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Review

Infrared thermal imaging associated with pain in laboratory animals

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Abstract: The science of animal welfare has evolved over the years, and recent scientific advances have enhanced our comprehension of the neurological, physiological, and ethological mechanisms of diverse animal species. Currently, the study of the affective states (emotions) of nonhuman animals is attracting great scientific interest focused primarily on negative experiences such as pain, fear, and suffering, which animals experience in different stages of their lives or during scientific research. Studies underway today seek to establish methods of evaluation that can accurately measure pain and then develop effective treatments for it, because the techniques available up to now are not sufficiently precise. One innovative technology that has recently been incorporated into veterinary medicine for the specific purpose of studying pain in animals is called infrared thermography (IRT), a technique that works by detecting and measuring levels of thermal radiation at different points on the body's surface with high sensitivity. Changes in IRT images are associated mainly with blood perfusion, which is modulated by the mechanisms of vasodilatation and vasoconstriction. IRT is an efficient, noninvasive method for evaluating and controlling pain, two critical aspects of animal welfare in biomedical research. The aim of the present review is to compile and analyze studies of infrared thermographic changes associated with pain in laboratory research involving animals.
Key words: animals, animal welfare, pain, thermal images, vascular change

Introduction

The science of animal welfare has evolved over the years, and recent scientific advances have enhanced our comprehension of the neurological, physiological, and ethological mechanisms of diverse animal species, which are now addressed in research protocols involving animals [1–13]. Fundamental concerns in experimental research involving animals include striving to reduce stress and unnecessary suffering and minimizing the pain caused by scientific procedures [9, 10]. The protocols for managing postoperative pain in laboratory animals are still the cornerstone of approaches to animal welfare and clinical medicine, so diverse lines of research have

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explored the efficacy of analgesics. However, specific difficulties have arisen in the evaluation methods adopted that have impeded or inhibited achievement of the goals proposed in such projects. One factor that has contributed to poor results in studies of the efficacy of analgesia in translational medicine is the broad gap that exists between clinical and preclinical measurements of pain in humans [14, 15], a situation that has encouraged the use of animal models to support assessments of pain and effective treatment for it [4]. Rodents are the animals most often used to study the physiopathological mechanisms of pain [16]. Nevertheless, these conditions underscore the importance of one fundamental requirement of *in vivo* research, namely, to prevent, minimize, and/or relieve pain in laboratory animals [17]. This great scientific and bioethical interest in developing strategies to reduce the pain experienced by research animals has led to the implementation of an innovative technology called infrared thermography (IRT). This technique has been in use for several years, but recently a novel focus has emerged that seeks to use IRT to associate changes in vascular microcirculation with pain. The temperature readings obtained from different corporal regions utilizing this method are reliable and allow researchers to estimate core body temperatures (T*core*) based on surface recordings. Modifications of body temperature in diverse animal species can be associated with such physiological changes as increased metabolic activity brought on by exercise, morbid infectious processes, lesions, and even stress. Recent studies have identified the existence of a correlation between the degree of pain and increases in temperature variation [18]. The IRT technique makes it possible to identify both local (regional inflammatory processes) and systemic lesions indirectly by detecting temperature increases (septicemia) [18]. It has also been widely utilized in studies of animal and human health [19]. This technique works by detecting and measuring levels of thermal radiation at different points on the body's surface with high sensitivity [20, 21]. In the field of pain medicine, specifically, the identification and interpretation of changes in infrared thermographic images provides an efficient, noninvasive means of optimizing the evaluation and control of pain, two critical aspects of animal welfare in biomedical research. For these reasons, the present review takes on an interesting challenge: its analyzes how the physiological control of temperature is achieved, how vascular changes related to cold and heat are modulated under pathological conditions, the thermographic responses associated with the cicatrization process in surgical techniques, alterations of dermal surface temperature due to the effect of anesthesia, and finally, how the thermal responses detected by means of IRT can be related to the pain experienced by laboratory animals.

Infrared Thermography in Research

Variations in body temperature can provide valuable information for biomedical research involving animals. However, evaluations of temperature have often been performed using invasive methods that cause stress in animals [22]. Traditionally, T*core* has been recorded in animals by means of sensors implanted internally or by rectal devices. While such methods can be useful for obtaining readings over long intervals, they are invasive and, therefore, have the potential to affect the behavior and physiology of the animals by altering temperature measurements and/or requiring a surgical procedure before use [23]. In this sense, IRT provides an option that is noninvasive because it consists of detecting the intensity of infrared radiation that, as we know, correlates directly with the distribution of temperature in a defined region of the body [24–26]. IRT converts this infrared radiation into digital images that can be interpreted as a function of color and a numerical scale [18]. This method is increasingly being used to detect and monitor peripheral vascular disorders in laboratory animals [22] and small animal species [20, 27] that may well have important applications in biomedical research [28]. But, of course, analyzing and interpreting thermographic images requires understanding the fundamental concepts upon which thermoregulation operates in homeotherm species.

