The use of infrared thermography in the evaluation of acute stress in three breeds of domestic rabbits during tattooing

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Abstract

Infrared thermography is a relatively new tool used in the evaluation of stress and health disorders in animals. This study aimed to assess changes in rabbit temperature in response to acute stress. The mean surface temperature of the inner corner of the eye was measured using a thermal camera in 12 rabbits of 3 breeds (four individuals of Mecklenburger Piebald Rabbit, Castor Rex Rabbit, and Large Light Silver Rabbit each) exposed to handling and tattooing of the ears. The mean surface temperature and the standard deviation in the inner corner of the eye in rabbits measured in their home cage was 36.75 ± 1.02 °C and was significantly lower ($P < 0.01$) than that measured during subsequent handling, tattooing and restraint. It was also lower in comparison with the mean temperature measured 30 min after tattooing when the rabbits were returned to the home cage (37.78 ± 0.54 °C). The surface temperature increased ($P < 0.01$) in response to handling when the rabbits were removed from the cage (38.22 ± 0.42 °C), and reached a maximum during tattooing (38.62 ± 0.52 °C). No significant difference was determined between the breeds ($P > 0.05$) at any stage of measurement. Changes in the surface temperature of the inner corner of the eye in rabbits took the same course in all three studied breeds of rabbits. An increase in temperature occurred during the initial handling of rabbits unaccustomed to human handling, and this increase persisted throughout the entire period of handling associated with tattooing.

Thermal camera, surface temperature, handling, welfare

The domestic rabbit (*Oryctolagus cuniculus* ssp. *domesticus*) is a species kept widely for the production of rabbit meat as well as for experimental and scientific purposes or as companion animals. During their lifetime, rabbits are subjected to various stress factors associated with housing conditions, such as temperature (Dalmau et al. 2015), excessive noise (Elwasife 2015), transport (Mazzone et al. 2010) and slaughter (Nakyinsige et al. 2014). Additional stress arises during their use in experiments as rabbits often serve as animal models when testing new interventional treatments before clinical trials (Pánková et al. 2021; Nguyen-Thanh et al. 2022). Despite efforts to improve their living conditions, certain aspects of rearing still remain unsatisfactory for rabbits (Trocino and Xiccato 2010) that are associated with the occurrence of behavioural disorders resulting from unsuitable social situations (Chu et al. 2004) or space restrictions (Gunn and Morton 1995) or even pathological changes as detected during a postmortem veterinary inspection (Ninčáková et al. 2022). Various methods are used for the identification of rabbits reared as pets, or as laboratory or farmed animals. These methods of marking rabbits include the use of ear tags, microchips, and tattooing (Oxley and Saunders 2015). Their application is generally associated with stress and pain in the animals, as was demonstrated in, for example, mice (Taitt and Kendall 2019) and rats (Kasanen et al. 2011). The application of microchips and tattooing also induces a pain response in rabbits, although its intensity may differ depending on the method used (Keating et al. 2012). Tattooing is still used

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given advantages such as low cost and the immediate identification of the animal at a glance, and tattooing is also required as compulsory identification of animals at some animal exhibitions.

In view of efforts to ensure an acceptable level of welfare for animals (Švestková et al. 2024), research focuses on the factors that affect these animals and the possibilities for monitoring the effect of stress or diseases on animals using the most gentle methods (Mousumi et al. 2022). One of the possible ways of assessing the level of animal welfare is the detection of stress. There are multiple ways of determining the stress in rabbits. Laboratory determination of levels of glucocorticoids in the blood (Hudson et al. 2011), the droppings (Prola et al. 2013; Volfová et al. 2022) or the saliva (Munari et al. 2020), for example, can be used. Another possibility is the use of a thermal camera capable of converting infrared radiation emitted from the rabbit's body into values of surface temperature. Acute stress was shown to cause changes on the surface of the bodies of animals (Herborn et al. 2015; Almeida et al. 2018; Bartolomé et al. 2019) as a result of increases in the levels of stress hormones in the blood (Cabezas et al. 2007). Temperature changes resulting from stress may differ depending on the selection of the area on the surface of the rabbit (de Lima et al. 2013). The area of the ear flap or the eye would seem to be the best for the measurement of temperature changes during acute stress in rabbits (Luzi et al. 2007; Ludwig et al. 2010). The ear area and measurement in this area also correlate better with the values of rectal temperature (Jaén-Téllez et al. 2020). When the measurement is conducted over a longer period, the temperature is seen to increase to a level higher than the baseline value as a result of vasodilation (Agea et al. 2021). A similar trend was also found in, for example, birds (Jerem et al. 2015) and goats (Bartolomé et al. 2019).

