

Relation between Infrared Thermometry and Thermal Imaging for Assessment of Rodents' Body Temperature: A Way to Improve Animal Welfare

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ABSTRACT

Animals have been used for biomedical research since ancient times. Humane endpoints have been increasingly implemented during experimental protocols to prevent or alleviate animals' suffering. Body temperature is an indicator of stress or anxiety. Infrared thermometer (IRT) and infrared thermal imaging camera (TIC) are commercially available for temperature measurement. This study aimed to determine mice and rats' body temperature using IRT and TIC, and to establish a mathematical correlation between both approaches, to understand if it is possible to infer TIC readings from IRT readings in animals' back and tail. Thirty Wistar rats (15 females and 15 males) and thirty FVB/n mice (15 females and 15 males) were used. The body temperature of each animal was measured for ten consecutive days using IRT (UT300) and TIC (FLIR E8, Model: E6390). The Root Mean Square metric was applied to obtain a measure of the degree of fitness. The model fit was validated using several metrics, with the Root Mean Square Error metric. TIC readings were 1.0 to 1.5°C higher when compared with IRT readings ($P < 0.0001$). The back temperature registered using IRT was higher in male rats when compared with female rats ($P < 0.0001$). In mice, the tail temperature measured using IRT was slightly higher in females when compared with males ($P < 0.0001$). We found that it is possible to fit, by linear regression, a straight line to the data, to obtain a linear transformation that allows inferring the TIC readings from the IRT readings, obviating the use of TIC readings.

Key words: Mice, Rats, Refinement, Temperature, Welfare.

INTRODUCTION

Animal models have been used for biomedical research since ancient Greece (Ericsson et al. 2013). The use of animals for scientific purposes led to numerous ethical concerns related to the suffering and stress caused to animals (Francois et al. 2022). This concern was the trigger to the English Biologists Russel and Burch to implement the 3Rs principle (Replacement, Reduction, and Refinement), in 1959, to guide animals' research (Tannenbaum and Bennett 2015). The monitoring of

Humane endpoints has been increasingly implemented during experimental protocols with animal models to prevent or alleviate animals' suffering, with a positive impact on the quality of the results. Body temperature is a physiological parameter that has been used for this purpose and can be considered an indicator of stress or anxiety through the hyperthermic response to stress (Gjendal et al. 2018; Van der Vinne et al. 2020).

An ideal temperature measurement technique must fit some points, including safety, easy execution, efficiency (in terms of time), accuracy (in the measurement of

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central body temperature) and not be influenced by external factors (Chung et al. 2010; Muhammed et al. 2019). The body temperature of mice and rats can be determined by invasive or non-invasive methodologies, each of them with advantages and disadvantages. Usually, the temperature is monitored in these animals using invasive techniques, such as rectal, tympanic, or esophageal catheterization, bladder or pulmonary artery catheterization, telemetry systems (radio transmitters and data loggers), and thermosensitive systems (implanted transponders and external receivers) (Dangarembizi et al. 2017; Fiebig et al. 2018; Mei et al. 2018). Considering that these methods require animals' handling, anesthesia, and/or surgery, and none of them is sufficiently accurate for routine use or continuous monitoring of body temperature, it is beneficial to seek and use non-invasive and more effective techniques (Fiebig et al. 2018).

Infrared thermometer (IRT) and infrared thermal imaging camera (TIC) are commercially available for temperature measurement in animals (Sellier et al. 2014), with advantages over traditional methods, including the speed with which the measurement is performed, not causing discomfort or stress to the animals (non-invasive method) and decrease in the risk of infections (without contact with the animals) (Soerensen and Pedersen. 2015; Muhammed et al. 2019). However, this equipment has very different acquisition and maintenance costs. The tympanic membrane, back, sternum, abdominal, and anogenital regions are the most common sites to measure surface temperature in rodents (Mei et al. 2018).

This study aimed to determine mice and rats' body temperature using two different devices (IRT and infrared TIC) and to establish a mathematical correlation between both approaches, in an attempt to understand if it is possible to infer TIC readings from IRT readings in animals' back and tail.

MATERIALS AND METHODS

Ethics Statements

All the experiments carried out were approved by the University of Trás-os-Montes and Alto Douro Ethics Committee (ORBEA - *Órgão Responsável pelo Bem-Estar e Ética Animal*) and the the Portuguese competent

authority (DGAV - *Direção Geral de Alimentação e Veterinária*; approvals no. 014139 and 021326). All biosafety standards specified for the use of animals for scientific purposes were followed (European Directive 2010/63/EU and National Decree-Law No. 113/2013).