Physiological control of body temperature

The process of thermoregulation in mammals is orchestrated by the central nervous system (CNS) through a series of endocrine, autonomous, and behavioral mechanisms that actively balance heat generation and loss [29–31].

Physiological responses

The fundamental mechanisms of thermoregulation in mammals consist of thermogenesis of brown adipose tissue (that is, the production of heat through the catabolism of specialized fatty brown tissue), blood flow in the skin (the rate of heat exchange between the skin and the environment, which depends on blood flow at the level of the skin), trembling (rapid movements of skeletal muscles to generate heat), and heat loss through evaporation (a thermoregulating strategy that dissipates heat) [32–35]. Neural control of temperature in humans is mediated primarily by two classes of neurons that are activated by heat at temperatures of 32–42°C or by cold

at temperatures of 14–30°C, respectively. The cellular bodies of these neurons are located in the trigeminal ganglion (which innervates the head and face) and at the root of the dorsal ganglion (which innervates the rest of the body).

Codification of temperature at the level of the spinal cord is performed by neurons that are sensitive to heat or cold and form synapses with neurons in the dorsal mast, which projects its terminations towards the hypothalamus. This information is sent to the somatosensory cortex, while the information that reaches the hypothalamus is sent through the parabrachial complex. At the central level, the hypothalamic preoptic area (POA) is located between the anterior commissure and the optic chiasm, a thermosensitive area that regulates responses to temperature changes and controls thermal sensitivity in the brain. The POA neurons also receive peripheral information through ascending neuronal pathways [33, 35]. Studies have shown that the GABAergic neurons that predominate in the hypothalamus are related to inhibition processes [33, 35], while smaller amounts of glutamatergic neurons intervene in excitation processes [33, 36].

Control of temperature in laboratory mammals

Unlike humans, who lose heat primarily through sweating, other species utilize distinct strategies to achieve thermal homeostasis. In animals, areas without fur, like the feet, hands, and face, may specialize in discriminating and regulating temperature variations. Among domestic species it is well known that dogs regulate excess heat by panting and sweating in interdigital spaces, while cats do this by sweating in zones like their foot pads and interdigital spaces, as well as by licking their fur [37]. Some species have specialized thermoregulation systems that utilize such physiological mechanisms as the vasodilatation of surface blood vessels, for example, the ears of rabbits and the tails of rats. The tail and paws of rodents are heat-exchanging regions involved in maintaining the animal's T*core*. In part because of this, they are the organs most often targeted in studies based on "models" of acute or chronic pain (Figs. 1 and 2). Another heat-reducing strategy in rodents consists of spreading saliva over the fur [38]. The rat's tail and paws lack fur and have a high surface/volume ratio (the tail and paws represent approximately 7% and 10% of the rat's total body surface, respectively), and the tail is highly vascularized. These characteristics give the tail and paws a crucial role in thermoregulation, since heat dissipation in arteriovenous