The advantages of evaluating stress with the use of a thermal camera are swiftness and the fact that it is an entirely contact-free method of measurement – it is possible to avoid affecting the final values by direct handling of the animals. This method also means the animal's welfare need not be affected in any kind of negative way. It is, however, necessary to consider certain factors that may affect the measurement, such as air currents, direct sunlight, and humidity (Travain and Valsecchi 2021).

This study aimed to assess the changes in surface temperature in the inner corner of the eye of rabbits in response to acute stress induced by handling and tattooing and to compare surface temperature changes in three breeds of rabbits (Mecklenburger Piebald Rabbit, Castor Rex Rabbit, and Large Light Silver Rabbit).

Materials and Methods

Rabbits

Measurements were performed on rabbits of three breeds – Mecklenburger Piebald Rabbit, Castor Rex Rabbit, and Large Light Silver Rabbit, aged 7–8 months and kept for exhibition and breeding purposes. The rabbits were housed individually in wooden cages with straw litter located beneath a shelter, fed *ad libitum* with hay and a feed mix for rabbits reared for exhibition purposes (Tetčický Mlýn, Tetčice, Czech Republic), and provided with water *ad libitum*. The rabbits were not accustomed to human handling and had previously been removed from their housing only rarely. Four individuals of each breed were chosen at random, so in total 12 animals were included. The rabbits were treated with care during handling and tattooing in accordance with the relevant animal protection legislation of the Czech Republic. Straight tattoo 10 mm pliers (Driml s.r.o., Brno, Czech Republic) and black tattooing ink (Raidex GmbH, Dettingen/Erms, Germany) were used to tattoo both ears of each rabbit. The measurements were carried out only once, as it was not possible to repeat the tattoo on the same rabbits.

Data collection

A Testo 890-2 thermal camera (Testo SE & Co. KGaA, Germany) with emissivity set to 0.97 was used to scan the rabbits. The camera is of scientific grade with 640 × 480 pixels, thermal sensitivity of < 0.04 °C and a temperature range of −30 to 100 °C. Images were taken at right angles to the rabbit's head at a distance of 0.5 metre in a shady environment on an examination table in the vicinity of the rabbit housing, with the same external conditions maintained during all measurements (outdoor temperature 24 °C and humidity 46%) without being affected by direct sunlight. The people handling the animals avoided touching the animals in the eye area during all handling so as not to affect the measured values.

For evaluation, measurement was performed in 5 phases of the process associated with the marking of the rabbits by tattooing (Table 1). Two images were taken in each phase. The surface temperature measured was recorded in the program IRSoft (Testo SE & Co. KGaA, Germany) as the maximum value measured in the inner corner of the rabbit's eye.

Table 1. Measurement design.

Rabbit condition	Description of measurement
Cage pre-treatment (baseline value)	In the cage before any handling (undisturbed)
Handling	After transfer from the cage to the table
Tattooing	On the table within 20 s of tattooing
Restraint on the table after tattooing	On the table, 2 min at the earliest after the completion of tattooing
Cage post-treatment	After returning to the cage, 30 min after tattooing

Statistical analysis

The mean of the temperatures measured and the standard deviation in all rabbits during the individual phases and in individual breeds in all phases of measurement was calculated in all phases of measurement (pre-treatment, handling, tattooing, restraint and post-treatment).

Statistical comparison of the data was performed in the statistical program UNISTAT 6.5 for Excel (Unistat Ltd., London, UK). A test of normality was first conducted for the statistical evaluation of changes in surface temperature between the individual phases (Shapiro-Wilk test). Based on the result of this test (normal distribution), the data were further evaluated by statistical ANOVA test and Tukey-HSD test. A value of $P < 0.05$ was determined as statistically significant.

Results

Table 2 shows the mean surface temperature of the inner corner of the eye in 12 rabbits measured in the individual phases of measurement. An increase ($P < 0.01$) in the surface temperature was found in all phases of measurement in comparison with the baseline value (36.76 ±1.02 °C). The maximum mean temperature was found after tattooing (38.62 ± 0.52 °C), though it did not differ from the values measured during handling (38.22 ± 0.42 °C) and restraint (38.40 ± 0.44 °C). A significant ($P < 0.01$) decrease was found 30 min after tattooing (37.78 ± 0.54 °C) in comparison with the temperature measured immediately after tattooing (Fig. 1).

No difference ($P > 0.05$) in the values of the surface temperature was recorded between the individual breeds at any phase of measurement (Table 2).

Table 2. Comparison of the surface temperature of the inner corner of the eye in three rabbit breeds (mean ± SD).

