from the human studies of Hugon (1973) that there are two distinct reflex components evoked by electrical stimulation of the sural nerve. The reflex which appears first, named the R<sub>II</sub>, is evoked by nonpainful mechanoreceptive stimulation and plays a role in the control of movement. This is not a protective reflex, but rather a "locomotion" reflex in the broadest sense of the term. It is evoked by stimulation of superficial mechanoreceptive and proprioceptive (muscular or articular) afferent fibers. The R<sub>III</sub> reflex has a longer latency and is a nociceptive defense reflex; indeed, there is a near-perfect coincidence between the development of painful sensations and the evolution of the R<sub>III</sub> component of the flexion reflex (Hugon, 1973; Willer, 1977). In general, electrical stimulation can activate the whole spectrum of cutaneous afferents and evoke several reflex components. Among these cutaneous afferents, only a

proportion can be considered as being involved in nociceptive phenomena. Among C fibers, this proportion is overwhelming, but it must be remembered that even some of these are thermoreceptive (Hensel, 1973; La Motte and Campbell, 1978, Darian-Smith et al., 1979, Duclaux and Kenshalo, 1980).

In conclusion, the appearance of a flexion reflex does not *ipso facto* mean that the stimulus is nociceptive or that it involves a nociceptive flexion reflex. For example, in the newborn, the flexor muscles show hypertonia in comparison with the extensor muscles, and this relates to the fact that exaggerated flexion reflexes can be brought about by harmless stimuli, without any suggestion that they are a sign of pain (Bodensteiner, 1992). In the newborn rat, one can easily produce a movement of the tail with temperatures that would be not be painful in the adult. However, this is not a suitable response because it results in the tail approaching the source of a potentially noxious stimulus (Fig. 16; Falcon et al., 1996; Holmberg and Schouenborg, 1996). In fact, what these observations show is that the central nervous system is immature; the logical sequence of development is that inhibitory control systems can develop only after the excitatory mechanisms, which they will modulate, are in place. To interpret such observations in terms of pain would be extremely difficult (Lloyd-Thomas and Fitzgerald, 1996). One could conclude that they represent a state of hyperalgesia only if one used the standard, oversimplistic hypothesis—albeit one that is never openly stated—that excitation equals pain, and inhibition equals analgesia. There is no doubt that the central nervous system does not work with such clearcut duality; rather, the excitatory and inhibitory mechanisms within the central nervous system work together and in competition to produce an overall effect (as witnessed, for example, by mechanisms operating throughout the visual system). In addition, in some parts of the human body such as the orofacial region, nociceptive reflexes predominantly involve inhibition of muscle activity, not excitation (Orchardson and Cadden, 1998).

### B. Not All Nociceptive Reflexes Are Flexion Reflexes

Just as flexion reflexes are not exclusively nociceptive, nociceptive reflexes are not always flexion reflexes. Indeed, electrical stimulation of some cutaneous nerves can activate extensor muscles (Hagbarth, 1952). For each of these muscles, there is a nociceptive cutaneous receptive field, stimulation of which provokes muscular contraction and an extension movement (Kugelberg et al., 1960; Engberg, 1964). This is why the term flexion reflex does not completely match that of nociceptive reflexes. In this context, Schouenborg introduced the notion of a "modular" organization of the "withdrawal reflex". His studies allow one to specify the organization of nociceptive reflexes: most muscles in the lower limb—be they flexor, extensor, or otherwise (supinator, pronator)—can contract during nociceptive stimulation

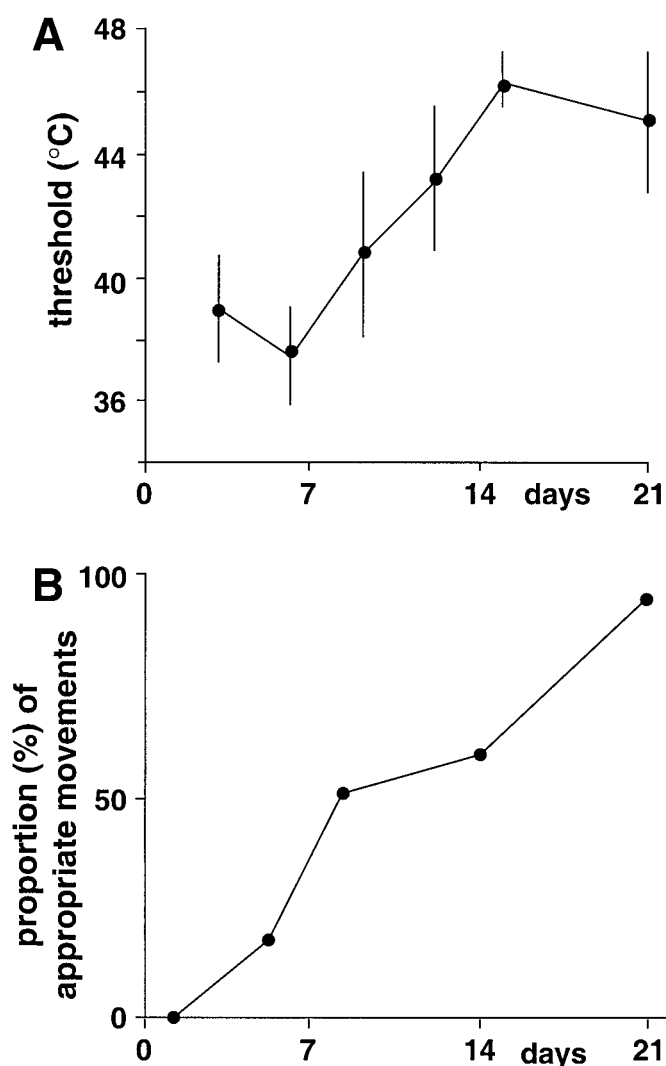


FIG. 16. Temporal evolution of the nociceptive reflex in the tail of the newborn rat. In developmental terms, the seventh postnatal day corresponds to birth in a human being. A, thresholds for obtaining the tail-flick by immersion of the tail in water (postnatal age, abscissa). In newborn rats, movement of the tail was produced by non-nociceptive temperatures. This threshold increased in the course of the second week after birth (after Falcon et al., 1996 with permission). B, Holmberg and Schouenborg (1996) observed movements of the tail produced by CO<sub>2</sub> laser thermal stimulation in young rats. At birth, the movements produced by stimulation of the distal part of the tail were systematically incorrect in the sense that they took the tail nearer to the source of the thermal stimulus. The proportion of movements that distanced the tail from the thermal source increased gradually and approached 100% at 3 weeks. Modified from Holmberg and Schouenborg, 1996 with permission.

of a well defined region of skin, and thus, each muscle has its “nociceptive cutaneous receptor field” (Schouenborg and Kalliomäki, 1990; Schouenborg et al., 1992). Indeed, there is a nearly perfect match between the fields of cutaneous receptors and the cutaneous territories that are removed from the nociceptive stimuli (Schouenborg and Weng, 1994). Bearing this in mind, it is possible to understand that it is not essential for a withdrawal reflex to be a flexion reflex. Schouenborg and Kalliomäki (1990) drew a map of excitatory receptive fields for most of the muscles of the rat hindlimb. As a result of the overlapping of these receptive fields,

stimulation of a given cutaneous zone can result in the contraction of several muscles. On the basis of these data, one can envisage a modular organization with several parallel chains of interneurons, each module leading to the activation of a single muscle (Schouenborg and Kalliomäki, 1990; Schouenborg et al., 1992; Schouenborg and Weng, 1994).

### C. Spinal Shock

In a number of experiments, the animals used have had their spinal cords sectioned at a cervical or thoracic level. This is done to remove supraspinal controls and thus permit the study of pure spinal mechanisms. However, the recording of a motor response as an indication of spinal nociceptive activity makes the use of these models precarious.

In fact, the excitability of spinal reflexes varies with time following sectioning of the cord. The spinal lesion is accompanied initially by a state of areflexia which, since the time of Sherrington, has been termed *spinal shock*. The duration of this state varies considerably between species, from a few seconds in the frog to a few months in humans. The areflexia is followed by a period of hyporeflexia, and then by a state of hyperexcitability. For example, in the rat, the areflexia is complete for 10 to 20 min after which the response reappears and increases over a 5- to 8-h period until it shows a significant level of hyperexcitability (Schouenborg et al., 1992). This level will stabilize only after about 2 weeks (Borszcz et al., 1992). Spinal shock does not directly affect ventral horn motoneurons but results from the lifting of descending facilitatory controls onto premotor interneurons in the ventral horn (Chambers et al., 1966; Spencer et al., 1966c; Zapata, 1966). Since spinal shock does not involve dorsal horn neurons, one should not read too much into any variations of the reflex in “spinal animals”.

### D. Excitatory Effects of Opioids on Motor Activity

The stimulatory effect of morphine or other opioids on motricity is well known to anesthetists who refer to it as “opioid-related rigidity” (Bowdle and Rooke, 1994). This adverse effect is exerted mainly by the activation of  $\mu$ -opioid receptors (Negus et al., 1993). In animals, morphine in low doses can cause signs of behavioral stimulation—hyperactivity, stereotyped movements—whereas larger doses (>10 mg/kg) cause catatonia together with akinesia and muscular rigidity (Fog, 1970; Babbini and Davis, 1972; Groppe and Kuschinsky, 1975; Turski et al., 1982). A spectacular form of catatonia in the mouse and the rat, Straub’s reaction, consists of lordosis of the whole body including the tail (Bilbey et al., 1960); it also is mediated by central  $\mu$  receptors (Nath et al., 1994).

These modifications may be caused by inhibition of GABAergic activity in striatonigral pathways (Turski et al., 1984). Furthermore, wide lesions of the periaqueductal gray matter (PAG) or low, precollicular decerebration

completely abolish this phenomenon (Lee et al., 1978). A more recent study (Weinger et al., 1991) provided evidence that the reticular formation, in particular nucleus raphe pontis, and the periaqueductal gray matter have leading roles in morphine-induced rigidity. Finally, Weinger and colleagues (1987, 1989, 1995) reported that  $\alpha_2$  adrenergic and serotonergic systems are implicated in the muscular rigidity induced by alfentanil.

In the rat and the monkey, biphasic dose-dependent effects of systemic morphine on nociceptive flexor reflexes have been described. At low doses, morphine facilitates these reflexes, whereas at higher doses it inhibits them (Cooper and Vierck, 1986a; Guirimand et al., 1995; Yeomans et al., 1995). Using a protocol incorporating variable stimulus intensities to construct recruitment curves, Guirimand and colleagues (1995) showed that morphine at low doses exerted an intensity-dependent facilitatory effect on the reflex; there was no effect on the threshold, but the effects became more and more marked as the stimulus intensity was increased. These effects have only a distant relationship to nociception, but they can perturb the tests that are used to study it.

## IX. The Sensitivity of the Tests

### A. Statement of the Problem

There is a recurrent problem in the basic pharmacology of analgesics (Table 1). This problem can be illustrated by considering morphine as an example. It has been known for a long time that in animals, when one uses most classic tests such as the tail-flick test, the hot plate test, or paw withdrawal from mechanical stimuli, the effective doses of morphine are much higher than those used clinically (Hammond, 1989). This could be explained by interspecies differences in susceptibility or in pharmacokinetics. However, although these differences are real, they are not sufficient to explain the magnitude of the discrepancy. Indeed, when other tests are used, such as the formalin, writhing, or vocalization tests, this disparity is significantly reduced. As for minor analgesics, their actions are generally not revealed by these tests except when very high (quasitoxic) doses are used.

One explanation for this paradox could be that it is related to the phasic or tonic character of the stimulus

used—analgesics being more effective on pains generated by the latter. However, such an explanation is unlikely given that morphine is efficient in humans on pains produced by very short-duration phasic stimuli but has a much lesser effect when the stimulus is more intense (Cooper et al., 1986). Such an inverse relationship between the intensity of the stimulus and the measured efficiency of analgesics has also been demonstrated with models of clinical pain (Laska et al., 1982). In animals, the sensitivity of behavioral tests of nociception has seemed to depend on the intensity of the nociceptive stimuli being applied, be they thermal (Gray et al., 1970; Luttinger, 1985; Carstens and Campbell, 1988; Carstens and Ansley, 1993; Carstens and Wilson, 1993; Dirig and Yaksh, 1995), chemical (Shaw et al., 1988), or electrical (Guirimand et al., 1995). For example, the apparent antinociceptive power of opioids increases when the temperature is decreased in the hot plate test (Ankier, 1974; O'Callaghan and Holzman, 1975; Hunskaar et al., 1986; Zimet et al., 1986) or when the slope of heating is less in the tail-flick test or paw withdrawal test (Bonnycastle, 1962; Granat and Saelens, 1973; Suh et al., 1992; Dirig and Yaksh, 1995; Abram et al., 1997). In certain cases, these observations can result in a calculation artifact (Fig. 24).

One can easily imagine that an analgesic will be that much more efficient when the stimulus produces weaker and less synchronous neuronal activity. This is quite likely to be what happens during the subcutaneous or intraperitoneal administration of relatively weak algogenic agents (having been chosen for ethical reasons and to minimize stress). For a given stimulus, the neuronal activities recorded in the spinal cord or brain will be more desynchronized, the slower the conduction velocities of the peripheral fibers responsible for the response. As a very direct result of this consideration, one must think about interactions between the type of fiber responsible for a response and the pharmacological effects that are exerted on it.

### B. What Types of Fiber Underlie the Responses?

It has long been known that experimental pain in humans is little affected by doses of morphine, which are effective analgesics for patients (Beecher, 1956a, 1957).

TABLE 1  
*ED<sub>50</sub> (mg/kg) obtained by one group using several tests on two species of animal and several analgesic substances*

	Mouse			Rat	
	Tail-Flick	Hot-Plate	Writhing	Writhing	Formalin
Morphine (s.c.)	3.3	3.7	<b>0.2</b>	<b>0.3</b>	<b>0.2</b>
Meperidine (s.c.)	10.3	10.6	<b>2.3</b>		
Codeine (s.c.)	28.9	33.6	<b>2.4</b>		
Pentazocine (s.c.)	>50	16.9	<b>0.8</b>	<b>0.7</b>	<b>2.4</b>
Aspirin (p.o.)	>200	>200	47.8	67.5	>150
Paracetamol (p.o.)			52	118	>300
Zomepirac (p.o.)	>100	>50	<b>2</b>		

The ED<sub>50</sub> values that approach the doses used in man are in boldface type. All the others are much higher (adapted from Hammond, 1989).