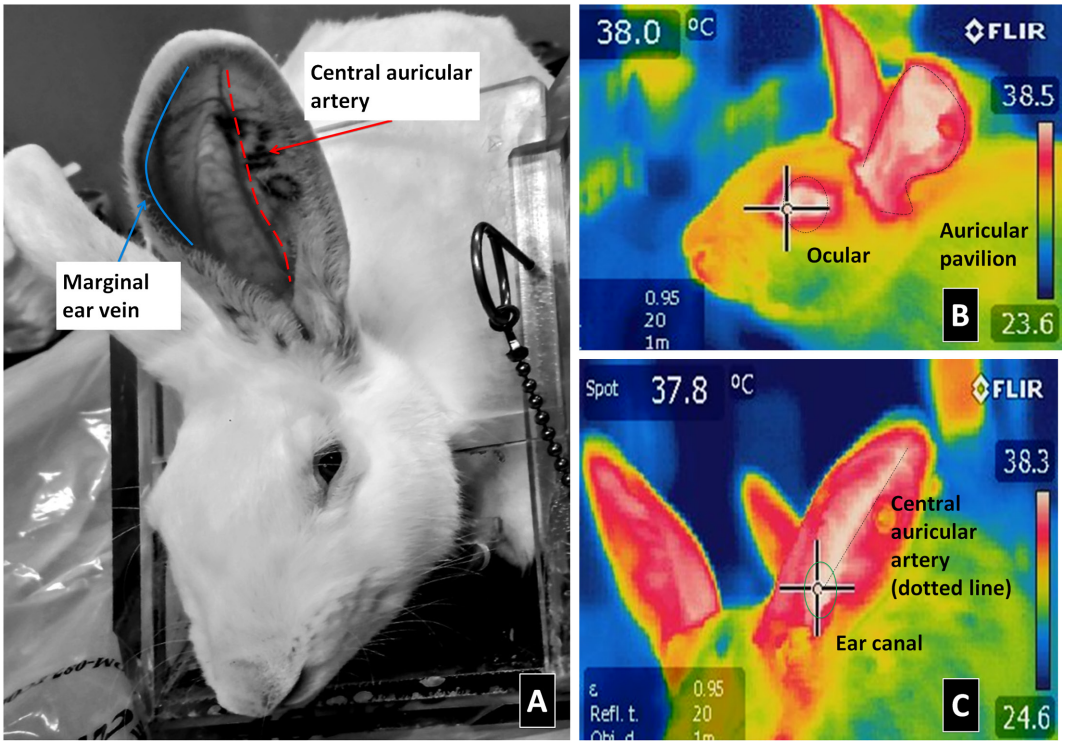

Fig. 1. A) Representative image of vasodilatation in the central artery (red) and marginal vein (lateral in blue) of a rabbit's ear (dotted line in red), one of this species' primary mechanisms for dissipating heat. B) Infrared thermographic image showing areas with temperatures above 38°C in the periocular and auricular regions. C) The left ear and, in white, the trajectory of the central auricular artery in longitudinal form from the base of the ear to the vertex. The auditory canal, marked in a green circle, indicates a temperature of 37.8°C.

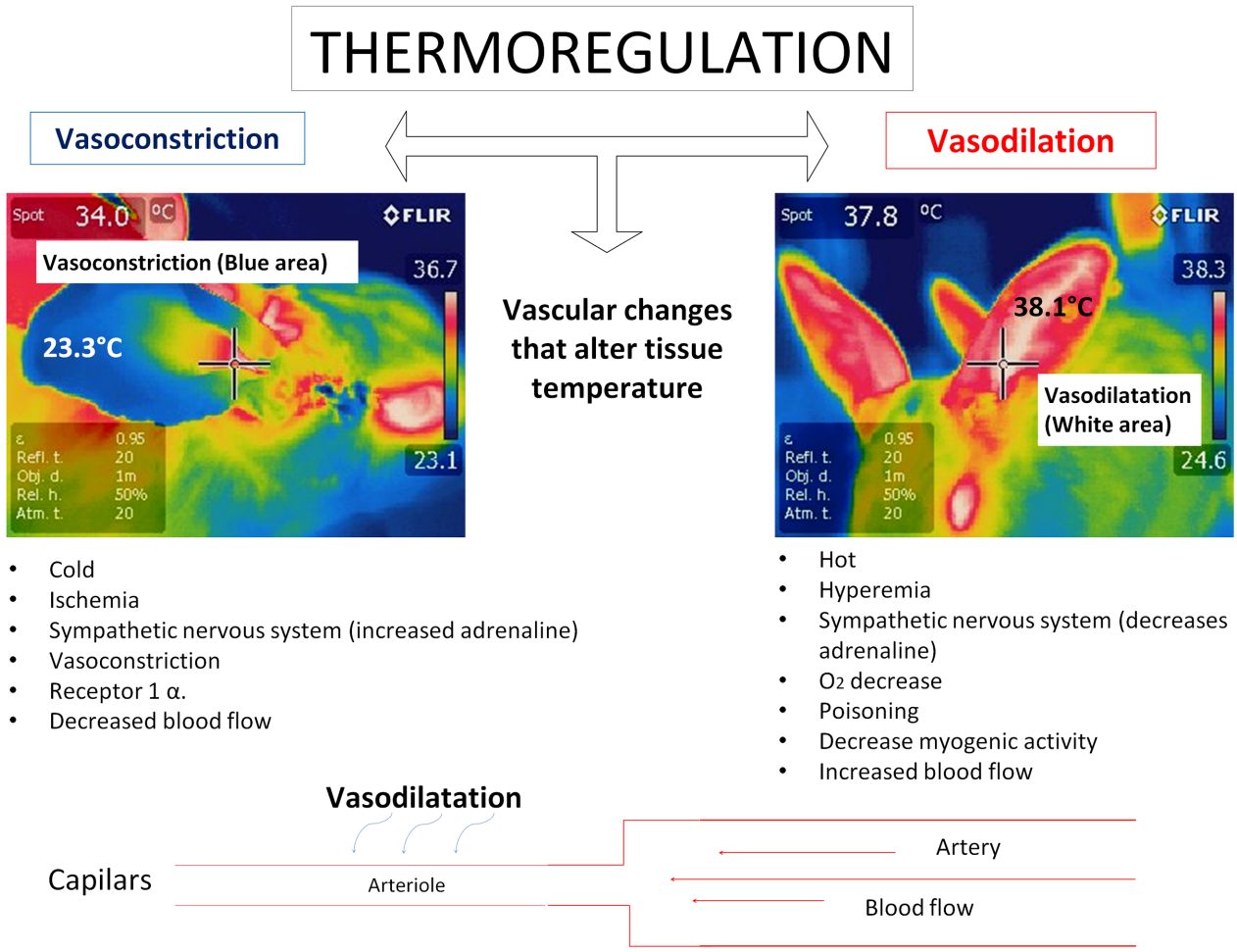

Fig. 2. External factors like significant changes in ambient temperature foster vasodilatation or vasoconstriction of blood vessels in the skin, mediated by afferent neurons of the noradrenergic type.

anastomosis systems is regulated by abrupt variations of blood flow [39]. In addition, the plantar surface of the rat's paws contains a high proportion of arterioles and venules, and observations have shown that vasodilatation is synchronized between the tail and paws during stress brought on by environmental heat [40]. These structures dissipate heat quickly because of their ample surface area. Moreover, studies by El Bitar *et al.* [39], have demonstrated that these thermoregulating mechanisms exert a critical effect on the responses triggered by thermal nociceptive tests in rats.