Animals and Housing

Sixty animals were included in the study: 30 Wistar rats (15 each of females and males), obtained from Charles River, with 12 weeks of age, and 30 FVB/n mice (15 each of females and males), obtained from a colony housed in the Animals' House of University of Trás-os-Montes and Alto Douro, with 12 weeks of age. The animals were kept under controlled conditions of temperature ($20\pm 2^\circ\text{C}$), relative humidity ($50\pm 10\%$), and light/dark cycle (12h light/12h dark).

The animals were housed in properly identified polycarbonate cages, with smooth surfaces and rounded corners. Rats were housed in Tecniplast® 1500UEurostandard Type II 1264C cages (Tecniplast, Buguggiate, Italy), while mice were housed in Tecniplast® 1500UEurostandard Type IV 1500U (Tecniplast, Buguggiate, Italy). Each cage contained five animals. Corncob was used as bedding (Ultragene, Santa Comba Dão, Portugal) and changed weekly. Polyvinyl chloride tubes and cardboard rolls were used to enrich the animals' environment. All animals had *ad libitum* access to tap water and a standard laboratory diet (Diet Standard 4RF21®, Mucedola, Italy) throughout the experiment.

Equipment

Two different types of equipment were used for temperature measurement: IRT (UT300) and TIC (FLIR E8, Model: E6390). The IRT had a temperature range from -32°C to 400°C , with a thermal sensitivity of 0.1°C . The TIC had a 320×240 pixels resolution, with a temperature range from -20°C to 250°C (recommended temperature 30 to 45°C) and thermal sensitivity $<0.06^\circ\text{C}$, according to the manufacturer. The main characteristics of the two equipment are summarized in Table 1.

Body Temperature Measurements

After two weeks of acclimatization, rats and mice were randomly assigned to the cages. Body temperature of

Table 1: Main characteristics of IRT and infrared TIC equipment (Sellier et al. 2014)

Characteristics	Infrared Thermometer (IRT)	Thermal Infrared Camera (TIC)
Temperature range	-32 to 400°C	-20 to 250°C
Thermal sensistivity	0.1°C	$<0.06^\circ\text{C}$
Response time	1 ms to 250 ms	
Calibration	Need to be calibrated with an object of known temperature or combined with a contact thermometer (when emissivity is unknown).	
Price	~100€	~1000€
Precautions to be taken to use correctly the sensor	Ratio distance/surface is mentioned for all-optical (technical datasheets) and must be respected (optical resolution). Angle: for a surface with an emissivity of 0.98, the associated temperature error is independent of viewing angle up to 30° but increased from 0.5 to 3°C at $30-70^\circ$ and is greater than 4°C at angle above 70° (McCafferty, 2007). Emissivity must be adjusted. Bare skin has an emissivity of 0.98 and the emissivity of dry fur is relatively uniform in mammals, in the range 0.98–1.0. The emissivity of the coat can also be changed by dirt or other materials (e.g., soil=0.93–0.96 or water=0.96) (McCafferty 2007). Wavelength: must be chosen according to the object of interest. Possible choice of the most suitable lens: wide-angle or telephoto lens.	
Environmental constraints	Influenced by ambient radiation, dust, local temperature and humidity, any obstacle on the path of radiation (ex: glass, window untreated).	

each animal was measured in the back and tail for 10 consecutive days (Fig. 1), using IRT and TIC. Both devices were placed 50 cm above the animal, being the minimum focus distance for TIC according to the manufacturer's instructions. The measurement using the IRT was performed by pointing the device at the desired measurement location (back and tail) and the temperature was recorded. For measurement with TIC, an emissivity correction value was set to 0.98 and the camera sensor unit was warmed up by turning on the camera for 30 minutes before taking the readings. Before beginning the temperature measurements, all animals were adapted one week, each day for five minutes, to a non-brilliant, black-lined cage. For the measurements, the animals were placed in a non-brilliant, black-lined cage (so as not to suffer sudden temperature changes) and the temperatures were recorded only when the animals remained immobilized (Fig. 2). Photographs were taken and saved through the TIC and were later analyzed using FLIR Tools® ThermaCAM Researcher Professional 2.10 software. Temperature readings were acquired always at the same spot. Animals handling was performed to minimize stress and discomfort, and both IRT and TIC measurements were performed by the same researcher. All temperature readings were acquired as quickly as possible to avoid any temperature increase arising from stress responses.