This is undoubtedly because experimental pain is usually produced by A $\delta$  fibers. We have already discussed the double pain produced in humans when a brief and sufficiently intense single stimulus activates A $\delta$  and C fibers, which have different conduction velocities; in these cases, the emotional component of the second pain is much stronger than that of the first (Handwerker and Kopal, 1993). When electrical stimuli are applied to distal parts of the body such as the lower leg, pain produced by activation of A $\delta$  fibers can disappear completely before the onset of pain because of the more slowly conducting C fibers (Cooper et al., 1986). Thus, in some experimental protocols, it is possible to identify and unambiguously measure each component of double pain and test the effects on these of morphine. It is found that the first pain is little affected by morphine, whereas the second shows clear sensitivity (Fig. 17; Price et al., 1985; Cooper et al., 1986; Price and Barber, 1987; Yeomans et al., 1996a).

Under laboratory conditions, the usual phasic stimulation methods predispose human subjects and animals to respond to the pain as soon as it occurs, i.e., at the moment the first pain produced by A $\delta$  fibers occurs (Dubner et al., 1977). The presence or absence of second pain will generally have no impact on the measurement (Lineberry, 1981). In any event, stimulation is stopped as soon as a response is obtained. One is therefore tempted to agree with Yeomans and Proudfit (1994, 1996) that most nociceptive tests—at least most of the usual ones involving mechanical and thermal stimuli—actually investigate only responses triggered by A $\delta$  fibers. This would be the reason why these tests would not be sensitive to morphine except at very high doses. It is known that morphine depresses responses of dorsal horn neurons, produced by C fibers, more easily than it depresses those produced by A $\delta$  fibers (Le Bars et al., 1976; Jurna and Heinz, 1979). Furthermore, Wikler (1950) noted long ago that in the chronic spinal dog, morphine depressed spinal reflexes only when they were characterized by long-lasting after-discharges; otherwise, the reflexes were not affected or were even facilitated. On the other hand, when a vocal response is clearly triggered by C fibers, it is very sensitive to morphine, with the ED<sub>50</sub> value being 5 times less than when it is triggered by A $\delta$  fibers (Fig. 10C; Jourdan et al., 1995, 1998). It is therefore tempting to state that in the small number of tests that show little sensitivity to morphine, it is a response (or threshold for a response) to the activation of A $\delta$  fibers that is being studied. Conversely, in the tests that are more sensitive to morphine, the response being studied is a supraliminal and durable one to the activation of C fibers.

The increase in pharmacological sensibility induced by an inflammatory agent could simply be caused by the fact that the C-fiber polymodal nociceptors are particularly susceptible to sensitization phenomena. Consistent with this, it was shown that C-polymodal nociceptors are

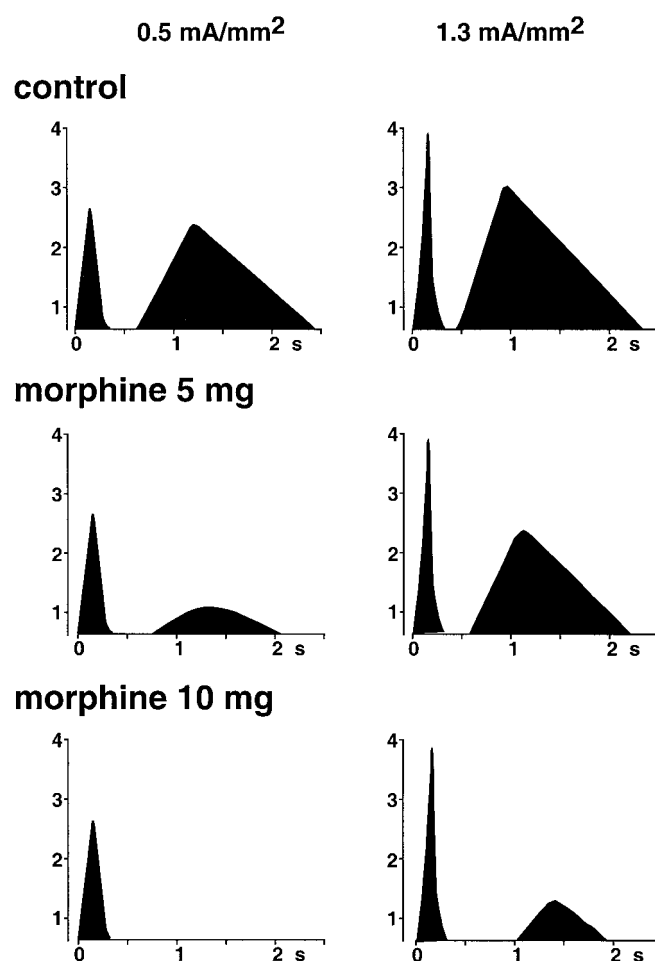


FIG. 17. Differential effects of morphine on first and second pain, evoked successively by A $\delta$  and C fibers. Cooper et al. (1986) applied brief electrical stimuli (AC currents, 60 Hz, 50 ms) to the calf of five trained healthy volunteers. Several stimulus intensities, including those shown here, were applied in random order. The volunteers estimated the resulting levels of pain by drawing lines on paper or giving verbal descriptors after each trial (ordinates, arbitrary units). In addition, they traced the time course of the second pain using a potentiometer (abscissae, time in ms). They all believed the double pain (top graphs; note that the time course shown for first pain was simply an estimate—it could not be followed with the potentiometer). One hour after the intramuscular administration of morphine, the first pain was hardly affected, whereas the second pain was significantly reduced, particularly if the dose was high and the stimulus was low. Indeed after 10 mg, the second pain evoked by a current of 0.5 mA/mm<sup>2</sup> was completely abolished. Adapted from Cooper et al., 1986 with permission.

much more sensitive to pH and bradykinin than are A $\delta$ -polymodal nociceptors (Khan et al., 1992; Steen et al., 1992). In addition, many unmyelinated afferent fibers are silent under normal conditions but respond to thermal and mechanical stimuli when the tissues are inflamed; this new class of C nociceptor has been labeled “silent” or “dormant” by various authors (Schmidt et al., 1994; Schmidt, 1996). These considerations prompt the conclusion that as a whole, C nociceptors are more sensitive to inflammatory phenomena than are A $\delta$  nociceptors. Neither should we forget that they are much more numerous (Ochoa and Mair, 1969; Scadding, 1980; Lynn and Baranowski, 1987; Povlsen et al., 1994; Fig. 18). Thus, sensitizing unmyelinated nociceptors, which is

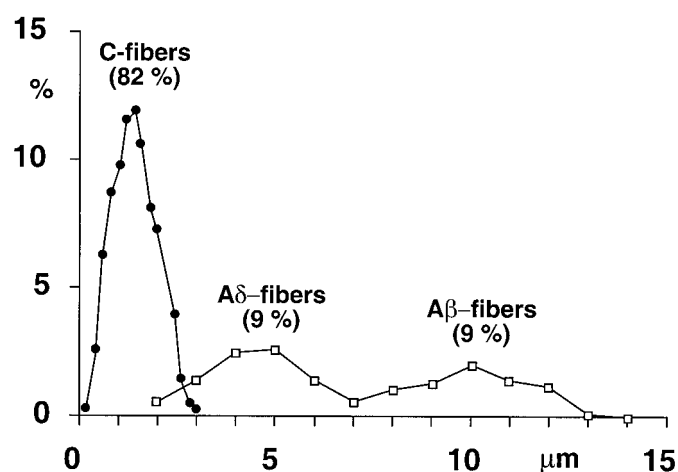


FIG. 18. Frequency distribution of the diameters of afferent fibers measured in histological sections from a biopsy of the sural nerve of a 15-year-old boy. Abscissa: diameters of fibers. Ordinate: percentage of the total fiber population within the section. Note the overwhelming preponderance of C fibers within this pure sensory nerve; some of these would be heat-sensitive, whereas the rest would be nociceptive (Fig. 7D). Modified from Ochoa and Mair, 1969, copyright 1969 Springer-Verlag.

undoubtedly responsible for lowering nociceptive thresholds, may transform a threshold for a response to Aδ fiber activation into a threshold for a response to C-fiber activation without the nature of the stimulus being changed. For example, in the case of paw withdrawal, one can see that the test may fall into the group that is sensitive or the group that is insensitive to morphine, depending on whether the skin has been sensitized or not by the preliminary administration of an inflammatory agent (Randall and Selitto, 1957; Winter and Flataker, 1965b; Hargreaves et al., 1988). One can check this observation on a single animal that has just one inflamed paw. It is possible that when sensitization of unmyelinated polymodal nociceptors is clearly responsible for lowering the nociceptive threshold, it may do so by transforming the measurements from ones of the threshold for a response to activation of Aδ fibers (insensitive to morphine) to ones for the threshold of a response to activation of C fibers (sensitive to morphine). Thus, under these conditions, one would record a response produced by Aδ fibers when the stimulus is applied to a healthy paw and a response produced by C fibers when the stimulus is applied to an inflamed paw. In this context, the applied physical stimulus was not changed by the experimenter who, in good faith, believed that he or she carried out the same test on both of the animal's paws. The experimenter did this even though the physiological "effective stimulus"—which can be defined in terms of the actual physical stimulus and the physicochemical and physiological (or pathophysiological) properties of the target tissue—would have appeared completely different. However, from a pharmacological point of view, one is faced with two different systems. A similar phenomenon may also occur during chronic inflammatory processes (Pircio et al., 1975; Kayser and Guilbaud, 1983).

Some paradoxical results obtained following neonatal administration of capsaicin in the rat could be explained by similar mechanisms. Nowadays it seems clear that this processing primarily destroys C fibers and sometimes, albeit to a lesser extent, destroys certain Aδ fibers (Lynn, 1990; Holzer, 1991; Szolcsányi, 1993; Szallasi and Blumberg, 1999). Such treatment must render the animals quasianalgesic. As far as tests of nociception in the rat and the mouse are concerned, one can summarize the effects of neonatal treatment with capsaicin thus: there is a consensus that responses produced by algogenic chemical stimuli are blocked, but mechanical and thermal stimuli have produced conflicting findings, with completely negative results being obtained from experimental protocols that lead to a large decrease in the number of unmyelinated afferent fibers (Holzer, 1991; Campbell et al., 1993; Winter et al., 1995). One can propose several explanations for these apparent discrepancies, notably differences in the stocks of animal and in the experimental protocols (Holzer, 1991). However, if one accepts Yeomans' and Proudfit's proposal (1994, 1996) that most of the tests using mechanical or thermal stimuli involve studying responses triggered by Aδ fibers, then there is a simpler explanation for this paradox: the negative results are what one would expect, whereas the positive results can be correlated with the percentage of Aδ fibers destroyed by the capsaicin treatment—a proportion that is not generally known. Although speculative and retrospective, this proposal is plausible and illustrates the urgent need for a better understanding of the tests of nociception that we use. When this is the case, interpretation will become easier. This would apply to the acute effects of subcutaneous injections of capsaicin that facilitate limb withdrawal when the heating slope is slow and activates C fibers but do not have any effect when the heating slope is fast and activates Aδ fibers (Yeomans et al., 1996b; Zachariou et al., 1997).

In practice, it is not easy to evaluate the respective contributions of these two groups of fibers for a given test of nociception. As already mentioned, Aδ fibers are less numerous than C fibers (Fig. 18) to such an extent that it is undoubtedly necessary to have more sustained activity in them if they are to provide sufficient information to evoke pain. On the other hand, their conduction velocities enable them to trigger a response well before the C fibers. In addition, when a stimulus is sudden, the resulting activity in Aδ nociceptors arrives at the spinal cord in a highly synchronized fashion, which counterbalances the numerical weakness of these fibers. Furthermore, the strong intensity and speed of application of the stimulus generally make it possible not to have to consider the fact that the thresholds of Aδ-polymodal nociceptors are higher than those of C-polymodal nociceptors (Treede et al., 1995). Finally there are various arguments which make it possible to think that by comparison with C-polymodal nociceptors,

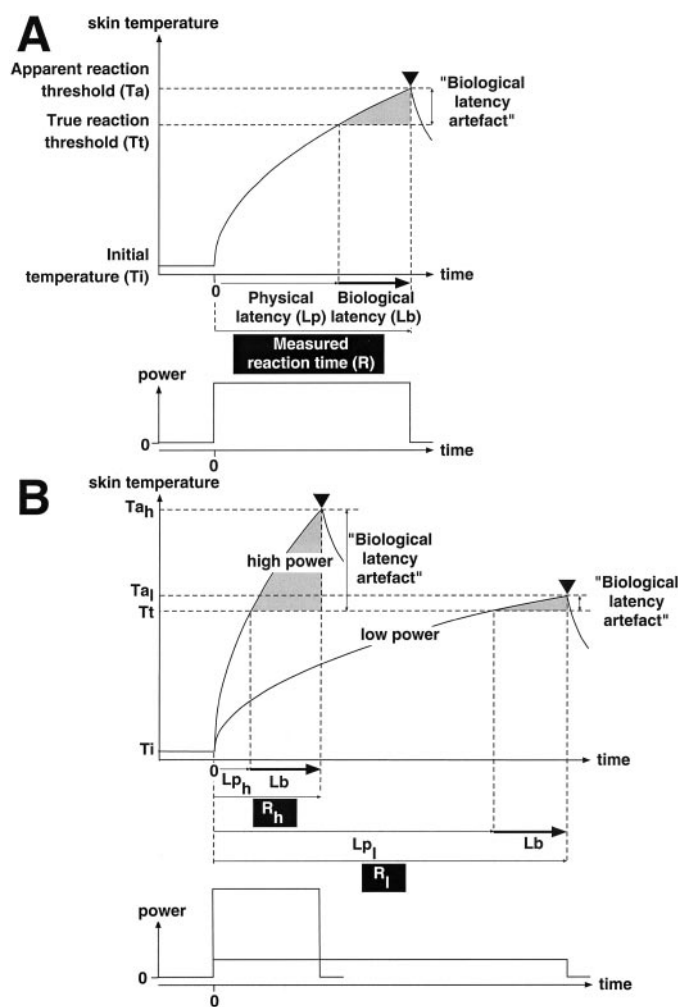


FIG. 19. Production of a nociceptive response by a constant source of radiation. A, when a stimulus with constant caloric energy is applied (lower graph), the initial baseline cutaneous temperature ( $T_i$ ) increases proportionally with the square root of time (upper graph). After a certain time ( $L_p$ ), the temperature reaches such a value that it generates sufficient activity in nociceptors to evoke a reaction and/or a sensation. It does this by transduction mechanisms. To take the example of a nociceptive reflex: the temperature that is the true threshold for the reflex ( $T_t$ ), once reached, generates information in the peripheral afferent pathways to trigger the reflex movement (the occurrence of which is represented by a black triangle). The time necessary to achieve the afferent, central, and efferent processes underlying this response constitutes the  $L_b$ . A priori, there is no reason why this reaction time should be influenced by the fashion (slow or fast) in which the temperature threshold  $T_t$  was reached. It may be noted that the measured reaction time ( $R$ ) is in fact the sum of  $L_p$  and  $L_b$ , although only the latter has any meaning in the context of nociceptive mechanisms. Since the ratio  $L_p/L_b$  becomes higher as  $L_p$  becomes longer, it can be deduced that measured reaction time is further divergent from biological latency the gentler the slope of the stimulus. B, the intensity of the applied caloric energy determines the apparent reaction threshold ( $T_a$ ) and, thus, the biological latency artifact. When the intensity is high ( $h$ ), the cutaneous temperature increases rapidly to reach the true threshold of the reflex ( $T_t$ ) in a time  $L_{p_h}$ . This results in the reaction (black triangle) being evoked at a latency  $L_b$ ; during this time  $L_b$ , the temperature continues to increase to the level  $T_{a_h}$ , thus generating a significant biological latency artifact ( $T_{a_h} - T_t$ ). When the intensity is low ( $l$ ), the temperature increases more slowly and takes longer to reach  $T_t$  ( $L_{p_l}$ ). The reaction is obviously triggered with the same latency  $L_b$ , which generates a smaller biological latency artifact ( $T_{a_l} - T_t$ ).

which can be rather static,  $A\delta$ -polymodal nociceptors can be dynamic receptors, e.g., more sensitive to fast variations in temperature than to absolute values (Yeomans and Proudfit, 1996). Taken together with their relatively

rapid conduction velocities, this property confers on them the role of being the outpost of the nociceptive system, giving early warning of highly phasic stimuli.