Vascular Changes Due to Heat or Cold: Normal and Pathological Conditions

Normal conditions

The skin is the thermoregulating organ *par excellence*, as the venous plexuses on its surface regulate the blood flow that controls and maintains body temperature. Two vasomotor mechanisms regulated by sympathetic vascular innervation explain this principle. Cutaneous vasodilatation reflects the activation of sympathetic nerves during hyperthermia that cause vasodilatation in the blood vessels of the skin. This permits increased blood flow at the periphery and, therefore, dissipates heat towards the environment. Cutaneous vasoconstriction, in contrast, results from temperature decreases through the skin and/or internally that trigger the reflex activation of the noradrenergic sympathetic nerves, resulting in vasoconstriction. This process decreases blood flow and, hence, maintains body temperature [33, 41]. To maintain their temperature, organisms activate systems that regulate cutaneous vasoconstriction, thermogenesis, and reductions of basal metabolism. When an organism needs to dissipate heat, it works to suppress thermogenesis and cutaneous vasodilatation and eliminate the excess heat through mechanisms that are species specific [33]. Self-regulation of blood flow is a physiological mechanism that is proportional to the demand for blood by tissues and/or organs, which depend on it for their physiological activity. This mechanism can be classified as either hyperactive or hypoactive.

Generally speaking, blood flow is regulated by oxygen (O_2) and carbon dioxide (CO_2) pressure, as well as by the concentration of cell residues. Upon receiving a specific signal, certain elements in the endothelial cells release distinct substances that are indispensable for regulating vascular tone, and therefore, blood flow towards tissues. Meanwhile, inactivated endothelial cells release vasoactive (vasodilator) substances such as nitrous oxide, prostacyclins, bradykinins, vasopressin, free catecholamines, and natriuretic peptides. Once activated, the endothelial cells produce vasoconstrictor substances like the endothelins, A2 thromboxane, angiotensin II, and free radicals [42]. It is important to note that any chemical substance (adrenalin, acetylcholine, histamine) that acts on receptors in the endothelium of blood vessels will alter their biomechanical properties by significantly modifying their functioning, including such actions as permeability and hemodynamics.

With respect to microvasculature, it is known that blood pressure decreases most as blood circulates between arteries and capillaries. Hence, the greatest resistance to blood flow takes place in the arterioles, while local blood flow is regulated at the arteriolar level [43]. Arteriolar microcirculation adapts to the requirements of individual organs or tissues. In metabolic diseases like diabetes and arterial hypertension, alterations in the microvasculature can be precursors of total organ damage. Minimal temperature changes are caused by oscillations of microcirculation and appear at the body's surface as variations in heat that can be captured by IRT on surfaces of 8–14 mm. Disorders of arteriolar function in systemic diseases that affect microcirculation (diabetes, hypertension, obesity, metabolic syndrome) have very similar patterns in different regions of the body and organs. Thus, microcirculation in the skin is a good referent for circulation in the principle organs [26].

Pathological conditions

In cases of traumatic brain damage, hyperthermia affects a high percentage of patients (70%) and, if prolonged, causes diverse neurological symptoms and lesions, including hemorrhages, edema, ischemia, encephalitis, and atrophic changes in various regions of the SNC, such as the encephalic trunk, cerebellum, hippocampus, and cerebral cortex. Under normal conditions, the blood-brain barrier (BBB) is highly selective and impedes the movement of large or hydrophilic molecules and toxic substances towards the brain. However, when body temperature rises, the permeability of the BBB increases significantly in a process that explains, in part, the development of cerebral edema in hyperthermic states.

Studies under laboratory conditions have demonstrated that rats with body temperatures above 38.5–39°C develop cerebral edema due to the increased permeability of the BBB [44]. When used to study tumors, IRT makes it possible to analyze blood flow through estimates based on temperature values. It appears that tumors have a greater volume of blood flow than healthy tissues but that the blood supply to tumors tends to be primitive and chaotic in nature, with some areas being deprived of nutrients and others having low oxygen concentrations. As a result, we know that some tumor cells are sensitive to the cytotoxic effects of heat and oxygen [45]. Thermography allows researchers to study the progression or involution of tumors. Studies have demonstrated that thermography can detect temperature changes as small as 0.1°C, so it is being used in surgical procedures to identify the margins of tumors for surgical resection [46].