Mathematical Model

Before proceeding to the use of linear regression, an exploratory analysis of the data was performed, through scatter diagrams and through boxplots. Using the Matlab software it was possible to obtain fitting models to the data, through linear regression, in order to obtain formulas that allow inferring TIC readings from IRT readings. The Root Mean Square metric was applied to obtain a measure of the degree of fit. The model fit was validated using several metrics, with the Root Mean Square Error (RMSE) metric, i.e., the square root of the root mean square error, being the preferred metric.

Statistical Analysis

Data from IRT and TIC measurements were analyzed using Statistical Package for the Social Sciences (SPSS

version 27 (IBM, Armonk, NY, USA). Temperature readings were compared between males and females and between IRT and TIC using Student's t-test. Data are expressed as mean±SE. Differences with P-values lower than 0.05 were considered statistically significant.

RESULTS

Body Temperature Measurement Using IRT and TIC

Looking at IRT and TIC readings recorded for 30 rats and 30 mice at two body sites (back and tail), it was possible to see that the TIC readings in both sites were 1.0 to 1.5°C higher when compared with IRT readings (P<0.0001) (Tables 2 and 3).

Some statistically significant differences were observed in the temperature between males and females. The back temperature registered using IRT was higher in male rats when compared with female rats (P<0.0001, Table 2). In mice, the tail temperature measured using IRT was slightly higher in females when compared with males (P<0.0001, Table 3).

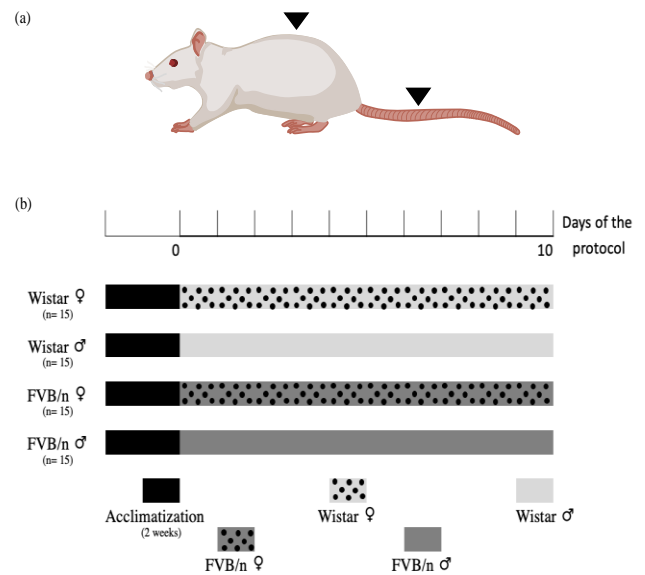


Fig. 1: a) Temperature measurement on the animal's back and tail, and b) Experimental protocol.



Fig. 2: Temperature measurement on the animal's back using infrared A) IRT and B) TIC.

Table 2: Back and tail temperature (°C) measured in female and male rats, using infrared thermometer and infrared thermal imaging camera

Animals	Back		Tail	
	IRT	TIC	IRT	TIC
Male (n=15)	27.40±0.04 a,b	28.29±0.06	22.76±0.05 b	24.47±0.06
Female (n=15)	27.04±0.06 b	28.23±0.05	22.72±0.06 b	24.38±0.07
All Animals (n=30)	27.22±0.05 b	28.26±0.04	22.74±0.04 b	24.43±0.05

a and b with values (mean±SE) indicate statistically different from females and TIC measurement ($P < 0.001$), respectively.

Table 3: Back and tail temperature (°C) measured in female and male mice, using infrared thermometer and infrared thermal imaging camera

Animals	Back		Tail	
	IRT	TIC	IRT	TIC
Male (n=15)	24.70±0.09 b	27.04±0.21	21.06±0.05a,b	23.04±0.09
Female (n=15)	24.81±0.06 b	27.33±0.05	21.44±0.07b	22.89±0.10
All Animals (n=30)	24.75±0.05 b	27.19±0.11	21.25±0.05b	22.96±0.07

Values (mean±SE) bearing alphabets indicate statistical differences from females and TIC measurements ($P < 0.001$), respectively.