Breed	The surface temperature of the inner corner of the eye (°C)				
	Pre-treatment	Handling	Tattooing	Restraint	Post-treatment
Mecklenburger Piebald Rabbit	36.71 ± 0.52[a]	38.17 ± 0.26[a]	38.55 ± 0.48[a]	38.38 ± 0.44[a]	38.00 ± 0.57[a]
Castor Rex Rabbit	36.58 ± 0.68[a]	38.06 ± 0.40[a]	38.35 ± 0.54[a]	38.15 ± 010[a]	37.35 ± 0.36[a]
Large Light Silver Rabbit	36.96 ± 1.72[a]	38.44 ± 0.55[a]	38.95 ± 0.45[a]	38.67 ± 0.58[a]	38.00 ± 0.51[a]

[a] Values with the same superscript in a column are not significantly ($P > 0.05$) different (ANOVA)

Discussion

The results of our study demonstrated that the surface temperature in the area of the inner corner of the eye of rabbits increases significantly during handling and tattooing. An increase in the surface temperature in the eye area, similar to that seen during tattooing in rabbits, was also found during handling and acute stress in goats (Bartolomé et al. 2019),

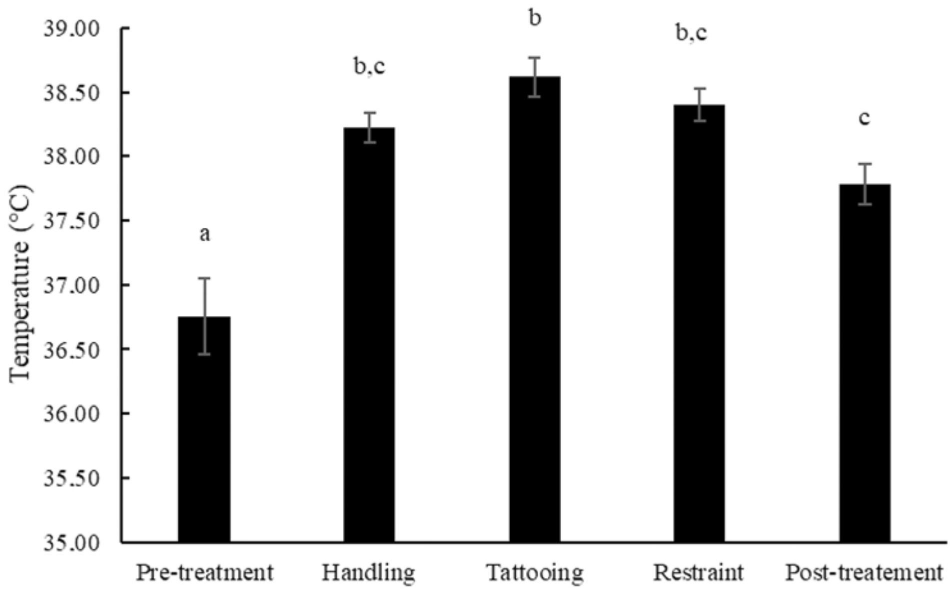

Fig. 1. Mean surface temperature of the inner corner of the eye of rabbits (n = 12)

[a-c] Values with the same superscript in a column are not significantly ($P > 0.05$) different (ANOVA)

horses (Yarnell et al. 2013), and dogs, in which the effect of stress on the change to surface temperature was evaluated during examination by a veterinary surgeon (Travain et al. 2015). The values of the surface temperature in the dogs were higher during the examination itself than the values before and after the examination. Although the highest value in rabbits was measured immediately after tattooing (38.62 ± 0.52 °C), i.e. a procedure associated with pain, no significant difference in comparison with the mean values of surface temperature before and after tattooing was recorded (38.22 ± 0.42 °C and 38.40 ± 0.44 °C, respectively). All these values were, however, significantly higher than the initial value that was measured before any handling occurred and that was considered the baseline value (mean 36.76 ± 1.02 °C). Similar results were also obtained by Jerem et al. (2019) in their evaluation of acute stress in blue tits during short-term capture when the temperature in their eye area rose by more than 1 °C during acute stress.

A decrease in temperature in certain parts of the body associated with vasoconstriction during acute stress was also reported in some studies during measurement with a thermal camera, in addition to an increase in surface temperature. Jerem et al. (2019), for example, detected a short-term fall in temperature in blue tits, followed by a significant increase. Similarly, a fall in temperature immediately following exposure to a stressor was also reported by Stewart et al. (2008) in calves. Lower values during acute stress are caused by vasoconstriction in the given area (Herborn et al. 2015) as a response to hormones in the acute phase of stress, though the effect on vasoconstriction or vasodilation may vary depending on the site of measurement on the body of the animal, as was found in rats (Vianna and Carrive 2005) and primates (Chotard et al. 2018). It is possible that vasoconstriction occurs in rabbits immediately following exposure to the stressor and is not so easy to record, though a more important factor would appear to be the area measured on the body of the animal. Vasoconstriction was demonstrated during exposure to a stressor in rabbit ears (Blessing 2003), which are a peripheral part of the body. It means that it is also possible that this phenomenon does not occur at all in the eyes, as it was not recorded during tattooing. A slight fall in the surface temperature in the area of the eyes of rabbits was also described by Luzi et al. (2007) who found an average temperature of 35.4 °C when measuring it in the eye area in cages and 35.1 °C after exposure to stress. In their study, however, images were not taken until 15 min after exposure to the stressor, and it is possible that the temperatures may have fallen again during this time. Our results show a decrease in temperature in rabbits after their return to the cage following the completion of all handling. In contrast to the results published by Luzi et al. (2007), however, the values measured in our study in the cage half an hour after tattooing (37.78 ± 0.54 °C) were still significantly higher than the baseline values (36.76 ± 1.02 °C).