Infrared Thermography in Relation to a Discomfort Condition

Infrared thermography consists of detection of the intensity of the infrared radiation in a defined corporal region and direct correlation of it with the temperature distribution in that region [26]. In clinical diagnostics, infrared images are utilized as a test to measure temperature changes possibly associated with contusions, fractures, burns, tumors, dermatological diseases, inflammatory processes, diabetes mellitus, and pathologies involving deep venous thrombosis, liver disease, and bacterial infections, among other ailments (Table 1). These conditions often appear in association with regional processes of vasodilatation, hyperthermia, hyperperfusion, hypermetabolism, hypervascularization, and hyperemia (that is, areas with large amounts of infrared emissions), all of which generate high temperatures in the tissues involved [28].

A related area of research has succeeded in estimating the activation of the sympathetic nervous system in bovines. Stewart *et al*. [57, 58] demonstrated the existence of temperature alterations in the tear caruncle region of the eye of bovine cattle in response to stressful or painful procedures (e.g., punctures, epinephrine administration, castration). In veterinary medicine, IRT has emerged as an effective, noninvasive tool for measuring stress in production animals of various species. Yañez-Pizaña *et al*. [21] described stress and temperature modifications in piglets associated with social disorders and environmental enrichment, while Herborn *et al*. [59] demonstrated temperature elevations related to stressful conditions in the handling of poultry. In similar work,

Table 1. Original publications on infrared thermography (IRT) associated with pain in laboratory animals

Laboratory species	Category	Contribution	Author(s)
Rat	Infrared thermography as a support tool for monitoring human health	Demonstrated the usefulness of thermography (even in anesthetized animals) and that related experiments (rat model of neuropathic pain) can contribute to our understanding of the role of alterations of skin temperature and sympathetic activity in the pathogenesis of neuropathic pain in humans.	[47]
Rat	Infrared thermography as a support tool for monitoring human health	Administration of SJHXT (a mixture of 17 herbal plants) in an arthritis model in rats (chronic pain). An increase in the temperature of the surface of the tail appeared to improve peripheral circulation. Increased locomotor activity is attributable to the elimination of pain.	[48]
Rat	Infrared thermography as a support tool for monitoring human health	Rats with transection of the tibial and sural nerve (TST) showed behaviors characteristic of neuropathic pain. Resistance to bilateral surgical sympathectomy was observed. Hence, the TST model can be a useful, easily reproducible model of sympathetically independent pain (SIP).	[49]
Mouse	Infrared thermography as a support tool for monitoring human health	Demonstrated that while a surgical incision does not cause hyperalgesia due to cold, it does cause inflammation and an increase in temperature. This suggests that distinct mechanisms are involved in surgical inflammatory pain.	[50]
Pig	Infrared thermography as a support tool for monitoring animal health (Validating the use of thermography)	Determined the emissivity of adult pig skin from the shoulder, the base of the ear, and the caudal part of the udder, as well as the effect of the villus on blood perfusion in emissivity.	[51]
Pig	Infrared thermography as a support tool for monitoring human health (Validating the use of thermography)	This study demonstrated the capacity of infrared thermography for monitoring the control of circulation and blood perfusion in a swine animal model (systemic inflammatory response syndrome [SIRS] or sepsis). Developed indices to quantify the course and severity of the disease.	[52]
Rat	Analgesic action	Modulation of the temperature of damaged tissue (model of a partial lesion of the Achilles tendon in rats). Offers evidence of the participation of LLLT (low-level laser therapy) in controlling these inflammatory agents, since the mediators are directly involved in fostering a temperature increase in the tissues at the site of the injury.	[53]
Mouse	Evaluating the welfare of rodents	The use of thermographic images can contribute to refining studies with animals, basically by monitoring the respiratory frequency and locomotor activity that contribute to the detection of stress or pain.	[54]
Pig	Evaluating the welfare of piglets	Shows the capacity of infrared thermography to precisely measure cardiorespiratory signals in anesthetized piglets, in which an increase in heart rate and respiratory frequency (RF) may be associated with pain, fear, anxiety, and panic.	[55]
Mouse	Analgesic management of pain Evaluating the welfare of rodents	Evaluated acute surgical pain in a mouse model of a spinal cord lesion (LSC) using the Mouse Grimace Scale infrared thermography, and administration of multimodal analgesia with buprenorphine (opioid + AINE) and carprofen *vs*. buprenorphine. The former was more effective.	[56]

Bartolomé *et al*. [60] analyzed these concepts in goats, while De Lima *et al*. [61] did so with rabbits in habitats marked by high temperatures or cattle in habitats associated with tissue damage [62]. Luzi *et al*. [63] showed that control of the conditioners of stress and use of IRT are valuable in terms of ensuring the welfare of laboratory animals. In this regard, IRT was used as an indicator of tissue damage and discomfort in a comparative study of the extension and duration of inflammation observed in production animals after two branding procedures, one with a hot branding iron and one based on freezing. Both methods caused tissue damage, but the hot branding sites remained significantly warmer than the freezing sites one week after branding. The inflammatory response, tissue damage, and discomfort or pain were more prolonged in the animals branded with the hot tool [64].