Model Fit

In Fig. 3 it is possible to see the dispersion of the temperatures determined by IRT and TIC in rats and mice' backs and tails. Looking at the temperature dispersion in males and females, and in all animals (males and females together), we found that it is possible to fit, by linear regression, a straight line to the data, to obtain a linear transformation that allows inferring the TIC readings from the IRT readings.

The process of linear transformation is represented in Fig. 4. In the initial phase of the experiment, calibration was carried out using the IRT and TIC. With the recorded data, it was possible to use parameter estimation algorithms to fit mathematical functions to the data. In this case, a simple straight line, in the sense of least squares. Once the daily calibration/calculation time had elapsed, it could be used exclusively in the IRT, and the estimated parameters were used to correct the readings through a linear transformation (Fig. 5 and 6). Thus, to convert the temperature determinations made by IRT to TIC we can use the formulas shown in Table 4.

DISCUSSION

Animal welfare is still a great concern in animal research and humane endpoints have been increasingly implemented during experimental protocols to prevent or alleviate animals' suffering. Temperature is among the parameters evaluated to assess animals' welfare, being an indicator of stress or anxiety (Chung et al. 2010; Mei et al. 2018; Muhammed et al. 2019). The implementation of non-invasive techniques is fundamental in reducing animals' stress and, considering the costs of the equipment and their maintenance, the validation of cheap equipment is crucial.

The body temperature is regulated by the hypothalamus and the core temperature, and the temperature of the organs placed deep within the body (close to the main organs, heart, brain, and viscera) is considered the true standard to represent the basic state of the animal at rest or as a sign of disease in the event of an abnormality (Zeng et al. 2022). The surface temperature results from a balance established between environmental and physiological conditions (Chung et al. 2010; Sellier et

al. 2014). Temperature can be monitored by invasive or non-invasive approaches. The insertion of a temperature probe into the rectum is the most commonly used method to measure temperature in rats, once it involves the use of inexpensive and easy-to-handle equipment with reliable results (Chung et al. 2010; Mei et al. 2018; Muhammed et al. 2019). The core temperature should be measured by vaginal, rectal, vascular, tympanic, intra-peritoneal, or digestive tract sensors (Chung et al. 2010; Sellier et al. 2014). When measured rectally, accuracy and repeatability can be affected by distance from the upper chest, muscle strain, and the presence of feces. The tympanic membrane is vascularized by the internal carotid arteries that infuse the hypothalamus and, therefore, a good alternative to the traditional invasive method (Kunkle et al. 2004). Besides causing animal suffering, the probe insertion can lead to the body temperature increase due to the stress, providing wrong information about the animals' physiological state. The repeated insertion of these probes can also cause problems in animals' health, among which the mucosa rupture leading to septicemia and death stands out (Saegusa and Tabata 2003; Kunkle et al. 2004; Dangarembizi et al. 2017; Mei et al. 2018; Franco et al. 2019; Van der Vinne et al. 2020). This method of temperature measuring is considered time-consuming, invasive, and with the possibility of transmitting microorganisms among animals (Muhammed et al. 2019).

The non-contact and non-invasive methods, like IRT and TIC, have been sought and used with advantages over traditional methods (Shimatani et al. 2021). IRT was originally developed for human use, where it used pyroelectric sensors to detect the heat emanating from the tympanic membrane and the internal surface of the external auditory canal. It is a cheap, non-invasive, and fast measurement method (Saegusa and Tabata 2003; Kunkle et al. 2004; Chung et al. 2010). This equipment uses only one point that measures the average body temperature, in contrast to the TIC. The sensors are made of a lens that focuses on infrared thermal radiation and makes its conversion into an electrical signal that is displayed in temperature units (Sellier et al. 2014; Soerensen and Pedersen et al. 2015).