### C. What Is the Significance of Measurements of Reaction Time When the Stimulus Intensity Is Increasing?

In reality, stimuli are often applied in a gradual fashion. Under these conditions, the question arises as to what is the significance of the measurements that are taken.

The measurement of a reaction time is conceptually very simple: the time between the application of the stimulus and the start of the evoked response is measured; undoubtedly, this constitutes a biological parameter. This concept does not produce any problem when short-duration stimuli are used (e.g., electrical stimulation, laser thermal stimulation). However, the situation is more complex when the intensity of a stimulus is gradually increased while the stimulus is being delivered. Here there is a potential confusion between the concepts of reaction time (often referred to as "latency") and "threshold", with the former being regarded as a covariant of the latter. We examine this problem below.

Let us consider the measurement of the reaction time of a response initiated by radiant heat. Energy can be delivered continuously from a constant caloric source coupled to an obturator. In physics, one could regard such a stimulus as producing an increase in the temperature of the target tissue, whatever that may be, which would be proportional to the square root of time (Fig. 19A). That is effectively what experiments confirm when one measures cutaneous temperature in humans (Buettner, 1951; Hendler et al., 1965; Stolwijk and Hardy, 1965) or the anesthetized rat (Yeomans and Proudfit, 1994). Achieving a given temperature triggers the response. We then note that the measured reaction time ( $R$ ) is the sum of the physical ( $L_p$ ) and biological ( $L_b$ ) latencies. Physical latency corresponds to the time taken to increase the temperature of the skin. This increase will depend on the properties of reflectance, transmission, and absorption of the epidermis and dermis, all of which will depend on the wavelength of the radiant source (Hardy et al., 1956; Hardy, 1980). Reflectance is very significant in the visible and adjacent infrared fields (Fig. 3A). However, the electromagnetic emission spectrum of a lamp varies with the intensity of the electrical current that is applied to it (Fig. 3C). Thus, a variation in intensity will result in a concomitant variation in all these parameters, which relate to the physical properties of the skin. As a result, there is a tricky problem of how to interpret these phenomena when considering the physiological activation of receptors.

The  $L_b$ , which is a parameter that really interests us, results from phenomena all of which have a finite duration: transduction, conduction in peripheral afferent fibers, conduction and integration in the central nervous

system, conduction in efferent fibers, and the response itself. It is somewhat surprising that authors usually implicitly regard the measured reaction time ( $R = L_p + L_b$ ) as being the same as the  $L_b$  of the movement. In addition, the use of a reaction time as an index of nociception and, consequently, of its increase as an index of hypalgesia implicitly presupposes that the increase in cutaneous temperature is proportional to time, but that is something which is never verified.

Figure 19B shows the theoretical evolution of cutaneous temperature during the application of two different intensities of radiant heat to the skin (with the thermal radiation being stopped on the appearance of a movement). It can be seen that the faster the heating, the earlier the response—the temperatures reached being that much higher. This observation is explained by the fact that the peak temperature corresponds to the movement that occurs after a given latency ( $L_b$ , by definition). During this time  $L_b$ , the temperature continues to increase, which means that the movement was triggered by a temperature lower than that which was noted at the time when it occurred. This “biological latency artifact”, which is also referred to as the “reaction time artifact” (Yarnitsky and Ochoa, 1990; see also Dirig et al., 1995), is greater the steeper the heating slope.

There is no reason to believe that the temperature threshold which must be reached on the surface of the skin to trigger a movement corresponds to the threshold for activating nociceptors (Tillman et al., 1995a). Indeed, it is actually the temperature that activates a minimum number of nociceptors to a level sufficient to transmit the barely adequate volume of information to produce the movement (Fig. 20). This in turn depends on complex central excitatory and inhibitory processes. This temperature threshold has to be higher than the thresholds of individual nociceptors and concerns the true threshold for the reaction ( $T_t$ ). All these considerations have functional consequences because once the  $T_t$  is reached, one must wait some time ( $L_b$ ) before seeing the reaction. During this period, the stimulus continues to grow and activate nociceptors. The integration of the response time artifact and the duration  $L_b$  (gray zones in Fig. 19) will determine the “total volume of nociceptive information” that elicits the strength of the response. Thus, independent of its threshold, this response will be more vigorous with a shorter reaction time the steeper the heating slope. In this respect, it is interesting to recall that the classic tail-flick is described as a brief movement of the tail observed within few seconds, with the reaction time being shorter and the movement more vigorous when the intensity of the source of radiant heat is more intense (see *Section V.A.1.a.*).

The temperature measured on the surface of the skin gives only an approximation of the temperature reached at the level of the nociceptors, which are hidden in the surface layers of the skin at the dermoepidermal junction. In humans and monkeys, the heat-sensitive noci-

ceptors would, on average, be located at a depth of 200  $\mu\text{m}$  (Stoll and Greene, 1959; Stolwijk and Hardy, 1965; Tillman et al., 1995b). The systematic character of the resulting error renders it relatively unimportant when considering experiments using constant thermal stimuli. On the other hand, as soon as the stimulus varies during the experimental protocol, this approximation can be the source of erroneous interpretations. Thus, because of thermal inertia, the heat achieved at the nociceptors is close to the surface temperature when the heating slope of a thermode is gentle, but it is less and is shifted in time when the heating slope is steep (Tillman et al., 1995b).

The intensity of stimulation can determine which type of fiber starts the reaction. We have seen that strong stimulation predisposes a reaction triggered by  $A\delta$  fibers. That is true with regard to radiant heat when it is applied abruptly (Fig. 21A). On the other hand, when the stimulus is applied very gradually, the response may be triggered by C fibers since the threshold for activation of C-polymodal nociceptors is lower than that of the  $A\delta$ -polymodal nociceptors, particularly with thermal stimuli in which the difference in threshold is about  $5^\circ\text{C}$  (Treede et al., 1995). For example, with a heating slope of  $1^\circ\text{C/s}$ , it takes approximately 5 s to pass from the threshold of activation of C-polymodal nociceptors to that of the  $A\delta$ -polymodal nociceptors; this 5-s period is ample to permit activation of the C fibers to trigger a reaction even before  $A\delta$  fibers have been activated (Fig. 21B). The experiments of Yeomans and Proudfit (1994) illustrate this concept perfectly since the mean paw withdrawal threshold of  $47.2^\circ\text{C}$  was achieved in 13.4 s with a low intensity lamp (presumably by activating C nociceptors), whereas the mean paw withdrawal threshold of  $51.7^\circ\text{C}$  was achieved in 2.6 s with a high-intensity lamp (presumably by activating  $A\delta$  nociceptors). Note that the  $4.5^\circ\text{C}$  difference between thresholds fits perfectly with the  $5^\circ\text{C}$  difference between the mean thresholds of individual  $A\delta$ - and C-polymodal nociceptors (Treede et al., 1995).

It should be noted that some of these experiments are not easy to interpret when a conventional source of energy is used to apply radiant heat. We have already mentioned that the electromagnetic emission spectrum of a lamp varies with the intensity of the electrical current (see *Section III.A.1.a.*; Fig. 3C). Since the radiation properties of the skin depend on the wavelength emitted by the source of radiation, it follows that low and high intensities of a given lamp will affect different volumes of skin. In other words, in such experiments both the intensity and the stimulated volume vary when one changes the current applied to the incandescent bulb.

Thus, total ignorance of physical factors contributing to a reaction time renders the measurements of the latency somewhat illusory from a biological point of view. Because the aim of these experiments is not to



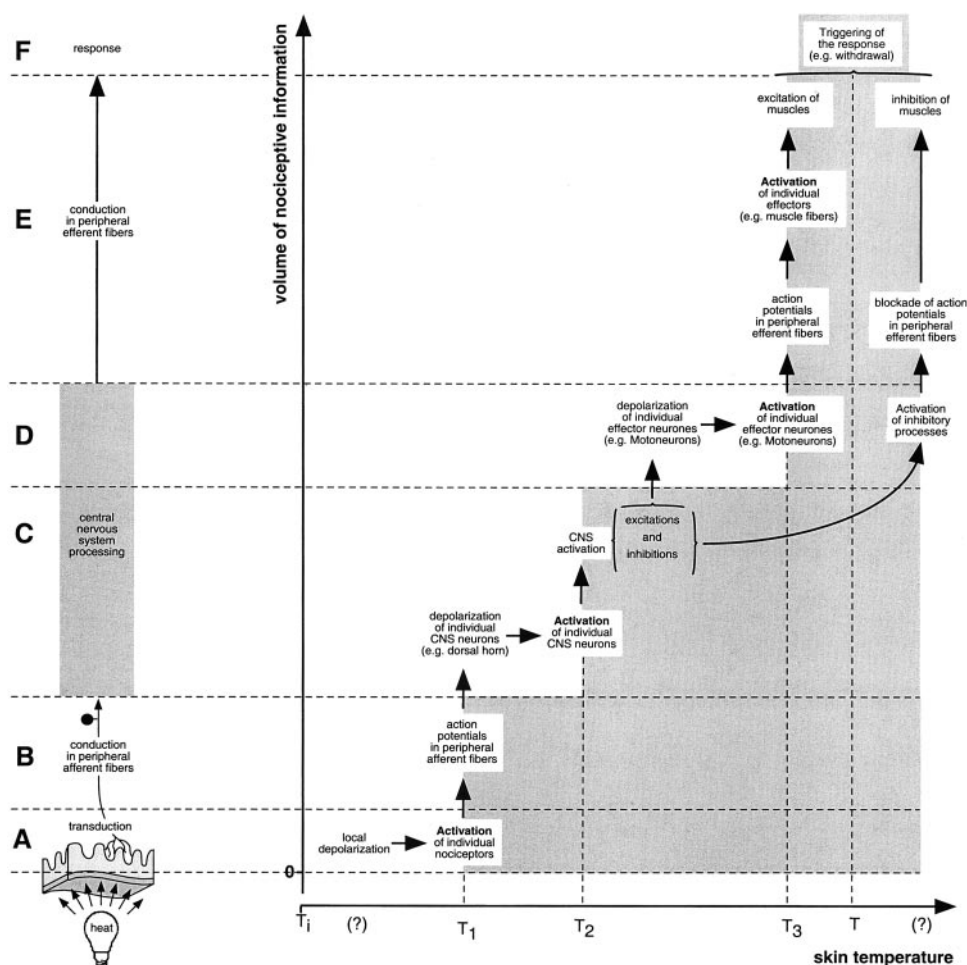


FIG. 20. Schematic representation of the sequences of events that produce withdrawal-like responses to a nociceptive thermal stimulus. This is a simplified diagram that does not show several phenomena such as convergence between adjacent pathways. Starting from an initial skin temperature of  $T_1$ , several events occur. A, transduction mechanisms produce a local depolarization of nociceptors that triggers the firing of action potentials in peripheral afferent fibers as soon as a threshold temperature  $T_1$  is reached. B, these spikes are conducted through the nerves toward the dorsal horn of the spinal cord or the trigeminal system. C, to activate second-order neurons, the central summation that results from increased peripheral afferent activity is required. This is achieved by higher temperatures and thus has a notional threshold of  $T_2$ . Activity in these second-order neurons then triggers a complex series of excitatory and inhibitory processes within the central nervous system. D, more summation (and thus afferent activity) is required before the motoneurons are activated (at  $T_3$ ). E, because of the one-to-one coupling of action potentials between motoneurons and muscle fibers, the muscles are also activated at this threshold ( $T_3$ ). F, it is likely that several motor units will have to be excited (or inhibited in antagonist muscles) before a detectable movement will occur—hence this will occur at a higher threshold still—denoted here as T. This schematic representation would be easy to interpret and test experimentally if short-duration stimuli of graded intensities (e.g., electrical shocks) were applied sequentially. However, if the withdrawal-like response is elicited by an increasing-intensity, nociceptive thermal stimulus, then the situation becomes far more complex at an experimental level as temperature is linked to time (although time is not represented on this graph). In fact, the triggering of the reflex means that a sufficient volume of nociceptive information was generated some time ago at the periphery—in other words that the temperature of the skin had reached T. This time is the physical latency required for increasing the temperature from  $T_1$  to T. When T is achieved at the periphery (i.e.,  $T = T_t$  in Fig. 19), then a biological latency is required to send the information to the effectors. The withdrawal occurs later, with an apparent threshold ( $T_a$  in Fig. 19), even higher than T. Note that such reasoning could be applied to all the events illustrated in the diagram, each exhibiting a true and an apparent threshold with  $T_{t1} < T_{t2} < T_{t3} < T_t$  and  $T_{a1} < T_{a2} < T_{a3} < T_a$ . However, at the experimental level, the former are difficult to determine. Note also that, a priori, an agent with unknown pharmacological properties might have an effect at any level in such a sequence.

make absolute measurements but to identify and measure variations in the parameter being considered, one has to acknowledge that there is a systematic (but unknown) error in the measurement by a magnitude of  $L_p$ . If we accept this problem in this way, then to get meaningful results we must be certain that the magnitude  $L_p$  is invariable and consider only differences in reaction times and never express these differences in the form of percentage variations. The first of these requirements is never verified because the temperature of the skin is not recorded; indeed, there are a number of reasons to be-

lieve that  $L_p$  is not always constant, particularly because the basic cutaneous temperature can vary (see *Section XII.E.*). The second requirement is rarely even considered. Authors often calculate a “percentage of the maximum possible effect”, and this is discussed in the next section.