Stubsjøen *et al*. [65] detected a temperature reduction in the eyes of sheep subjected to moderate levels of pain (tourniquet and application of medications), as well as thermal changes in such behaviors as licking the lips, increased vocalizations, and forward movements of the ears. In that study, heart rate proved to be more sensitive than IRT. Schaefer *et al*. [66] determined significant

infrared thermal changes in the temperature of the eyes of calves with viral bovine diarrhea, even on days prior to the onset of the clinical signs associated with this infection. In a mouse model of spinal cord injury, Redaelli *et al*. [56] measured facial expressions associated with pain using infrared thermography to verify that drugs provided enhanced analgesic effects. They further demonstrated a possible relation between pain and temperature variations. Unlike thermographic measurements of the tail, BAT (brown adipose tissue) provided the most consistent measurements for describing the thermoregulating response associated with surgery and pharmacological analgesic treatments.

Thermographic Responses Associated with the Cicatrization Process/Wounds in Surgical Techniques

In dogs and cats, analyses of foot pads and footprints by thermographic methods could be used to evaluate weight burden, symmetry, and displacement, before or after a surgical treatment [37]. Postoperative monitoring with IRT is important because it permits analysis of the vasculature of flaps to corroborate the surgical technique at that moment, as well as follow-up on angiogenesis processes through a sequence of thermal images [67]. Ferreira *et al*. [68] evaluated the second intention cicatrization process in calves by recording thermographic images from the moment a lesion was generated to day 21 post-injury. However, they did not find conclusive evidence that would have allowed them to differentiate between the first and second intention cicatrix.

Całkosiński *et al*. [20] administered carrageenan (a pro-inflammatory substance) to the right side of the lower lip and left and right paws of rats. They detected diverse changes, including increases and decreases in regional temperatures. Their findings are related to sympathetic activation, hemodynamic changes, carbon dioxide concentrations (CO_2), and the synthesis of pro-inflammatory chemical mediators like histamine, the quinines, and prostaglandins. They concluded that infrared thermography can be employed as an objective, noninvasive quantitative tool for determining the dynamics of inflammatory processes. Their results led them to propose reducing the number of animals used in this type of research.

Alterations of Dermal Surface Temperature Due to the Effect of Anesthesia

Under normal conditions, the T*core* of homeothermic animals is relatively constant (normothermia) despite the continuous production of metabolic heat and variations in ambient temperature. T*core* depends on the factors that balance the production and loss of heat (endothermic organisms) [69, 70]. In relation to temperature, we can divide organisms into two compartments, one core (central) compartment that produces heat and one peripheral compartment regulates heat loss. These regulatory mechanisms exist to protect the core compartment at the expense of the peripheral one (surface skin temperature) [71]. Again, under normal conditions, body heat is generated by the basal metabolic rate of such internal organs as the brain and those in the thoracic/abdominal cavity. Blood is heated as it passes through those organs before being distributed by convection through the cardiovascular system from the core region to the cutaneous area [31, 71]. It is important to understand that body heat is not distributed uniformly throughout an organism. For example, T*core* is often several degrees (2–4°C) higher than skin temperature (Fig. 3).

As the principle regulator of T*core*, the preoptic hypothalamic area–where the temperature-sensitive neurons are located–receives and integrates afference from the ascending neuronal pathways at the periphery and is responsible for triggering the diverse mechanisms that maintain thermal equilibrium. It is important to point out that although this area performs a very similar role in almost all mammals, the specific means through which it achieves normothermia vary from one species to another [29].

The body temperature of a patient under anesthesia is critical because anesthetic drugs can alter the range in which compensatory responses to body temperature occur, due to the environmental alteration applied [72]. During anesthesia, deviations from normothermia are common, with hypothermia being the predominant thermal disorder and hyperthermia occurring less frequently [69]. Bayter-Marían *et al*. [71] pointed out that both general and regional anesthesia equally deteriorate the mechanisms that protect against hypothermia (Fig. 4). General anesthesia involves administering drugs that inhibit the first defense mechanism of thermoregulation, leaving the autonomous defense system to compensate for environmental alterations [69]. Inhaled anesthetics, however, deteriorate the autonomic responses that react to hypothermia under exposure to hypothermic conditions during surgery.

General anesthesia lowers metabolism and the production of energy and heat, so the normal regulatory mechanisms are not activated until the heat loss reaches 2–3°C. Vasoconstriction begins as the first compensating mechanism against the fall in temperature (first phase). Both epidural and spinal anesthesia cause hypothermia