A significant increase in temperature in the corner of the eye was found in our study during initial handling consisting of the rabbit being moved from the cage to the table, which corresponds to the fact that the rabbits were not accustomed to contact with humans and even gentle catching and handling was stressful for them. Rabbits accustomed to some degree to the presence of humans show less fear of persons in their vicinity (Pongrácz et al. 2001). A non-significant increase in surface temperature was observed after tattooing. The effect of pain, which was previously demonstrated during tattooing in other animal species (Kasanen et al. 2011; Taitt and Kendall 2019) was not seen in our study. The surface temperature did not change during a supposedly painful intervention in comparison to the preceding handling. The results suggest that animals that are unaccustomed to human handling perceive handling itself as a stressor comparable to the pain associated with tattooing. Thus, the question remains, how an increase in surface temperature can reflect the degree of stress experienced. When comparing the values measured by a thermal camera with the increase in stress markers in the blood or saliva, the authors of studies on mammals do not agree on whether changes in surface temperature correspond to changes in the stress markers evaluated (Stewart et al. 2010; Valera et al. 2012; Jakubas et al. 2022). Thus, although the increase in surface temperature may not correspond to the degree of stress experienced, the duration of the stress response is measurable by the return of values to basal. Thus, it is possible that by comparing the length of the surface temperature increase, a conclusion can be drawn about how strongly the stressor is affecting the individual.

The return to the baseline surface temperature values post-stress differs in various species of animals. In rats, for example, it occurs after roughly 30 min (Vianna and Carrive 2005). As far as the difference in the increase in temperature in various individuals is concerned, a role may be also played by experience with the given stressor (Valera et al. 2012) or by the thermoregulatory properties of the organism – studies on rats, for example, show that after the animal was exposed to a stress stimulus the tail is the organ that dissipates excess heat accumulated during stress (Vianna and Carrive 2005). In calves (Stewart et al. 2008) and blue tits (Jerem et al. 2019) it was similarly found that a fall in temperature is followed by an increase, with the surface temperature remaining at values higher than those before exposure to stress for some time after the stressor subsided.

No significant difference was found between individual breeds of a rabbit at any phase of measurement. It is known that there may be differences between certain breeds of the rabbit not merely in their weight and morphological characteristics, but also certain hormones or enzymes. For example, the levels of insulin, superoxide dismutase and immunoglobulins in the blood may differ in individual breeds (Abdel-Hamid and Dawod 2020). Certain markers of oxidative stress also differ between breeds of rabbits during heat stress, as was found by Jimoh (2019). Another study focused on the measurement of temperature in the rabbit breeds Fauve de Bourgogne, Chinchilla, New Zealand White and British Spot revealed a permanently lower body temperature in the New Zealand White, though no difference was found in temperature fluctuations in response to external conditions (Jimoh and Ewuola 2018).

There is no known study devoted to the evaluation and comparison of changes in surface temperature in response to the same stressor in various rabbit breeds. However, some other factors affecting the surface temperature in rabbit breeds have been evaluated. A differing surface temperature has, for example, been found depending on the age of rabbits, with temperature increasing with increasing age (Jaén-Téllez et al. 2021). Lewden et al. (2017) demonstrated in their study of acute stress in black-capped chickadees that temperature changes may vary in the same breed in response to the same stressor depending on external conditions. Furthermore, body temperature may be influenced by the animal's temperament and the environment in which it lives, as was found in cats (Foster and Ijichi 2017). A thermal camera can provide precise data on changes in surface temperature that can, as is confirmed by the results of our study, be used in the evaluation of acute stress in rabbits, regardless of breed. Changes in the surface temperature of the inner corner of the eye in rabbits took the same course in all three studied rabbit breeds. An increase in temperature occurred during the initial handling of rabbits unaccustomed to human handling, and this increase endured throughout the entire period of handling associated with tattooing. No direct dependence between surface temperature and the level of stress/pain experienced can, however, be deduced from the results of the study because the temperature measured did not differ before and after tattooing.

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