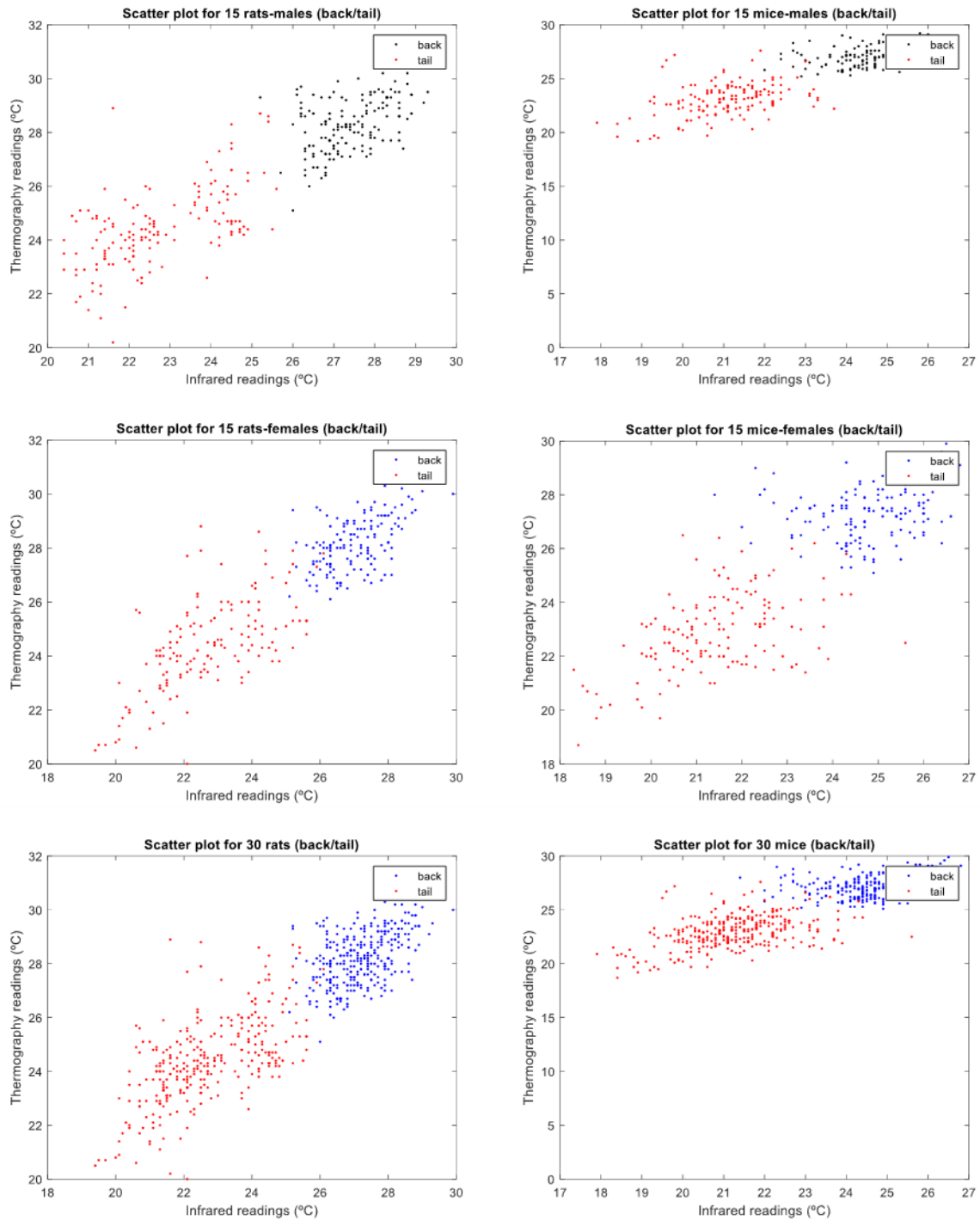


Fig. 3: Dispersion of temperature measured on the animal's back and tail, using infrared thermometer or infrared thermal imaging camera. Data for rats (on the left) and data for mice (on the right).

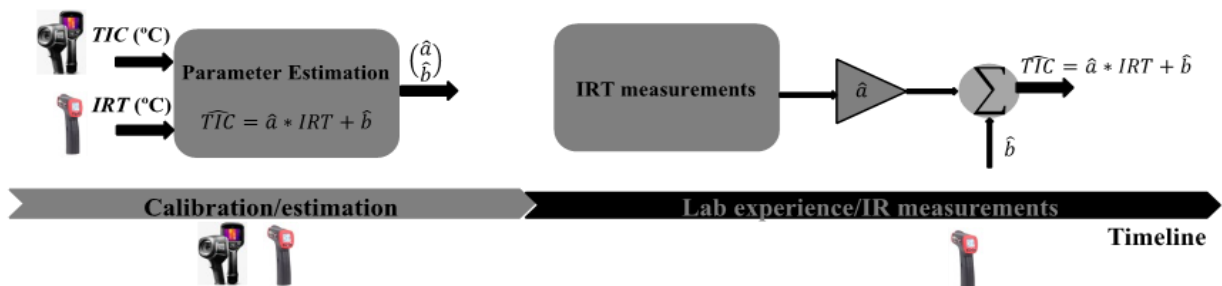


Fig. 4: Procedure to infer infrared thermal imaging camera readings from infrared thermometer readings, through linear transformation and using estimated parameters (least squares).