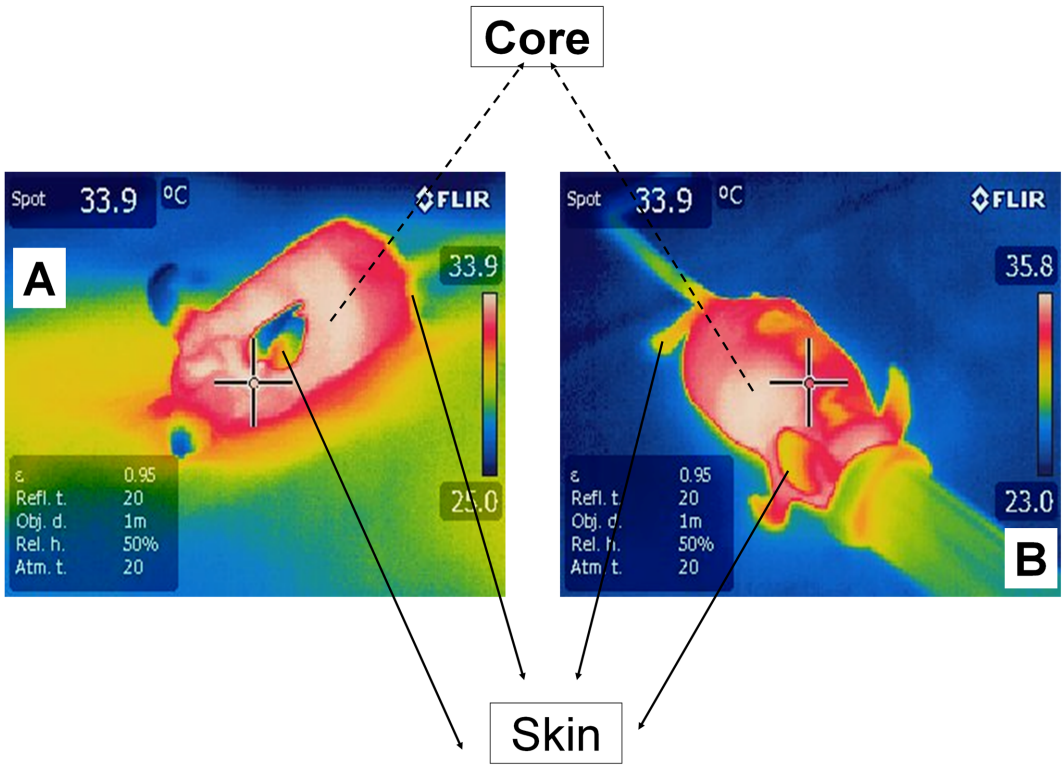

Fig. 3. Nude mouse thermogram. A) Different temperature gradients between the skin surface and core (i.e., the T*core* visible in the white zones in the range of 34–36°C) of the mammal's body. B) Anesthetics that cause indiscriminate vasodilatation result in a mixing of core and peripheral blood that reduces the T*core*.

by redistributing core heat towards peripheral tissues. In the second phase, a reduction of basal metabolism occurs and triggers a linear temperature decrease. In the third phase, vasoconstriction is activated once again with the closing of the shunts of the hands and feet that leads to a reduction in heat loss, but with no reheating of the body. Under regional anesthesia, the third phase is critical because the compensating mechanisms are not reactivated with the consequent decrease in temperature. This reaction may increase in severity depending on the precise dermatomes that are blocked (sympathetic block). This means that blood perfusion may deteriorate due to the blood's viscosity caused by hypothermia [73].

Scope of Evaluation of Infrared Thermography in Laboratory Animals

Research with animals has made it possible to obtaine broad knowledge in both basic and clinical science in human and veterinary medicine by making fundamental discoveries during intense searches for the causes and treatment of diseases, search that have confronted numerous challenges, one of which is "pain." On the one hand, pain must be recognized from its early or subtle signs; on the other, options for suppressing or relieving it must be explored [74, 75]. Pain is likely the most common symptom seen in clinical practice, so ongoing treatment to relieve it must be the cornerstone of clinical medicine [9, 10]. Our ability to correctly characterize pain is fundamental to both diagnostics and treatment choice [76]. In experimental research with animals, body temperature is used not only to evaluate physiological functions but also as a parameter that helps determine whether an animal involved in a research process experiences periods of excessive stress or suffers pain.

In surgical practice, follow-up on the processes of thermoregulation of patients by means of IRT should be an obligatory practice, since recording their physiological and metabolic parameters can enable response to the events that occur during recovery. In addition, achieving more precise control of physiological constants and other parameters related to temperature, blood flow, and vasomotor aspects, to name a few, would support the goal of reducing the number of animals used in research protocols and, therefore, the concept of the 3Rs.

Today, in fact, IRT is no longer only a support method because its objectivity is garnering greater importance in diagnosing disease, identifying signs of, for example, diabetes and inflammatory processes, and validating surgical techniques. In the near future, researchers in the field of biotechnology will develop more sophisticated IRT equipment and cameras equipped with specialized