We mention without comment two lesser sources of uncertainty. The first results from the fact that many thermal stimulators do not have an obturator—the timer being started at the moment of the powering of the lamp. A new physical factor is thus introduced: the time

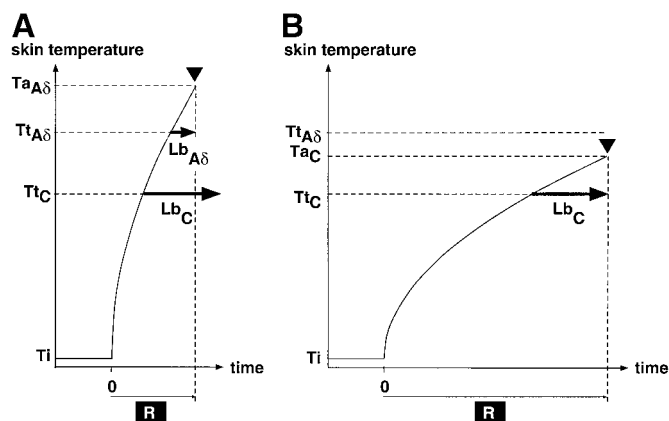


FIG. 21. The intensity of applied caloric energy can determine the type of fiber that evokes the reaction. This observation is based on the different properties of  $A\delta$  and C fibers.  $A\delta$  fibers conduct impulses more rapidly (4–30 m/s) than do C fibers (0.4–2 m/s), but their thresholds for activation are higher (45 versus 40°C; Treede et al., 1995). Thus, when the intensity of applied energy is greater (A), the true threshold  $T_{t_{A\delta}}$  for the reflex evoked by  $A\delta$  fibers is reached rapidly and the movement (black triangle) can be triggered before the arrival of impulses in the C fibers because the latency  $L_{b_{A\delta}}$  is small. From a sensory point of view, such a scenario would produce the classic double pain (see Section III.A.1.). On the other hand, if the stimulus intensity is less (B), the true threshold  $T_{t_C}$  for the reflex evoked by C fibers is reached slowly and the reaction time artifact is too small to achieve an adequate temperature to produce an  $A\delta$  reflex. The movement (black triangle) is triggered by the arrival of impulses carried by C fibers following the  $L_{b_C}$  latency. This observation is only apparently paradoxical. In Figs. 22 and 23 and the corresponding text, for clarity of presentation, the reasoning will not take into account such problems. However, it is clear that they could be a further source of difficulties in the interpretation of some data.

of heating of the lamp, which will be related to its thermal inertia and the fact that the electrical resistance of the filament depends on the temperature. The second problem relates to old equipment that does not have an automatic device for stopping the timer when the movement occurs, e.g., an photoelectric cell. In this case, it is the experimenter who handles the timer, and the measured reaction time integrates his or her response time, which may be more or less constant.

Measured reaction time can vary for many reasons (see Section VII.). In the situation in which the antinociceptive activity of a drug does not affect the baseline cutaneous temperature, an increase in reaction time can result from two mechanisms (Fig. 22). The threshold for producing the reaction can increase ( $\Delta T$ ), which will lengthen the physical latency and consequently the measured reaction time ( $\Delta R$  in Fig. 22A). The biological latency of the reaction itself can also increase ( $\Delta L$ ) to lengthen the measured reaction time ( $\Delta R$  in Fig. 22B). In general, authors generally regard an increase in reaction time as revealing an increase in threshold even though it is probable that these two complementary mechanisms often coexist. However, the relative role of the first mechanism will be always overestimated compared with that of the second as time variations vary with the square of variations in temperature. Furthermore, one can understand that it is the intensity of stimulation that will determine the relative influences

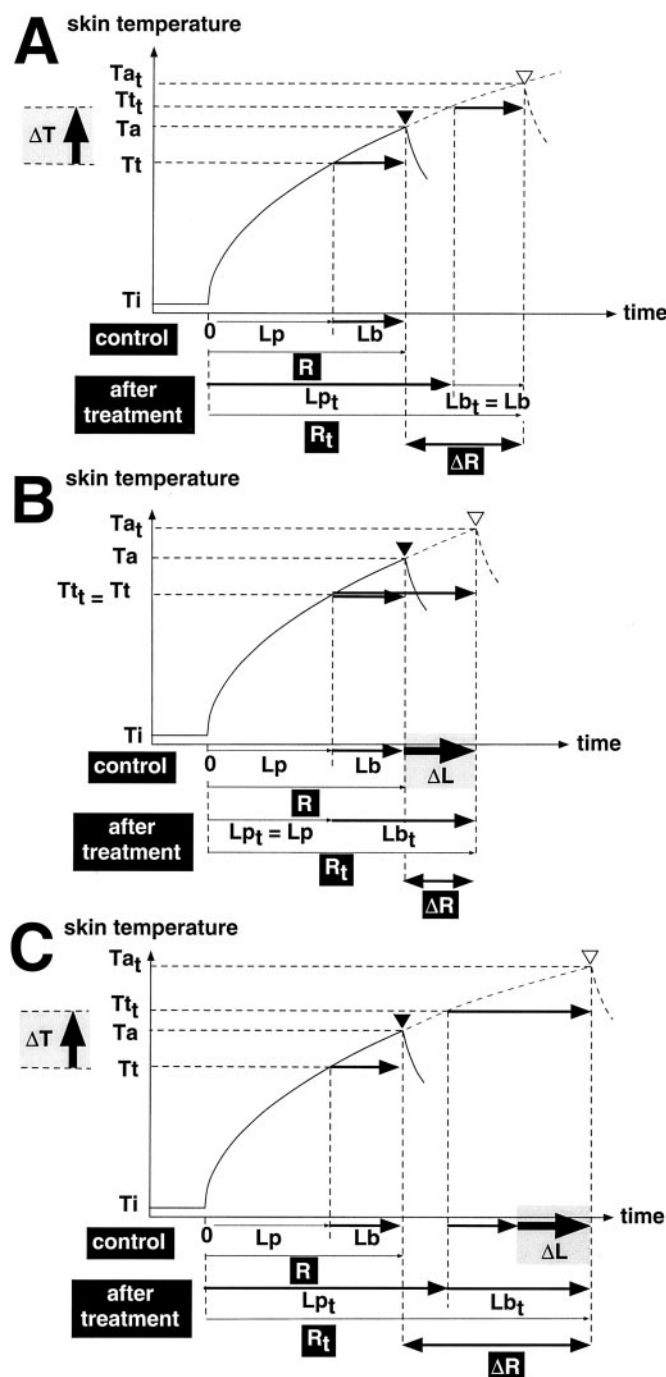


FIG. 22. The administration of an analgesic drug can increase the measured reaction time to radiant heat by increasing the true threshold of the reaction (A:  $\Delta T$ , shaded area) and/or by increasing its biological latency (B:  $\Delta L$ , shaded area). In the control period, when the temperature reaches the true threshold ( $T_t$ ), the reaction (black triangle) is produced with a latency  $L_b$  which, when added to the  $L_p$ , corresponds to the measured  $R$ . We are envisaging cases in which drugs do not affect the initial baseline skin temperature  $T_i$ . After administration of the drug, the occurrence of the reaction is represented by an open triangle. A, if the threshold is increased by  $\Delta T$  to reach the value  $T_{t_t}$  after the pharmacological treatment, the physical latency will lengthen to reach the value  $L_{p_t}$  and the reaction time  $R_t$  will be increased by the same amount ( $\Delta R = R_t - R$ ). B, if the biological latency increases by  $\Delta L$  to a value  $L_{b_t}$  without any variation in the threshold ( $T_{t_t} = T_t$ ), the measured reaction time  $R_t$  will also increase compared with the reaction time  $R$ , without there having been any change in this physical latency ( $L_{p_t} = L_p$ ). C, in practice, the values  $R$  and  $R_t$  are obtained in such a way that one cannot determine which of these mechanisms is prevailing. They probably often coexist, but with the former assuming greater importance since reaction time varies with the square of variations in temperature.

of the two mechanisms on measured reaction time. Indeed, we know that the increase in physical latency is negatively correlated with the energy applied to reach the temperature threshold (Figs. 19B and 24B), whereas the increase in biological latency is independent of this. Consequently it follows that the respective share of the increase in the threshold for producing the reaction, compared with the increase of the reaction time itself, will always be greater when the applied energy is weaker. For a very weak source of energy, a weak variation in threshold will translate mathematically into a large variation in reaction time.

The usual experimental protocols do not make it possible to solve these problems. Furthermore, we have assumed here that the reactions were all started by just one group of fibers, be they A $\delta$  or C. However, as we discuss below, in certain circumstances this may not be the case. Under those circumstances, the interpretation of certain experiments becomes even more complex.

#### D. Influence of Methods of Analysis

In several tests, the investigator defines a limit for how long the animal should be exposed to the stimulus (the cutoff time). This limit is absolutely necessary when the intensity of the stimulus is increasing and could rapidly damage the exposed tissues (Carroll, 1959). However, it can be a source of difficulty. As a result of such imposed limits, some analgesic effects will show up as an increased number of animals reaching the time limit. Many authors normalize results as percentages of the maximum possible effect with this being the time limit that itself is arbitrarily chosen. This percentage of the maximum possible effect (%MPE; Fig. 23A and 24A) is defined (Harris and Pierson, 1964) by the following equation:

$$\begin{aligned} \%MPE &= \frac{\text{reaction time after treatment} - \text{control reaction time}}{\text{cutoff time} - \text{control reaction time}} \times 100 \\ &= \frac{R_t - R}{C_o - R} \times 100 \end{aligned}$$

The calculation of this index introduces a new arbitrary ratio that is dependent on the choice of time limit by the investigator (Carmody, 1995). Moreover, the term *maximum possible effect* is ambiguous because it is not actually a biological maximum but an arbitrary value. Thus, simply reducing the time limit can “improve” the efficiency of a substance. In the tail-flick test, the time limit varies from 6 to 20 s. Thus, an increase of reaction time from 3 to 6 s will give an analgesic index of 18, 43, or 100% depending on whether a time limit of 20, 10, or 6 s has been chosen (Fig. 23B). “Maximum decided effect” would seem to be a much better statement than maximum possible effect, which suggests that there is a real pharmacological maximum effect.

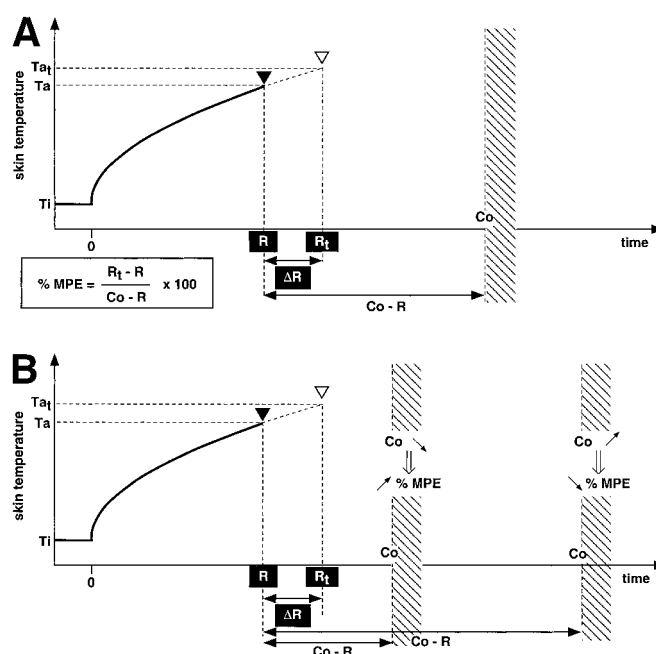


FIG. 23. The calculation of the percentage of the maximum possible effect of a treatment that alters a reaction time is dependent on the predetermined cutoff time. In these diagrams, the occurrence of the control reaction is represented by a black triangle and that of the reaction after treatment, by an open triangle. Note that the treatment increases the measured reaction time by  $\Delta R$ . A, calculation of the index. B, example of how the choice of the cutoff time determines the index. Note that the %MPE is negatively correlated with the cutoff time. %MPE, the percentage of the maximum possible effect; R, measured control reaction time;  $R_t$ , measured reaction time after treatment;  $C_o$ , cutoff time.

In addition, if a given cutoff time ( $C_o$ ) is considered, the percentage of the maximum possible effect will vary with the energy of the source of thermal radiation for strictly physical reasons (Fig. 24). If we consider a drug that increases the reaction time by  $\Delta R$ , it probably means that the apparent temperature threshold increased from  $T_a$  to  $T_{a_t}$ . When caloric energy is increased, the temperature increases more rapidly with the inescapable consequence of a decrease of  $\Delta R$ , an increase in  $C_o - R$ , and hence a decrease in their ratio, the %MPE. When caloric energy is decreased,  $\Delta R$  increases,  $C_o - R$  decreases, and the %MPE increases. In this kind of experimental protocol, the effectiveness of a treatment seems to increase when one lowers the intensity of the heat source. It is actually a pure artifact related to physical properties and the means of calculating the effect.

In view of these considerations, it must be emphasized that in the course of these tests, as in all others based on the principle of measuring a reaction time, the duration of the stimulus and consequently its intensity are determined by the reaction of the animal, which may or may not bring to an end the period of stimulation. This in turn emphasizes the difficulty, if not impossibility, of uncoupling the input and output of the systems studied in this way. As we have seen, the measured reaction time results from physical and biological factors. The

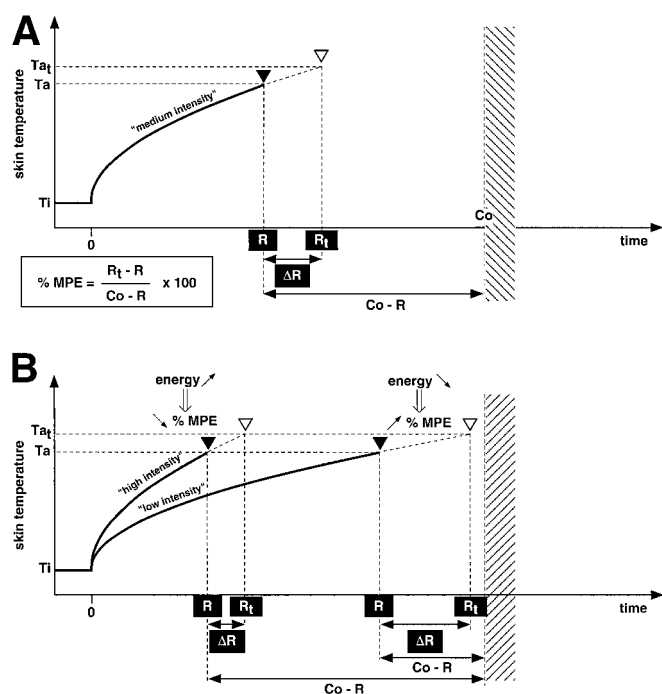


FIG. 24. The calculation of the percentage of the maximum possible effect of a treatment that alters a reaction time is dependent on the intensity of the applied stimulus. In these diagrams, the occurrence of the control reaction is represented by a black triangle and that of the reaction after treatment, by an open triangle. Note that the treatment increases the measured reaction time by  $\Delta R$ . A, calculation of the index. B, the intensity of the stimulus energy determines the index. The %MPE is negatively correlated with stimulus intensity for simple physical reasons. With a powerful stimulus, the temperature increases rapidly, which results in a decrease in  $\Delta R$  and an increase in  $(Co - R)$ , i.e., a reduction in the ratio between these terms. When the caloric energy is reduced,  $\Delta R$  increases while  $(Co - R)$  decreases, and thus, the ratio between them is greater. %MPE, the percentage of the maximum possible effect; R, measured control reaction time;  $R_t$ , measured reaction time after treatment; Co, cutoff time.

presence of a reaction signifies that at some prior point in time, the peripheral receptors reached a sufficient level of activation to trigger it (Figs. 19A and 20).