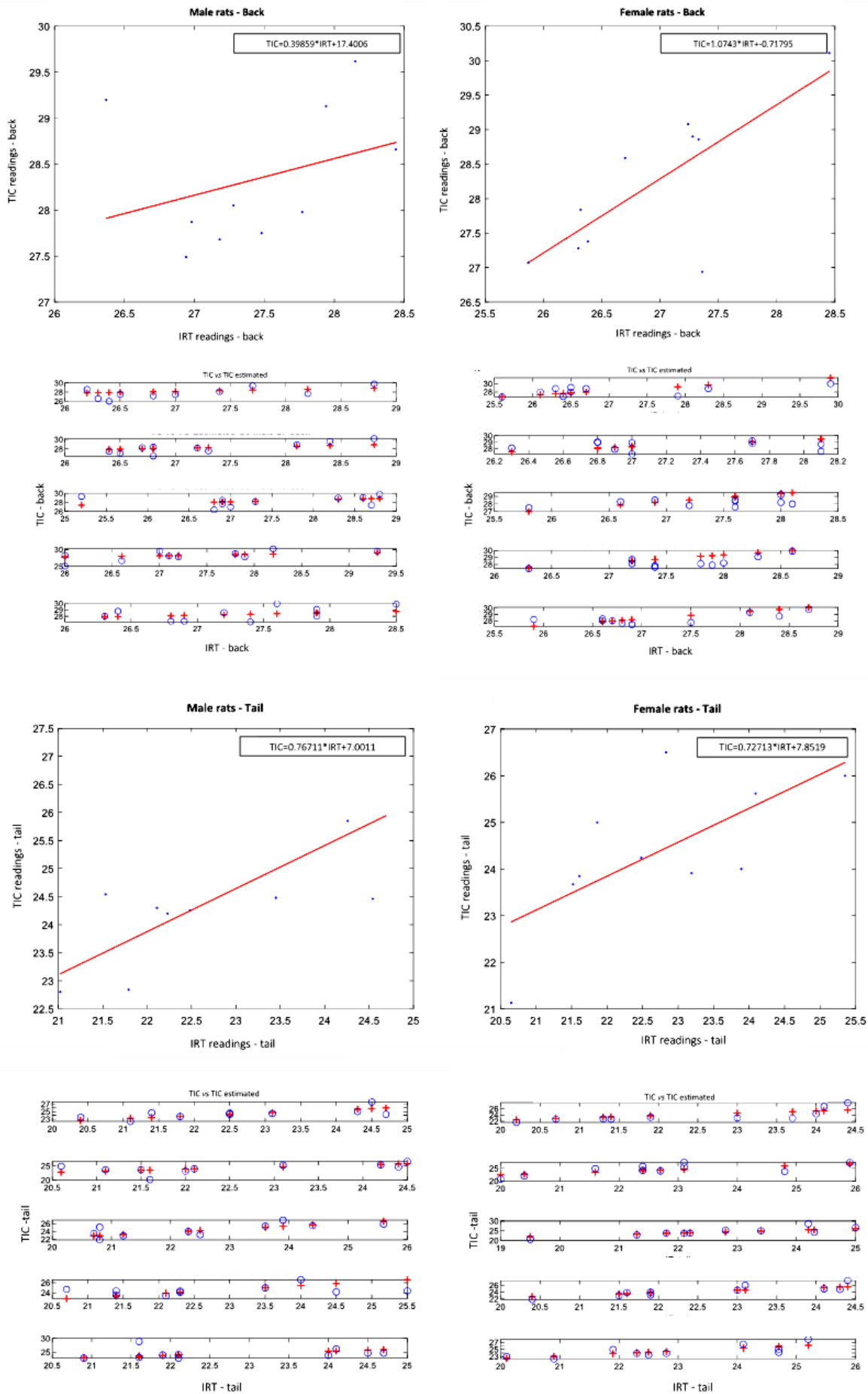


Fig. 5: Inference of infrared thermal imaging camera readings from infrared thermometer on male and female rats' back and tail through linear transformation and using estimated parameters.