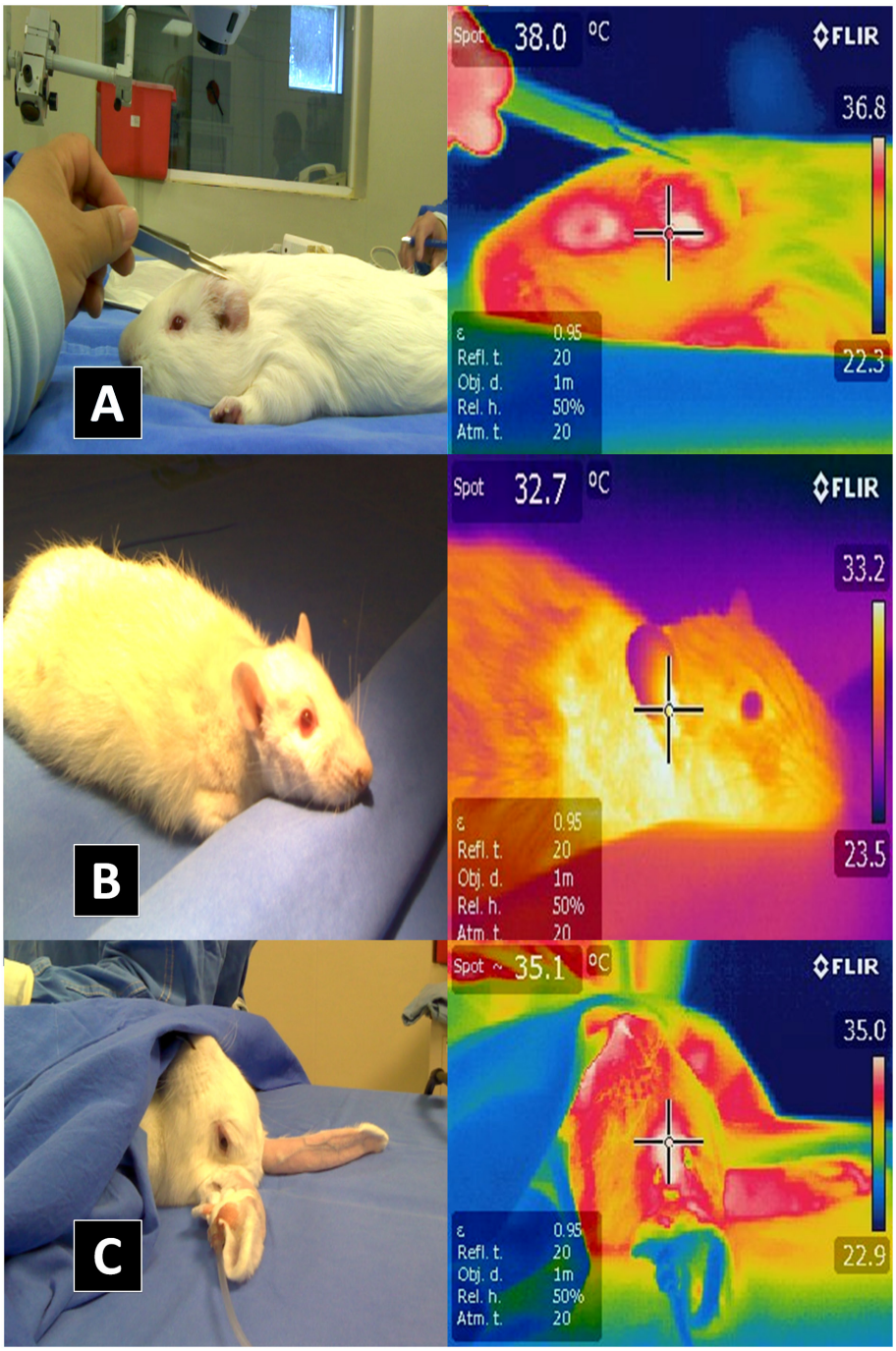

Fig. 4. The use of thermograms under anesthesia during a surgical process allowed adequate monitoring of the condition of laboratory animals and verification of the correct anesthesia. A) Auditory canal of the guinea pig as a thermal window for thermographic evaluation. B) The auditory canal of the rat offers an excellent thermal window for thermographic measurement. C) The tear caruncle and auditory canal of rabbits can be used as thermal windows for thermographic measurement.

software that will allow researchers to conduct more detailed analyses of the images obtained. The results generated will be extremely valuable and interesting for science.

Noninvasiveness, real-time operation and emissivity (of the body) are three of the key features that allow infrared thermography to be used with all bodies that emit caloric radiation, from healthy subjects to those who are described as having a pathology. Another advantage of thermography is that it can be utilized for subjects that are under anesthesia, patients who are in critical condition, and even cases that are difficult to access and for which monitoring is difficult.

Conclusions

There are two critical themes in the use of laboratory animals. On the one hand, scientists need to experiment with animals; on the other is the issue of animal welfare, which has become highly controversial due to increasing social and political pressure. These circumstances have generated a situation in which recognizing and relieving pain in animals are crucial aspects for medicine, ethics, and animal welfare. As stated above, pain is probably the symptom most often seen in clinical practice, so developing and evaluating systematic methods, and employing reliable, valid measuring tools that allow researchers and veterinarians to identify and quantify pain, are now fundamental objectives of science. This is the area where IRT–an objective noninvasive method for measuring pain in laboratory animals–will aid in achieving early recognition of pain, while the variations in body temperature so detected will also be able to provide valuable information for biomedical research involving animals, guide researchers in selecting adequate pharmacological treatments, and support the refinement of *in vivo* and translational research.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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