This coupling between the input and output of the system can cause hidden bias when interpreting data. Thus very often in a “Materials and Methods” section of an article, there is a cryptic phrase explaining that the intensity of the lamp had been adjusted for each animal so that the latency of the tail-flick was around a certain value (for example, 3 or 4 s); one might suggest that this is entirely reasonable if you want to homogenize a group of animals. Moreover, the “Results” section of these same articles often starts by mentioning that latencies did not differ significantly between the groups; here, one might suggest that this is quite reasonable if you want to use simple statistical tests to compare treatments in different animals. However, this practice hides a serious error in reasoning. Passing over the anecdotal fact that the result is nothing but a direct fruit of the method, if you choose to adjust a stimulus so that the reaction time is 3 s, a significant difference between the groups would simply mean that you only casually followed the method you claim to have used. As long as after determination of

the baseline response the groups differ only in the treatments which are applied, this practice, although debatable at a theoretical level, has no substantial consequences. On the other hand, when the groups are different even before the determination of the baselines, we are confronted with results that cannot be interpreted because we do not know the effects of certain factors (e.g., preliminary pharmacological treatment, cerebral lesions, nerve lesions, and lesions in ganglia) on the response being studied. In reality, this very standard practice consists of biologically applying a relative correction, or one might say calculating the percentage contribution of a physical artifact—the adjustment of the intensity of the lamp. And it is thus that one can calculate percentages of percentages without realizing it. Once again, in this type of analysis, there can be serious confusion between the stimulus and the response, which can lead to erroneous conclusions. Figure 25 illustrates this problem by considering an experimental plan with a 2-by-2 factorial: the first (A) treatment corresponding to the administration of an analgesic substance which increases the latency of the tail-flick, and the second corresponding to a pretreatment (e.g., pharmacological manipulation or a lesion in the nervous system) that increases (B1 pretreatment) or decreases (B2 pretreatment) this reaction time. According to the assumption in this example, the effects of the treatment and the pretreatment are simply additive (the graphs on the left represent results obtained when the intensity of stimulation was identical for all the animals). When the intensity of stimulation was adjusted so that the reaction time was identical during the control period preceding treatment A, regardless of the group of animals (graphs on the right), one could conclude erroneously that there was an interaction between factors A and B.

### E. Influence of Species and Genetic Line

We indicate the importance of these factors with only a few examples. They would otherwise merit a long discussion that would be beyond the framework of this review, which is devoted primarily to the methodology of the most commonly used animal models of acute pain. Nevertheless, we must always bear these factors in mind because they can influence the pharmacokinetics and pharmacodynamics of administered substances just as much as the physiological mechanisms that underlie the recorded responses.

In this context, the study of 10 lines of mice subjected to a series of different tests of nociception revealed a strong genetic influence on the responses of the animals; for example, one stock of animals showed virtually no responses to the formalin test (Mogil, 1999). Similarly, in the context of the hypothalamo-hypophyseal axis, the responses to stress vary according to the stock of rats, with extremes like the Lewis and Fisher stocks, which have low or high sensitivities, respectively. This results secondarily in the opposite susceptibility for inflamma-

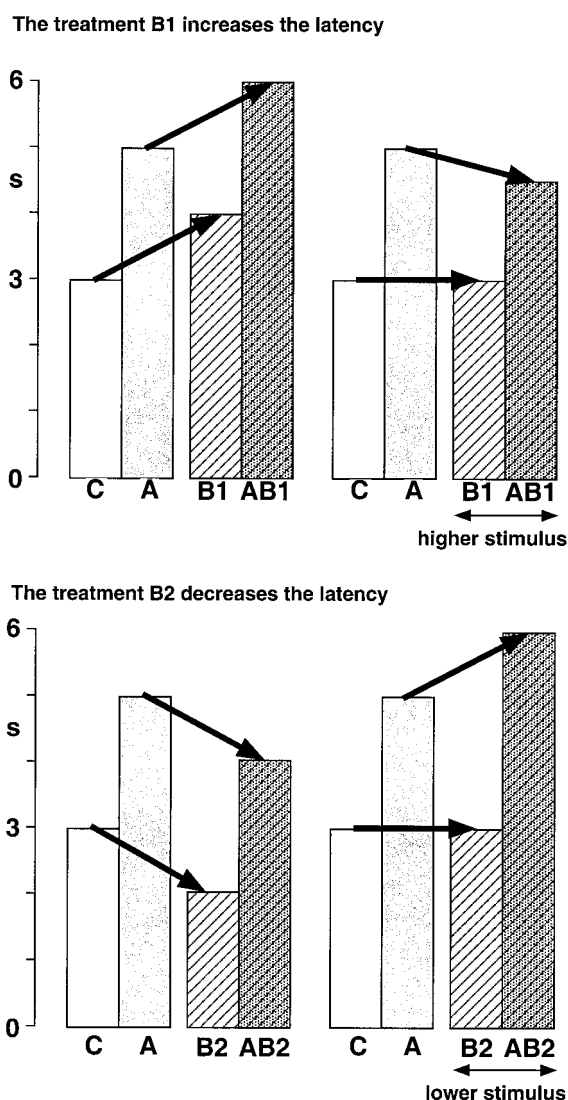


FIG. 25. In many experimental protocols, the intensity of thermal stimulation is adjusted empirically by the experimenter so that the measured reaction time has a predetermined value. For example, the intensity of the thermal source may be adjusted so that the tail-flick reaction time is 3 s. A priori, this practice is acceptable when this intensity is identical for all the animals used in a given protocol (four bars to the left of each graph). On the other hand, its use is more dubious when it is applied individually to each animal (four bars to the right of each graph). This can be illustrated with an example concerning the interactions between two treatments: A, the treatment (administration of an analgesic substance) increases the tail-flick reaction time by 2 s; B, the treatment (e.g., pharmacological pretreatment or a lesion to the nervous system) increases (B1) or decreases (B2) this reaction time by a second (top and bottom graphs, respectively). In addition, in these cases, we assume that these effects are simply additive. The bars to the left represent the results obtained when the intensity of stimulation was identical for all the animals. In accordance with the additivity of the effects, the combination of the two treatments A and B resulted in an increase in reaction time of three seconds (AB1, upper graph) or one second (AB2, lower graph). The bars to the right were from similar experiments except that the intensity of stimulation was adjusted so that the reaction time was 3 s during the control period preceding treatment A, this practice does not influence the real effects of treatment A: the reaction time changes from 3 to 5 s. In the animals pretreated with B1, the strength of the stimulus was increased so that the control response was brought back to 3 s: treatment A then seems less effective, which can lead to the erroneous conclusion that pretreatment B1 thwarted the effects of A. Conversely, in the animals pretreated by B2, the strength of the stimulus was lowered so that the control response was lengthened to 3 s: treatment A now seems more effective, which can lead to the erroneous conclusion that pretreatment B2 facilitated the effects of A.

tory diseases (Cizza and Sternberg, 1994). Variability can also relate to the anatomy of the nervous system: noradrenergic neurons from the locus coeruleus project toward the dorsal or ventral horn, depending on whether Sprague-Dawley rats belong to the Harlan or the Sasco stock (West et al., 1993). At a pharmacological level, the effects of morphine are also genetically determined, at least in the mouse (Mogil et al., 1996; Belknap et al., 1998; Elmer et al., 1998).

Interspecies variability is undoubtedly even greater. For example, NK1 receptors in humans are identical to those in the guinea pig but different from those in the rat and mouse (Watling et al., 1994). The pharmacological effects can also vary radically from one animal species to another. Even if physicians and biologists often ignore the fact, veterinarians have known for a long time that the properties of morphine vary radically with species. If we consider just domestic mammals, we can distinguish two groups of species in which, despite all their other differences (see below), analgesia is a common denominator (Aitken, 1983; Brunaud, 1986; Benson and Thurmon, 1987). The first group responds by being sedated and showing an increase in vagal tone comparable to what is seen in humans; this group includes the rat, the guinea pig, the rabbit, and the dog. The second group responds differently with an overall excitation and increase in sympathetic tone; this group includes the horse family, cattle, sheep, goats, pigs, cats, and mice. The first group is subject to respiratory depression, whereas the second is not. These differences can have significant consequences if one takes account of the possibilities of interference between certain physiological functions and nociception (see also Section XII.E.2.) One might emphasize the fact that the effects of morphine on thermoregulation are determined to a large extent at a genetic level (Belknap et al., 1998).

## X. The Specificity of Tests

Regarding the specificity of tests for exploring the nociceptive system, there is a question of whether we are always measuring a nociceptive threshold. To make tests more sensitive, some investigators have been tempted into decreasing the stimulus intensity, most notably the rate of heating in the tail-flick and hot plate tests. As previously mentioned, by doing this it is possible to increase the tail-flick reaction time from the usual 2 to 4 s to 6 to 9 s (Jensen and Yaksh, 1986; Ness and Gebhart, 1986). Under these circumstances, the reaction time is related to the gentler temperature gradient, which results in a reduction in the rate of heating within the tissues; it then becomes possible for very weak variations in temperature to result in large changes in reaction time. Although cutaneous thermoreceptors are activated before nociceptors, it is unlikely that the heat rather than the nociceptive character of the stimulus produces the reflex responses, at least under normal

physiological conditions. The essential physical parameter for producing this response is the actual skin temperature, which has to reach a critical value. This value is defined as the nociceptive threshold. However, for technical reasons, this temperature is not very easy to measure. In the rat, Hardy (1953) estimated it to be 44.7°C regardless of the initial temperature, the time of exposure to the stimulus, and the rate of heating. He then corrected this temperature to take account of differences in some of the physical properties of skin in the rat and in humans; as a result, the estimate became 47.6°C (Hardy et al., 1957). Estimates of this temperature have varied from one laboratory to another in the range 40 to 45°C. However, it always seems to be constant in a given laboratory (Jackson, 1952; Ness and Gebhart, 1986; Tsuruoka et al., 1988). Whereas the results of Hardy et al. (1957) suggested that it is very much the nociceptive character of the stimulus that generates the response, the wider range of lower temperatures found in other laboratories suggests that, in some cases, the threshold being measured could be for a prenociceptive or quasinociceptive reflex (Walters, 1994). However, it is difficult to be certain of this, given that measuring the temperature of an interface—in this case, the skin—is always a difficult technical problem to overcome. Nevertheless, it must be remembered that the thermal thresholds of individual nociceptors in the rat tail range from 40 to 55°C (Mitchell and Hellon, 1977; Fleischer et al., 1983).

If everyone is intuitively capable of understanding the difference between tepid, warm, hot, painfully hot, and burning sensations, it is less easy to define the transitional phases between these. The same problem exists in the experimental situation despite the fact that the term “phase” is avoided by the use of actual temperatures (Handwerker and Kobal, 1993). Some investigators have even disputed whether it is possible to determine a nociceptive threshold for thermal stimulation with any precision (Yarnitsky and Ochoa, 1990). This is reminiscent of the problems of trying to describe electrical stimuli in humans—where we speak, for example, of dental (and other) “pre-pains” (e.g., Shimizu, 1964; Brown et al., 1985). One could argue that these are reactions having the biological role of alerting the organism before a natural stimulus becomes really noxious. The triggering of these reactions may arise from the fact that many nociceptors, be they somatic or visceral, can be activated by nonpainful electrical, thermal, or, even more likely, mechanical stimuli (Handwerker and Kobal, 1993).

A similar situation may exist in animals, although we have no evidence to prove that this is the case. On the other hand, there is evidence that learning phenomena may affect the outcome of many of the tests (see *Section XII.D.*).

In summary, the specificity of a nociceptive test depends on the nature and temporal characteristics of the applied stimulus—the “input specificity”—and the type

of response being recorded—the “output specificity” (see *Section IV.*). However, it seems extremely difficult or even impossible to ensure such specificity. The reader will probably have realized that neither we nor anyone else has been able to avoid what borders on circuitous reasoning on this matter. Indeed, the problem is made worse by the possibility that further complications may result from the intercurrent pharmacological effects of the substance being studied (see below).

## XI. Comparison with Clinical Situations and Predictiveness of the Tests

Predictiveness—in terms of clinical applicability—is an absolute requirement in nociceptive tests for two reasons. First because it is necessary when searching for new molecules with therapeutic value to avoid false positives and false negatives (Collier, 1964; Chau, 1989). For example, the writhing test, which is very sensitive but only weakly predictive, has to be reserved for initial pharmacodynamic screening so that potentially analgesic substances are not missed (Hendershot and Forsaith, 1959; Loux et al., 1978; Dubinsky et al., 1987). Another reason is far more fundamental and related to situations in which one is trying to understand the basic mechanisms underlying pain and analgesia. What credence can be given to a line of reasoning that relies on manipulations (pharmacological, neurological, genetic, etc.), the results of which are based on variations of a relative parameter in a nonselective, and consequently nonspecific, test?

The most predictive of the models of acute pain are undoubtedly the formalin test (Dubuisson and Dennis, 1977) and the Randall and Selitto test (1957). A mathematical formula has even been proposed to devise directions for use of an NSAID agent in humans using as its starting point the ED<sub>50</sub> value from the Randall and Selitto test in the rat (Dubinsky et al., 1987). On the other hand, the tail-flick and hot plate tests are only predictive for substances that are morphinomimetic in the strictest sense, with very few effects for partial agonist compounds and none at all for mild antalgics from step 1 on the World Health Organization pain ladder (Taber, 1974; Dewey and Harris, 1975; Chau, 1989).

Here, there is a paradox that deserves a little thought. Certain tests, which are not very predictive in terms of identifying analgesic molecules, become useful when drawing up directions for the therapeutic administration of substances in humans. This is the case with the writhing test (Siegmund et al., 1957; Taber et al., 1964; Romer, 1980; Chau, 1989) for which a mathematical formula was proposed to predict directions for use in humans from the ED<sub>50</sub> value determined in the mouse (Pong et al., 1985). There are no satisfactory explanations for this inconsistency in the predictability of this method—weak at identifying a substance as analgesic

but strong at predicting its therapeutic capacities once it has been identified as being analgesic.