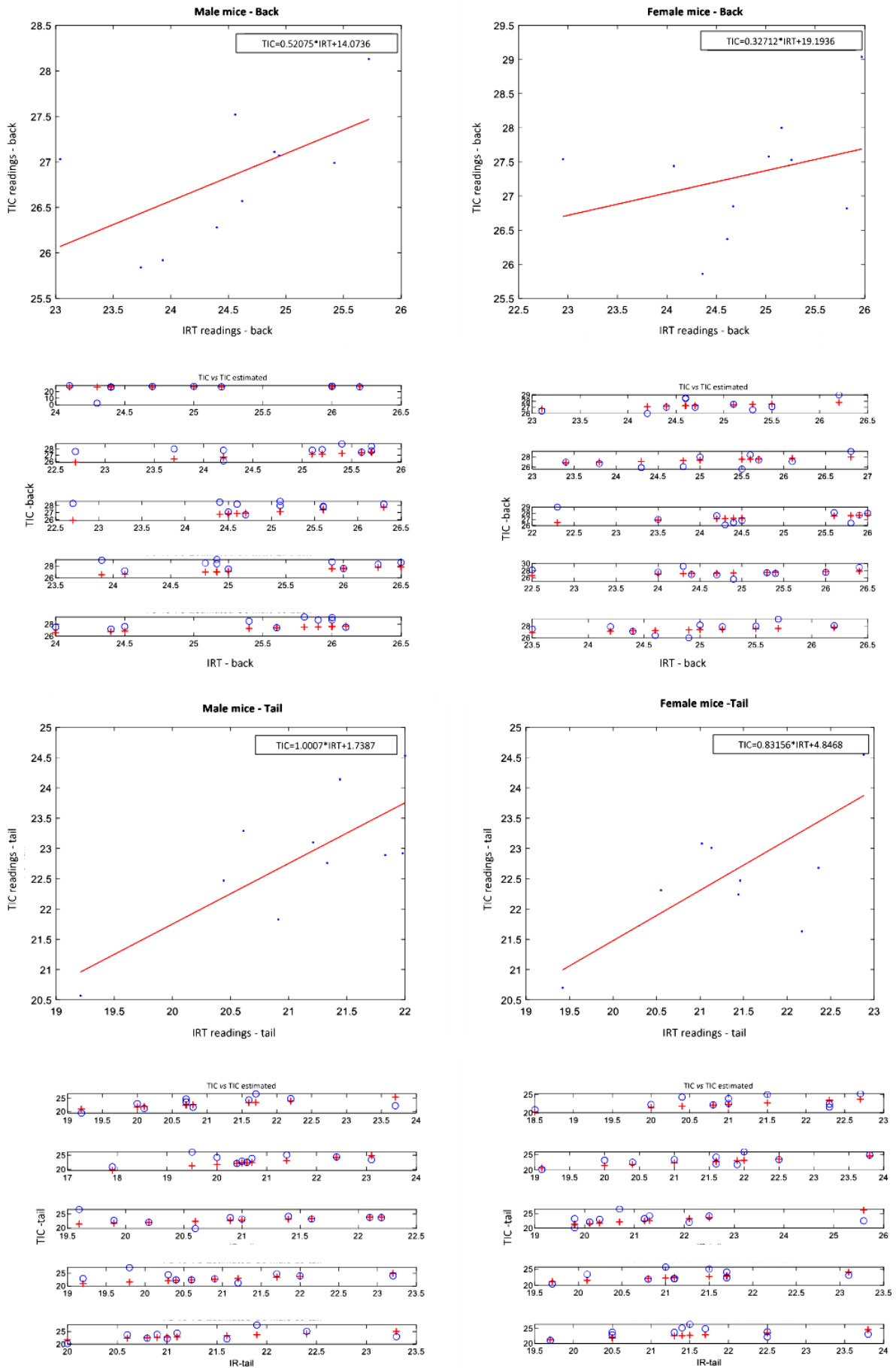


Fig. 6: Inference of infrared thermal imaging camera readings from infrared thermometer on male and female mice' back and tail through linear transformation and using estimated parameters.