## XII. Perturbing Factors

### A. Factors Linked to Pharmacokinetics

Pharmacokinetics can be very different in humans and different species of animals for a variety of reasons, notably the bioavailability, the tissue distribution, the metabolism, and the rate of elimination. Even in a single species, it can be radically altered by experimental manipulation. Thus, in the rat, section of the neuraxis at a thoracic level (producing a spinal animal) does not modify the plasma concentration of systemically administered morphine but reduces its concentration in the brain and spinal cord to about one-third (Advokat and Gulati, 1991). This prompts the question of how one can compare effects of morphine in intact and spinal animals (Irwin et al., 1951; Bonnycastle et al., 1953; Sinclair et al., 1988).

### B. Interactions Between Stimuli

The simultaneous application of stimuli to several topographically distinct parts of the body can introduce bias to a study by triggering diffuse noxious inhibitory controls with supraspinal origins (Le Bars et al., 1984, 1989). This applies particularly to the hot plate test and to tests using electrical stimulation through a grid that constitutes the floor of a cage. In both cases, the four paws and perhaps the tail of the animal may be stimulated simultaneously.

The importance of this factor has undoubtedly been underestimated. Indeed, some experimental situations can shed light on this potential problem. It has been shown several times in the rat and the mouse that intraperitoneal injections of irritant agents (the writhing test) produce an increase in nociceptive thresholds in distant somatic structures, e.g., the tail and the paws (Winter and Flataker, 1965a; Hitchens et al., 1967; Komisaruk and Wallman, 1977; Hayes et al., 1978; Kraus et al., 1981; Chapman and Way, 1982; Calvino et al., 1984; Wright and Lincoln, 1985; Kraus and Le Bars, 1986). Similarly, it can be shown that the insertion of electrodes into the tail provokes a net decrease in abdominal cramps induced by an intraperitoneal injection of acetic acid (Le Bars et al., 1984). Injection of formalin into the forepaw increases the threshold for vocalization evoked by mechanical pressure on the hind paw (Calvino, 1990). Finally, a burn on the back raises the tail-flick threshold (Osgood et al., 1987). These observations in experimental animals reflect something that has been known in humans since ancient times, namely that one pain can mask another (reviewed by Le Bars et al., 1984, 1989). Because opioids interfere with these phenomena (Kraus et al., 1981; Kraus and Le Bars, 1986), one must consider the possibility that bias is occurring in tests

that involve stimulation of several parts of the body—as typified by the hot plate test.

### C. Environmental Factors

The clinician knows by instinct and experience that pain, be it acute or chronic, is multidimensional, and thus, any evaluation of pain must be in a general context. In this way, anxiety is regarded as an aggravating factor for clinical pain (Beecher, 1956b; Sternbach, 1974). The same applies to experimental pain, including experimental pain in animals. Furer and Hardy (1950) described an increase in the reaction to a painful stimulus in anxious subjects. It is not always easy to reconcile such factors with the usual use of the classic tests described above. For practical reasons, such tests are often made on restrained animals and almost always on animals that are being confronted with a new environment. One has to imagine the bland environment in which generation after generation of laboratory animals are raised and then realize what a shock it must be to one of these animals when it is confronted with an experimental set-up. This shock is expressed in measurable variations in physiological parameters such as a lowering of the temperature of the rat's tail (Wright and Katovich, 1996).

It undoubtedly follows that the effects of morphine on the tail-flick test are greatly facilitated by the restraint and/or the novelty of the environment (Kelly and Franklin, 1984a,b; Appelbaum and Holtzman, 1986; Franklin and Kelly, 1986; Calcagnetti and Holtzman, 1992; d'Amore et al., 1992; Menendez et al., 1993; Montagne-Clavel and Oliveras, 1996; Sutton et al., 1997) or by more severe forms of stress (Sherman et al., 1981, 1984; Hyson et al., 1982; Lewis et al., 1982; Rosellini et al., 1994). Indeed, the facilitatory effect of anxiety on the action of morphine on a pain threshold was identified a long time ago in both humans and animals (Hill et al., 1954; Kornetsky, 1954).

Animals are very often placed individually in cylindrical containers that have an orifice to allow the tail to stick out. The time given to animals to habituate to these conditions before the experimental protocol is started varies from one laboratory to another. This confinement in a cylinder results in increases in core temperature and the temperature of the tail. These effects are counteracted by morphine in a dose-dependent fashion (Vidal et al., 1984; Tjølsen and Hole, 1992). These last effects probably result from the thermal environment in which the animals are confined and thus are actually caused by thermoregulatory mechanisms (see *Section VII.E.1.*). In this respect, it should be recalled that the temperatures in animal houses are very well controlled (at 19–21°C for rats and mice) under penalty of not being approved by the veterinary services if they are not. This is not always the case for the laboratories in which the experiments themselves take place or, of course, for the containment boxes that are usually used.

To minimize the stress caused to the animal by the procedures and not cause hyperthermia, some investigators prefer to manipulate the animal gently with a cloth to orient its tail toward the source of heat. When the two methods have been compared, it has been noted that the reaction times are shorter with the first than with the second (Ramabadran et al., 1989). Those most concerned with minimizing stressful conditions manipulate the animal daily before undertaking the actual test; this shortens the tail-flick reaction time (Milne and Gamble, 1989, 1990). Others have studied the tail-flick in the anesthetized rat (Fields et al., 1983; Ness and Gebhart, 1986). It is not easy to compare results obtained under such different experimental conditions.

Just for completeness, we also cite the potential influence of circadian rhythms that, as with other biological functions, are likely to interfere with measurements taken during various tests of nociception (Morris and Lutsch, 1967; Labrecque and Vanier, 1995). These considerations bear heavily at an experimental level even though they are not specifically related to this field of research.

#### *D. Related Psychophysiological and Psychological Factors*

We all know that major analgesics have serious side effects. Among these are some that give rise to subjective phenomena which in turn can disturb the response that is being studied. Thus, in the context of experimental pain in healthy human subjects, it is quite pointless to compare the effects of morphine with those of a placebo since the subjects will be able to distinguish these two substances without any ambiguity. Smith and Beecher (1959) gave a good description of these phenomena, which are characterized above all by lethargy ("mental inactivity") and confusion ("mental clouding") as well as by somatic symptoms (dizziness, nausea, pruritus, migraine, heat flushes, etc.). Although none of these subjective phenomena can be assessed in animals, one cannot a priori reject the hypothesis that all or some of them are produced by morphine (Watkins, 1989) and, therefore, could perturb the response being measured. This is all the more so true in those cases in which cognitive functions are called on by a test. These thoughts concerning morphine, a substance that is well known in a clinical context and is taken here as a reference, have to be considered in the light of the immense array of substances appraised by pharmacologists, some of which have and some of which have not been identified as psychoactive.

Learning can be extremely rapid. There is evidence of this from the second presentation of the stimulus in the hot plate test (see *Section V.A.3.* and Fig. 8), and it is also true in the Randall and Selitto test (Taiwo et al., 1989). In a test using heat, the heating is progressive and results in thermoreceptors being activated before nociceptors are recruited (Fig. 7D). Just as there is this

inevitable sequence of activation of thermoreceptors then nociceptors, there is a sequence of a hot sensation then pain. The same applies to tests using increasing pressures: there is a sequence of activating mechanoreceptors and then nociceptors. Furthermore, exactly the same is found when using an experimental paradigm with a conditioning stimulus before a conditioned stimulus to study phenomena related to the anticipation of pain (Vierck and Cooper, 1984; Cooper and Vierck, 1986a).

This is particularly obvious when using Semmes-Weinstein fibers (also called von Frey hairs) to test mechanical sensitivity. This method consists of applying to the skin a fiber of a certain diameter which, when made to bend, produces a constant pressure (Handwerker and Brune, 1987). The use of a range of such fibers with increasing diameters makes it possible to determine the threshold for evoking a response in the animal (e.g., a flexion reflex). This test is rarely used in healthy animals except when they are being used as controls. On the other hand, it is a prized tool in models of chronic pain (Kim and Chung, 1992). Here we simply wish to emphasize that responses are obtained with pressures that rapidly become a little elevated but undoubtedly are non-noxious even in healthy animals (Möller et al., 1998). It is not a matter of doubting whether this pressure can be lowered further after peripheral or central sensitization, but of emphasizing that anticipatory or training phenomena are likely to blur the response, which consequently cannot be interpreted other than in terms of pain. This consideration cannot be ignored, and its importance can be shown, for example, by the fact that morphine blocks various responses conditioned by non-nociceptive stimuli (Fig. 26; Cook and Weidley, 1957; Holtzman, 1976) and interferes with the cognitive capacities of the animal (Schulze and Paul, 1991).

The use of an electric bulb to deliver a thermal stimulus can produce training phenomena whereby without the knowledge of the experimenter, the animal associates the visual stimulus with the simultaneous nociceptive stimulus. Thus, King and colleagues (1997) showed that the application of weak stimulus intensities, which lengthens the reaction time of the tail-flick, increases the duration of the "conditioning" visual stimulus. As a result, after several tests, the animal responds more rapidly. If one chooses the parameters of conditioning visual (or auditory or mechanical) stimuli judiciously, the animal can respond even before the application of the thermal stimulus (a "negative reaction time" by anticipation). These effects disappear when the animal cannot see the source of light or when it is "spinalized". Moreover, the effects are blocked by low doses of morphine (1 mg/kg; i.p.), which are ineffective in this test when the control reaction time is brief. It can be concluded that the movement of the tail may result from a simple spinal reflex or training, depending on whether the stimulus is short or long.



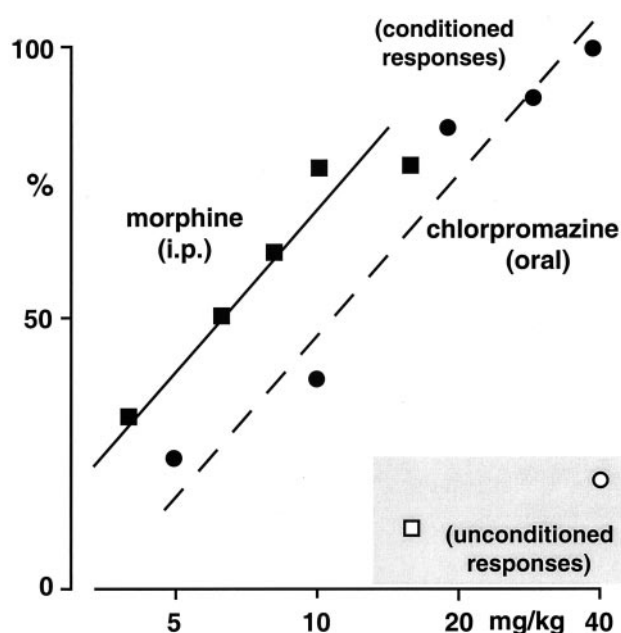


FIG. 26. Morphine blocks conditioned responses. In the experiments of Cook and Weidley (1957), rats learned to avoid electrical shocks delivered from the floor of the cage as soon as they heard a ringing sound. The oral administration of chlorpromazine (5–40 mg/kg) or the intraperitoneal administration of morphine (4–16 mg/kg) blocked this response without any effect on the response to the nonconditioned electrical stimulus. The ordinate represents the proportion of animals in which the conditioned (filled symbols) or nonconditioned (open symbols) response was blocked. Circles, morphine; squares, chlorpromazine. Modified from Cook and Weidley (1957) with permission.

In principle, this possibility that learning phenomena will bias the results is always present. The presence of a control group in a series of experiments, although indispensable for other reasons, is not *ipso facto* a guarantee against this problem. For example, when the test consists of adding a sharp stimulus to an inflammatory lesion several times, the allodynia will be exaggerated by anticipatory responses from the animal. Obviously, this problem is even more crucial in models of chronic pain, in which the animal undergoes a learning process throughout the duration of the syndrome even when it is in its cage and is not being observed. This is shown by the fact that the animal rapidly acquires analgesic behaviors and postures.

#### E. Related Physiological Functions

There is an almost insoluble problem. The strength as well as the weakness of scientific research resides in the way we reduce a problem to the simplest form in which it can be tested with the means at our disposal. This reductionist, but necessary, approach occurs in all areas of scientific research and sometimes excites historians of science. Interplay between the somesthetic and vegetative systems at anatomical and functional, peripheral, and central levels is such that it is sometimes difficult to decide what is a cause, what is a consequence, and what is simply a covariant. Once again, morphine, our reference substance, illustrates this point since it not only

has multiple physiological effects, but these vary between species.

We know that morphine causes sedation, respiratory depression, and an increase in vagal tone in a comparable way in humans and certain species of animals, but it causes excitation and an increase in sympathetic tone in others (see Section IX.E.). It is as a direct result of these observations that the physiological functions likely to interfere with tests of nociception can be completely different from one species to another. In this respect, we emphasize similarities between humans and the rat but differences with the mouse.

Problems linked to intercurrent physiological functions are often difficult to identify, analyze, and take into account. On several occasions, we have commented on the possibilities of intercurrent vegetative reactions with the responses of animals to nociceptive tests. One can illustrate the magnitude of this problem by listing the secondary effects of morphine in the dog—a “morphine-sensitive” species. All these effects are blocked by antagonists (Dewey, 1974). Some have hardly any influence on nociceptive tests (myosis, salivation, increased intestinal transit time), but it is very unlikely that that is the case for others (sedation, ataxia, depression of postural reflexes, respiratory depression, hypothermia, emesis, bradycardia, hypotension).

For example, hypercapnia increases the pain threshold in humans (Stokes et al., 1948) and the reaction time of the tail-flick in the rat (Gamble and Milne, 1990). Experimental protocols that involve spontaneously breathing animals do not take this parameter into account. When one knows that opioids are depressing breathing, one must take account of the interference produced by that parameter when interpreting the results. For example, in the awake rat, there is a very significant correlation between respiratory depression and the increase in tail-flick reaction time produced by morphine (Rauh and Osterberg, 1966).

1. *Thermoregulation.* The possibility of interaction between nociception and thermoregulation requires comment because it will never be possible using the normal tests to be free of the physiological consequences of thermoregulation, which are the basis of variations in cutaneous temperature. In humans, for example, the hypothermia associated with a decline in cutaneous temperature will provoke an “artifactual” increase in the thermal nociceptive threshold (Hardy et al., 1952; Andrell, 1954). Indeed, Winter and Flataker (1953) showed that, in the dog, a decrease in cutaneous temperature provoked by hypothermia is sufficient to explain, at least in that species, the morphine-induced increase in nociceptive threshold (see below). As a consequence, it is necessary to put pharmacological results in the physiological context of the test that is being used and consider that a drug could alter heat transfer and, hence, afferent input.

Indeed, rodents do not sweat, and their main peripheral organs of thermoregulation are the tail and, albeit to a lesser extent, the parts of the paws not covered by fur. The dissipation of heat is regulated at the level of the tail by abrupt variations (on-off) of blood flow in a system of arteriovenous anastomoses, which form a double ladder (Fig. 27; Rand et al., 1965; Gemmel and Hales, 1977; Dawson and Keber, 1979; Young and Dawson, 1982); this flow can increase by a factor of 35 when the arteriovenous anastomoses are open (Raman et al., 1983; Aukland and Wiig, 1984) and tail skin temperature may increase by as much as 10°C (O'Leary et al., 1985). As a result, one can observe a positive correlation between room temperature and the cutaneous temperature of the tail (Berry et al., 1984) and a negative correlation of each of these and the tail-withdrawal reaction time (Berge et al., 1988; Milne and Gamble, 1989; Tjølsen et al., 1989a). From a physiological point of view, this negative correlation between the tail-withdrawal reaction time and room temperature (Ren and Han, 1979; Schoenenfeld et al., 1985; Han and Ren, 1991) means it will decrease abruptly during drastic increases in peripheral blood flow and cutaneous temperature (Milne and Gamble, 1989), which occur spontaneously as part of the bistable character of this regulatory system (Young and Dawson, 1982). As it happens, the fact that fluctuations in room temperature are an important source of variations in thermal threshold has been known for a long time (Winder et al., 1946; Geller and Axelrod, 1968).

From a pharmacological point of view, during experiments undertaken in a constant-temperature environment, a decrease in cutaneous temperature will translate into an increase in the tail-withdrawal reaction time and will be falsely interpreted as a sign of hypalgesia (a false positive in the study of analgesics). An increase in cutaneous temperature will translate into a decrease in the tail-withdrawal reaction time and be falsely interpreted as a sign of hyperalgesia (Eide and Tjølsen, 1988;

Tjølsen et al., 1989a,b; Roane et al., 1998). On this basis, Tjølsen and Hole (1997) attributed the entire reduction in tail-flick reaction time after section of the spinal cord, lesions of the raphe-spinal serotonergic system, and systemic or intrathecal administration of serotonergic blocking agents to the increase in skin temperature. The physical stimulus that is applied has not changed, but conversely, the effective stimulus, which is constituted by the actual physical stimulus together with the physical properties of the skin, is completely different. However, on the basis of experiments undertaken in the mouse, Lichtman et al. (1993) maintained that the tail-flick reaction time is independent of cutaneous and central temperatures.

It should be noted that these considerations concern not only tests based on the use of thermal stimuli. For example, the formalin test is equally sensitive to room temperature, especially during the second phase. Behaviors monitored in the course of the second phase are exacerbated when the room temperature increases within the range of 20 to 28°C; indeed, at 26 to 28°C, the two phases merge (Rosland, 1991). Tjølsen et al. (1992) recommended that this test should be performed in a room at 22 to 23°C to produce clear and reproducible responses.

When considering the pharmacological effects of a substance, one must think about the limited number of reports that have taken account of basal cutaneous temperature, central temperature, and energy from the thermal stimulus which combine with the elevation of cutaneous temperature that ultimately evokes the behavior. Thus, one must remember that, under standard temperature conditions in the rat, morphine in weak doses produces hyperthermia but in larger doses produces hypothermia (Adler et al., 1988), with these effects being mediated by  $\mu$  and  $\kappa$  receptors, respectively (Chen et al., 1995). As for the intrathecal administration of morphine, in the rat it seems to provoke a hyperthermia associated with a decrease in the temperature of the tail (Rudy and Yaksh, 1977).

**2. Vasomotor Tone.** These considerations of vascular phenomena in the tail also prompt us to take account of the vasomotor tone of the whole animal. We have already mentioned that under normal conditions, the temperature of the skin results from an equilibrium between heating by means of the arteriovenous capillary bed and loss of heat through the skin surface (Fig. 28Aa). During vasoconstriction, blood flow through the arteriovenous capillary bed is shunted and the skin temperature decreases, i.e., the "heater" is turned down (Fig. 28Ab). During vasodilatation, the heater is turned up and the skin temperature increases (Fig. 28Ac). This tone can vary independently of phenomena related directly to thermoregulation and nociception. For example it may vary due to pharmacological manipulations, to environmental factors, and possibly to stress. A vasoconstriction will result in an increase in the withdrawal

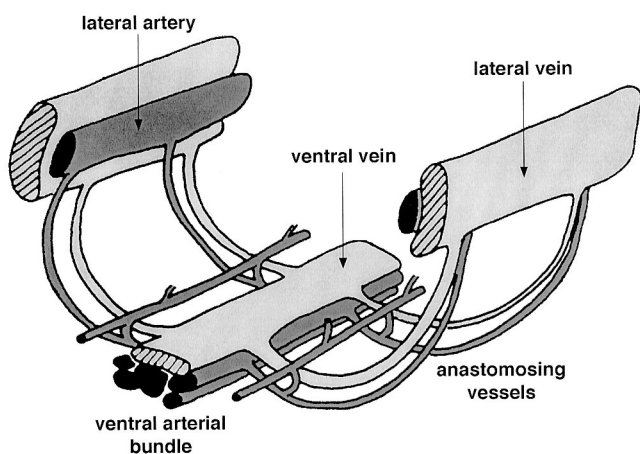
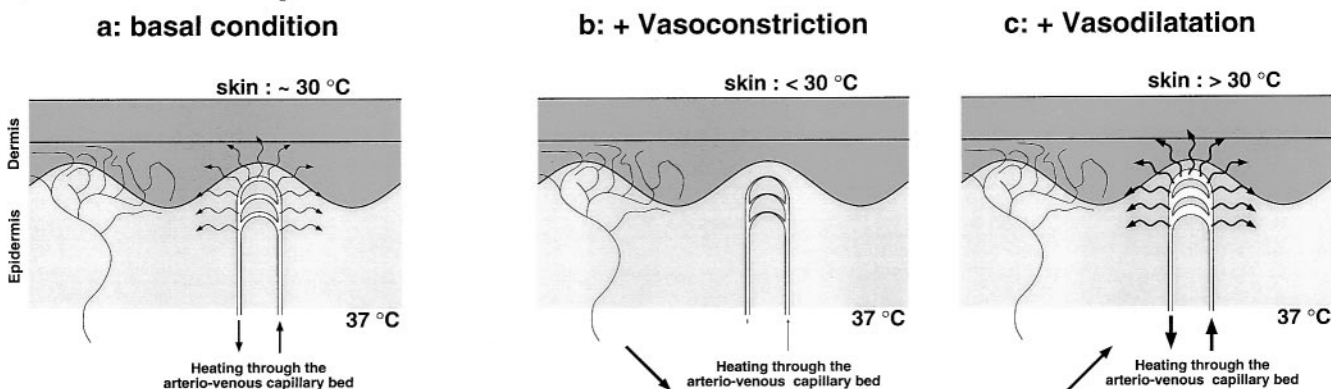


FIG. 27. Vascularization of the tail. Adapted from Young and Dawson (1982) with permission.

## A: Room temperature



## B: External heating

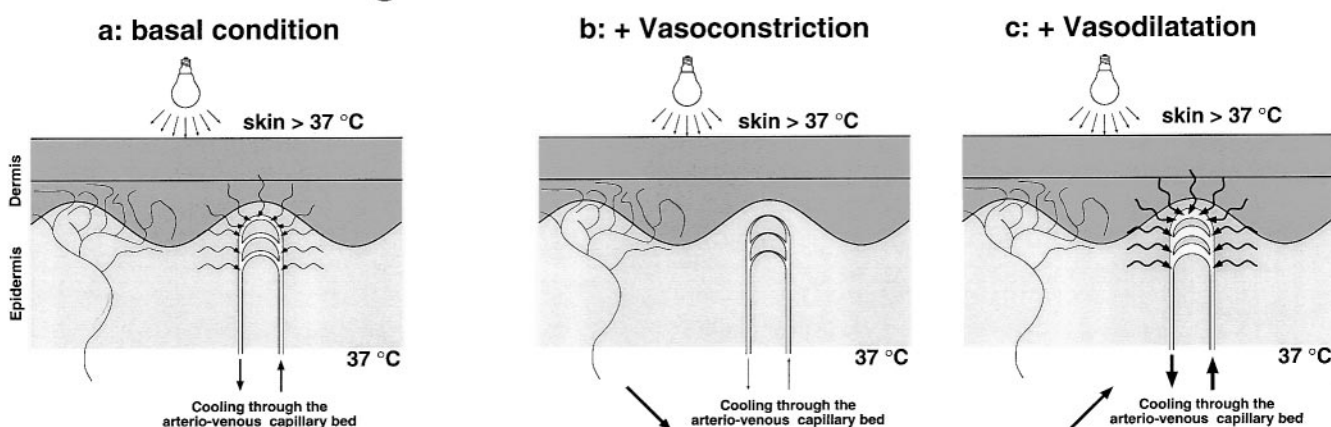


FIG. 28. The microcirculation serves to maintain skin temperature adequately high at room temperature (Aa) and to cool it when it is subjected to external heating (Ba). In both cases, the role is reduced by vasoconstriction (Ab and Bb) but enhanced by vasodilatation (Ac and Bc).

reaction time, whereas a vasodilatation will reduce it (Fig. 29). It is known that many stressful factors can provoke an increase in the tail-withdrawal reaction time (Akil et al., 1976; Lewis et al., 1980; Watkins and Mayer,

1982; Amit and Galina, 1986; Porro and Carli, 1988; Bodnar, 1993). This phenomenon is called “stress-induced analgesia”, a term that is possibly abused, not only because the very notion blatantly contradicts the daily practice of the clinician (Sternbach, 1974), but also because the phenomenon disappears and the opposite results may be found when tests other than the tail-flick are administered (Kelly, 1982; Vidal and Jacob, 1986; Huang and Shyu, 1987; Kiyatkin, 1989, 1990; Illich et al., 1995; King et al., 1996; Prentice et al., 1996, 1999). Furthermore, it is prone to strong individual variability (Jørum, 1988). The vasoconstriction released by stress could well, at least in part, explain increases in tail-flick reaction time obtained in some stressful conditions.

This problem of interactions between nociception and peripheral blood flow was identified long ago (Hardy et al., 1940; Beecher, 1957) and has been very well studied during recordings of lumbar spinal dorsal horn neurons in the cat (Duggan et al., 1978). It is known that an injection of norepinephrine will provoke a strong steady hypertension followed by a weak hypotension and that these events are accompanied by a decrease then an increase in cutaneous temperature. As a result of this, when a constant stimulus is applied, the temperature

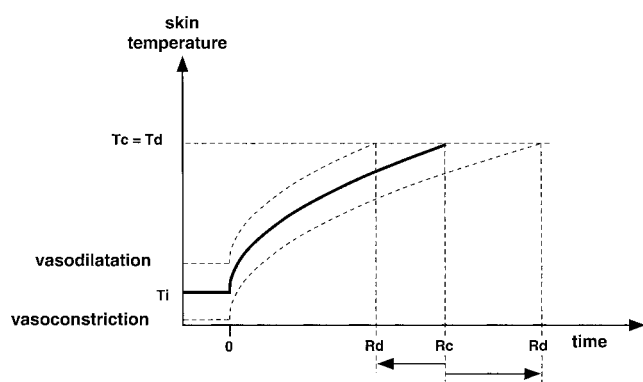
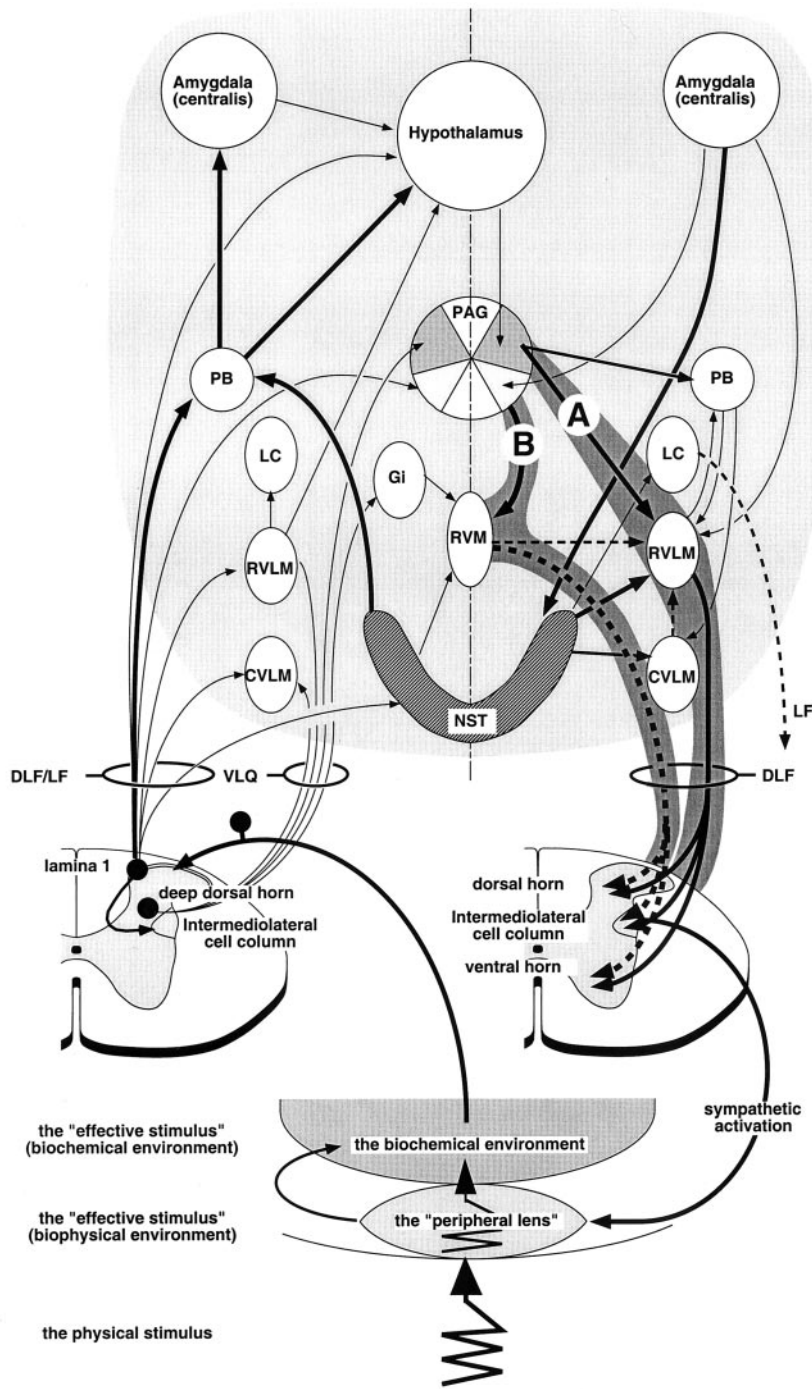


FIG. 29. A small variation in baseline temperature can produce a large variation in reaction time. The latter varies with the square of the variation in temperature. This is what can occur when a treatment has vasomotor effects. Vasodilatation and vasoconstriction can be interpreted as pro- and antinociceptive, respectively, even if the treatment is ineffective ( $T_d = T_c$ ).  $T_i$ , initial skin temperature;  $T_c$ , skin temperature at time of control response;  $T_d$ , skin temperature at time of response after drug treatment;  $R_c$ , control reaction time;  $R_d$ , reaction time after drug treatment.