Table 4: Formula to convert infrared thermometer into infrared thermal imaging camera

Animals	Rat		Mouse	
	Back	Tail	Back	Tail
Male	$TIC=0.399*IRT+17.400$	$TIC=0.767*IRT+7.001$	$TIC=0.520*IRT+14.073$	$TIC=0.371*IRT+19.194$
Female	$TIC=1.074*IRT+0.717$	$TIC=0.727*IRT+7.852$	$TIC=0.100*IRT+1.738$	$TIC=0.831*IRT+4.846$

The use of TIC allows the detection of infrared radiation emitted by the body and the data obtained are processed and presented in the form of temperature maps (thermograms), providing a detailed and precise analysis (Sellier et al. 2014; Wójcik et al. 2022). This allows the measurement of the temperature distribution over the surface, allowing the selection of specific areas of interest in thermal images, through points, lines, or areas in different ways, for data analysis. It is considered a modern and non-invasive approach. This method has several applications both in industry and in human and veterinary medicine (cattle, pigs, chickens, lambs), especially for diagnostic purposes. The measurement of body temperature via thermography requires equipment, conditions, and protocols, and depends on the environment (temperature and relative humidity), distance, the animal's skin, emissivity, and reflected temperature and radiation (Soerensen and Pedersen 2015; Vardasca and Mendes 2017). In laboratory animals, it has been shown to be useful in situations such as neonatal development, identification of stress, and monitoring of infections (Franco et al. 2019; Coe and Blackie, 2022). Despite its advantages, this practice is limited to laboratory animals due to the high cost of the equipment (Franco et al. 2019). Emissivity, that is the ability of an object to emit electromagnetic radiation, i.e. infrared energy when compared to a black body for the same temperature and wavelength, is an important parameter to be considered in the measurement of temperature by thermography (Harrap et al. 2018). Thus, the emissivity values are comprised between 0 and 1, with the value of 0 representing a perfect reflector and the value of 1 representing a black body (Avdelidis and Moropoulou 2003). In biological tissues, emissivity is generally high (between 0.9 and 1) and may depend on their composition. Furthermore, according to the literature, the emissivity of the skin varies between 0.95 and 0.99 (Całkosiński et al. 2015). Consequently, in an ideal case, emissivity should be calculated for each animal; however, in practice, it would become much more complicated. For this, our team has considered the same emissivity value for all animals/measurements (Fiebig et al. 2018).

Regression analysis is a statistical technique that estimates the relationship between a variable called the dependent variable and other variables called independent variables. This relationship is represented by a mathematical model called the linear regression model (Bzovsky et al. 2022). The model can be called the multiple linear regression model if several independent variables are incorporated (Uyanık and Güler 2013). Generically, linear regression is an equation in which the explanatory and outcome variables are linearly related, and the value of variable y can be estimated, given the value of variable x (Zar 2010).

Inversely to that observed in other studies using rats and mice, we observed that males' temperature was slightly higher when compared with females' temperature, which may be explained by the male's behavior (Sanchez-Alavez

et al. 2011; Smarr and Kriegsfeld 2022). Similarly, to that observed in other studies comparing IRT and TIC, in this study, the temperatures measured by TIC were slightly higher when compared with those measured by IRT, which may be explained by the device specificity (Nguyen et al. 2010, Mah et al. 2021). We observed that the order of magnitude between IRT and TIC readings in the worst case was 1.5°C. It means that this procedure is very efficient as it allows you to use an IRT simple and cheap to record readings and, through the proposed procedure, rectify the readings to values very close to the gold standard method, the readings through TIC. The distribution in Celsius using TIC and TIC "estimated" shows that the distribution of TIC readings is very close to the distribution of TIC inferred from IRT readings, corroborating the idea that the reading can be done using the cheapest equipment.

Conclusion

Once measuring the body temperature of animals is a common procedure, there is a need to find alternatives that reduce laboratory animals' stress, thus contributing to the procedures' refinement and animals' welfare. Through linear transformation, followed by a rectification process, it was possible to infer TIC readings, that is considered the gold standard for temperature measurement, from IRT readings. For the procedure to be reliable, it is recommended that calibration be performed using TIC readings so that the models are adjusted to the environmental conditions and the study in question. Without this prior calibration, the robustness of the model cannot be guaranteed, i.e., to provide accurate readings for all cases. After calibration and correct parameter estimation, this type of procedure can obviate the use of TIC readings. It is worth noting that IRT is a simpler, cheaper, and more accessible device when compared with TIC.

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Conflict of Interests

None to declare.

Author Contributions

Ana I. Faustino was responsible for statistical analysis, manuscript writing, and formatting. Paula A. Oliveira was responsible for study design and manuscript draft and writing. Lio F. Gonçalves was responsible for the model fit. Carlos Venâncio participated in study design and writing. Marina Gonçalves, Rita Reis, Tiago Azevedo, and Catarina Ribeiro were responsible for practical experiments, collected results, recorded values, and participated in writing. All authors contributed to the revision and review of the draft.

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