animal models of nociception : figure 30

FIG. 30. This figure emphasizes the anatomical and functional relationship between certain pathways that convey nociceptive signals (left) and those that regulate blood pressure (right). The diagrammatic presentation to the left and right is arbitrary and does not have any functional significance. Only relevant ascending pathways are represented: the spinothalamic and spinoreticulohypothalamic pathways are not. The represented ascending pathways, which include the outputs from neurons located in lamina 1 and deeper layers of the dorsal horn, will directly or indirectly activate many centers in the brain. The latter are implicated, as much by direct as by indirect mechanisms, in vegetative regulatory functions, especially cardiovascular controls (Lovick, 1993, 1996, 1997; Bernard and Bandler, 1998; Aicher et al., 2000). The principal effector of these controls lies in the ventrolateral brainstem and regulates preganglionic sympathetic neurons (the descending pathways in the dorsolateral funiculus, represented on the right). Thus, blood pressure and vasomotor tone are under the influence not only of baroreceptors and chemoreceptors but also of ordinary sensory systems. Furthermore, connections through the amygdala make them dependent on mental and emotional states. It may be noted that the pivotal point for distributing nociceptive information consists of the periaqueductal gray matter and the RVM. Thus, the role of these structures is not restricted to the control of neuronal activities in the spinal dorsal horn (descending inhibitory pathways in the dorsolateral funiculus represented on the right). Parasympathetic regulatory pathways centered on the nucleus of the solitary tract are not represented (afferents of the facial, glossopharyngeal, and vagal nerves—VII, IX, and X), nor are parasympathetic efferent pathways from the nucleus ambiguus and dorsal vagal motor nucleus. Moreover, the parabrachial area and the amygdala control certain hypothalamic activities, in particular the corticotrophic hypothalamo-hypophyseal axis (not represented). A and B represent pathways activated by microinjection of excitatory amino acids into dorsolateral and lateral

reached by the skin will be successively lower and then higher, which will result in a steady diminution followed by an increase in the neuronal response. Conversely, an injection of acetylcholine is known to provoke hypotension, which will result in an increase in cutaneous temperature and thus an increase in the temperature achieved by the thermal stimulus and an increase in the neuronal response. All of these variations are artifacts. When the stimulation set-up allows the baseline temperature to be kept constant by use of a feedback mechanism and phasic stimuli are applied on top of this, the response remains more or less constant after the administration of norepinephrine or acetylcholine, despite variations in blood pressure (Duggan et al., 1978). Indeed, in this case, the applied energy adapts to the uncontrolled variations in cutaneous temperature so that the stimulus is the desired effective stimulus. Tjølsen et al. (1989a, 1991) proposed a series of improvements to experimental protocols to minimize the overall effect of these variable factors. However, these improvements will work only within a limited range when “thermal clearance” phenomena take precedence over others (Duggan and Griersmith, 1979). Indeed, when the skin is heated above the central temperature, the arteriovenous heater will tend to cool the skin (Fig. 28Ba) and therefore facilitate the dissipation of heat, be it from an endogenous or an exogenous origin. To some extent, this will temper the effects of vasomotor variations (Fig. 28, Bb and Bc). However, we must stress that the time constant of such a regulation is generally longer than the conventional duration of application of thermal stimuli.

Setting aside pharmacological considerations, local vasomotor tone and, at least in certain species, sweating will help to stabilize the temperature of skin physiologically if it is subjected to a constant source of radiant heat for long enough. In fact, if a stimulus is applied to a normal limb or to one on which a tourniquet has been applied, the surface temperatures will increase in a similar way for the first few tens of seconds and then will diverge: in the normal limb, the temperature will stabilize gradually, whereas in the ischemic limb, it will continue to increase with the square root of time, in accordance with what one expects from an inert body (Lipkin and Hardy, 1954). In almost all tests, one can neglect this factor because the slopes of the temperature increases are steep, lasting only a few seconds.

Finally we must remember the interdependence of vasomotor tone and thermoregulation. Thus, painful intraneural stimulation or the occurrence of stress cause opposite effects in healthy volunteers depending on

whether they are being heated or chilled: vasoconstriction when they are “hot” and vasodilatation when they are “cold” (Oberle et al., 1988).

3. *Systemic Arterial Blood Pressure.* The relationships between nociception and blood pressure have been described by several authors (Randich and Maixner, 1984; Zamir and Maixner, 1986; Lovick, 1993, 1997). Above all else, it is important to emphasize the interplay between certain systems that modulate transmission of nociceptive signals and those that control blood pressure. Figure 30 summarizes this situation, which has been discussed in some excellent reviews (Bandler and Depaulis, 1991; Carrive, 1991; Lovick, 1993, 1997; Behbehani, 1995; Bandler and Keay, 1996; Blessing, 1997). The problem can be summarized schematically by identifying three great systems that interact together: the first two are based around the PAG and the third around the nucleus of the solitary tract.

Stimulation by microinjection of excitatory amino acids into dorsolateral and lateral parts of the PAG (Fig. 30A) triggers antinociceptive effects accompanied by hypertension, tachycardia, and vasoconstriction in the renal, mesenteric, and cutaneous vascular beds. On the other hand, it produces vasodilatation in skeletal muscles. Furthermore, hyperpnea, mydriasis, exophthalmia, piloerection, jerking of the facial musculature and rear limbs, vocalization, and escape behavior also occur. Such stimuli activate the preganglionic sympathetic neurons in the intermediolateral column via the rostroventrolateral medulla. Their effects mimic those of the “defense reaction”.

Stimulation of the ventrolateral part of the PAG (Fig. 30B) causes antinociceptive effects accompanied by extremely different vegetative phenomena: hypotension, bradycardia, vasodilatation in the muscles of the extremities, hyperpnea, and a type of immobility known as “hyporeactive” (because there is a noticeable lack of reactions by the animal to any stimulus—be it physical or in its vicinity). Such stimulation activates descending controls arising particularly from the nuclei raphe obscurus and magnus [the latter together with the adjacent reticular formation constitute the rostroventral medulla (RVM)]. These controls involve the rostroventrolateral medulla as much as the RVM and are exerted simultaneously on the dorsal and ventral horns and on sympathetic preganglionic neurons through axons that travel in the dorsolateral funiculus.

It is known that the nucleus of the solitary tract plays a central role in the regulation of blood pressure. Vagal stimulation causes “pro-” or “anti-” nociceptive effects, depending on the mode and parameters of stimulation. The tail-flick reaction time is reduced at low intensities

parts of the PAG and by stimulation of the ventrolateral part of the PAG, respectively (see text). The effective nociceptive stimulus is the result of 1) the physical stimulus itself; 2) the local biophysical conditions which themselves depend above all else on vasomotor tone; and 3) the biochemical environment that is particularly important during inflammatory processes. CVLM, caudal ventrolateral medulla; DLF, dorsolateral funiculus; GABA,  $\gamma$ -aminobutyric acid; Gi, nucleus gigantocellularis; LC, locus coeruleus; LF, lateral funiculus; NA, nucleus ambiguus; NST, nucleus of the solitary tract; PB, parabrachial area; RVLm, rostral ventrolateral medulla; VLQ, ventrolateral quadrant.

of stimulation producing a weak hypertension; however, it is increased at higher intensities of stimulation where it is accompanied by a decrease in blood pressure, bradycardia, and apnea. The antinociceptive effects would be exerted via the RVM and locus coeruleus, which themselves are the origins of serotonergic and adrenergic bulbospinal pathways (Randich and Maixner, 1984; Randich and Gebhart, 1992). However, it is known that vagal stimulation causes generalized depressor effects in the central nervous system, and anyone who has experienced vagal faintness can easily imagine that they would respond more slowly to any stimulus without being any less sensitive to pain.

**4. Nociception and Homeostasis.** In any event, we encounter a problem of the interface between pain and diverse functions such as anxiety, the cardiovascular system, and, in a more general way, the vegetative systems. However, our rather coarse methods for activating nerve centers are unable to separate these. It is sometimes quite difficult to come to a reasonable opinion about the real significance of certain experiments. Indeed, almost all studies concerning interactions between nociception and blood pressure are concerned only with responses to thermal stimuli, very often the tail-flick. Furthermore, a number of them were carried out on animals that were more or less deeply anesthetized—an essential factor in this kind of study. Thus, the hypertension produced by a noxious stimulus in the rat is transformed into hypotension when the concentration of halothane increases beyond the minimum alveolar concentration (Gibbs et al., 1989).

Just as a coincidence of events is in no way a conclusive sign of a direct causality between them, one must be wary of interpreting an effect as antinociceptive when it could simply be an indirect effect resulting from the modification of a wide variety of functions, particularly at the periphery and either linked to, or concomitant with, perturbations of the vegetative system. The anatomical observations summarized in Fig. 30 reveal an indisputable interplay between these systems, which suggests that nociception works in alliance with the much larger homeostatic system. This system makes it possible for the organism to react to modifications of the environment, in particular when it is confronted with noxious stimuli (Fig. 30, left side).

However, simple observation of this scheme also allows one to conclude that an imbalance in this arrangement, whatever its nature and origin, will result in concomitant modifications of several variables. One consequence will be peripheral adjustments, particularly at a cutaneous level. Thus, we are still faced with the concept of an effective stimulus, i.e., of a physical stimulus passing through a “peripheral lens” that will regulate its intensity as much for physical reasons as for those of biological origin (Fig. 30). The experimenter, using a constant thermal source, may be persuaded in good faith that the stimulus which it produces is invariable; in fact, as we have just seen, it can be inaccurate when the physical properties of the skin change for a variety of reasons. Thus, it is quite improper for anyone to decide that the antinociceptive effect of stimulating the dorsolateral and lateral parts of the PAG, with its drastic activation of the sympathetic system, has a central and/or a peripheral origin.

The existence of short- (spinal) and long- (supraspinal) latency somatovegetative reflexes has been known for many years. The latter are triggered preferentially when the limbs are stimulated; these reflexes are produced by spino-bulbo-spinal pathways that travel in the dorsolateral funiculus (Sato et al., 1997). In the anesthetized rat with a normal body temperature, a nociceptive stimulus causes increases in heart rate and in blood pressure. However, these responses are reversed if the animal becomes slightly hypothermic (Sato et al., 1976) or if, as mentioned above, the depth of the anesthesia is increased (Gibbs et al., 1989).

As evidenced by the effects of morphine, the intercurrent factors cannot be ignored at a pharmacological level. In the anesthetized rat, the vagal and sympatholytic actions of morphine evoke bradycardia, vasodilatation, and hypotension (Evans et al., 1952; Fennessy and Rattray, 1971; Gomes et al., 1976b; Willette and Sapru, 1982; Randich et al., 1991). On the other hand, in the nonanesthetized rat, hypertension is seen (Gomes et al., 1976a; Conway et al., 1983), although low doses can be without effect or even cause the opposite effect (Stein, 1976; Thurston et al., 1993). Obviously, prudence is necessary.

### XIII. Conclusion

As we have discussed in this review of behavioral models of acute pain in animals (Table 2), none is en-

TABLE 2  
Brief summary of the principal animal models of acute pain

	Thermal	Mechanical	Chemical	Electrical	
				Long Trains	Single Shocks or Short Trains
Phasic pain models	Tail-flick test Paw withdrawal test Hot-plate test	Randall and Selitto	Mechanical stimulation following chemical sensitization (carrageenin, capsaicin, etc.)	Tail Flinch-jump test	Tail Dental pulp Limbs
Tonic pain models		Distension of hollow organs	Intradermal injections (formalin test) Intraperitoneal injections (writhing test)		

tirely satisfactory. The first weakness lies in the stimuli used to trigger a nociceptive reaction. In general, the mastering of these stimuli has been mediocre. However, and undoubtedly more importantly, even when the physical parameters of the external stimuli are well controlled, that does not necessarily result in an equally well controlled effective stimulus. What we mean by effective stimulus is that the stimulus effectively activates the peripheral nociceptors—and this is dependent on the physiological state of the target tissues. We have illustrated many different sources of variability—some might call it plasticity—in the biological responses evoked by stimuli that are “constant” in strictly physical terms, but that may be very variable because of changes brought about in the immediate vicinity of the nociceptors by concomitant physiological factors. The second great weakness of these models lies in the nature of the dependent variable, generally the threshold of a motor reaction. Most of the models do not allow the study of stimulus-response relationships, although these are really an essential element of sensory physiology. Furthermore, it is often not the threshold itself that is measured but a response time to a stimulus of increasing intensity. It should go without saying that such a transformation is conceivable only if the intensity of the stimulus increases linearly with time. This is the case for example, with mechanical tests which use a regular weight attached to a lever or thermal tests using Peltier elements that are so well regulated that the temperature of the probe increases in a linear fashion. As it happens, the results of these tests are often expressed not as reaction times, but in the form of a physical measure corresponding to the threshold (force—itsself proportional to the pressure—or temperature, in the two quoted examples). It is curious, on the other hand, that in tests using radiant heat and in particular the very popular tail-flick test, it is not the temperature threshold that is measured but the response time, even though with a constant source of radiation, the temperature increases with the square root of time.

Finally, it turns out that nociceptive and vegetative systems have strong complex relationships as much at a central as at a peripheral level. These relationships can cause misinterpretations.

All these considerations invite prudence in the interpretation of results obtained using animal models of acute pain. Thus, one might wonder just how many different types of manipulation might increase the tail-flick reaction time—each of these could be interpreted as evidence for an antinociceptive system for controlling pain: “the presence of so many pain control systems is in itself a puzzle” (Lovick, 1993). This is all the more strange given that this test, as we have discussed, is not very sensitive to analgesics administered in quantities that are therapeutic in humans and active in other tests on animals.

However, the usefulness of animal models of acute pain is not in doubt. Consequently, it is important to under-

stand them better and to improve them. In this respect, it must be noted that the neural basis of the most often used tests is poorly understood. Furthermore, it is worth stressing that the considerations discussed in this review also relate to animal models of chronic pain insofar as the tests applied in these models are the same ones, or almost the same ones, as those we have described.

It is also important to consider the theoretical framework within which these models fit. Without entering a debate that would be necessarily long-winded, it does seem necessary to recall that pain is definitely not the result of the functioning of a single, highly isolated, individualized system. The pain system fits into a collection of subsystems—sensory, motor, vegetative, emotional, motivational—which by their very nature, the scientific, reductionist approach cannot study in their entirety. As a consequence, a result—whatever it might be—can be appreciated correctly only when it is viewed in this overall context. It is only by accepting this requirement that fundamental and clinical research can have a useful dialogue.

*Acknowledgments.* We thank Professor Arthur Duggan for critical reading of this manuscript and Professor Léon Plaghki for many fruitful discussions and suggestions during its preparation.

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