

Changes in medial canthus surface temperature and behaviour in guinea pigs during an exhibition

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Abstract

Animal exhibitions may represent a significant stressor for guinea pigs due to environmental changes, altered routines, and close proximity to unfamiliar people. This study aimed to evaluate changes in surface temperature of the medial canthus of the eye and behavioural responses of guinea pigs during an exhibition. Twenty US Teddy guinea pigs aged 1–2 years, each with one prior exhibition experience, were included. Surface temperature of the medial canthus was measured at five time points throughout the exhibition day. Behaviour was analysed from 15-min video recordings following each measurement using continuous sampling. Surface temperature changed significantly during the exhibition ($P < 0.05$). Higher temperatures were recorded immediately after human interaction, whereas lower values were observed during measurements not preceded by handling ($P < 0.05$). Behavioural analysis revealed significant differences in the distribution of behavioural categories across time points ($P < 0.05$). The occurrence of freezing decreased progressively over the course of the exhibition day ($P < 0.05$). Hiding behaviour was most frequent after handling. Abnormal behaviour was highest during the first measurement ($P < 0.05$) and declined significantly in subsequent measurements. In all observations, guinea pigs preferred lying along the enclosure wall rather than in the open area of the enclosure ($P < 0.05$). The results indicate that physiological responses were primarily associated with immediate handling, whereas behavioural changes reflected both acute stress responses and gradual adaptation to the exhibition environment.

Infrared thermography, behavioural analysis, animal exhibitions, animal welfare

Guinea pigs are commonly presented at public events as part of breeding activities, particularly exhibitions, where they may be exposed to a range of stressors such as noise, microclimatic conditions, and the presence of unfamiliar people. These stimuli may trigger an acute stress response, to which this species is generally highly susceptible (Foote 2024). Stress can be defined as a non-specific physiological response of the body to external or internal stimuli (Fink 2016). Activation of stress systems leads to behavioural and physiological changes that enable the organism to adapt (Johnson et al. 1992). Exhibitions may present a prolonged burden for guinea pigs, as they involve preparation, often long-distance transport, the duration of the event itself (which may exceed one day), and the trip back. Therefore, exhibitions cannot be considered short-term events (Zieliński et al. 2024).

The exhibition environment presents a combination of several major stressors. Animals are often housed in smaller enclosures than those they are accustomed to, transported over long distances, exposed to high visitor density, and may experience unsuitable thermal conditions. Confinement, improper handling, ambient temperature, and transport can negatively affect their welfare (Warwick et al. 2023; Fétiveau et al. 2024; Zieliński et al. 2024). Another important stressor in exhibition settings is the presence of unfamiliar odours and increased noise levels. Liu et al. (2019) demonstrated that even short-term exposure

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to high-intensity sound elicits a stress response in guinea pigs. At exhibitions where large numbers of animals are gathered, a social phenomenon known as stress contagion may also occur. Stressed individuals may unintentionally induce stress responses in others through behavioural or physiological cues. Other animals may respond with increased vigilance, elevated respiratory rate, or aggression. Stressed individuals may also actively signal the presence of a stressor, for example through alarm vocalisations or pheromone release, thereby activating stress responses in conspecifics, as reviewed by Brandl et al. (2022).

Several methods are available for assessing stress. In guinea pigs, cortisol concentrations have been measured in blood (Kaiser et al. 2023), saliva (Nemeth et al. 2016), and faeces (Schöpper et al. 2011; Walters et al. 2012; Powell et al. 2020). Behavioural analysis has also been used under laboratory conditions to evaluate responses to isolation (Hennessy et al. 2004) and to assess guinea pigs participating in animal-assisted therapy (AAT) programmes (Gut et al. 2018; Wirth et al. 2020). More recently, infrared thermography has been increasingly applied to assess stress load in animals (Kadlecová et al. 2024; Šebánková et al. 2025). This method has been successfully used in guinea pigs involved in AAT (Wirth et al. 2020) and in zoological gardens (Namiki et al. 2024). Infrared thermography is based on detecting changes in peripheral blood flow and their subsequent effect on body surface temperature (Giannetto et al. 2022). Numerous studies have documented an increase in body temperature during stress responses (e.g. Bartolomé et al. 2013; Jerem et al. 2018; Boileau et al. 2019). The ocular region is considered an optimal site for monitoring changes in surface temperature using thermography (Bartolomé et al. 2013), as it is hairless and richly vascularised, thereby reliably reflecting changes in local blood flow (Stewart et al. 2007; Johnson et al. 2011). For comprehensive welfare assessment, combining physiological and behavioural indicators is recommended (Pereira et al. 2018; Wirth et al. 2020; Namiki et al. 2024), enabling a more accurate evaluation of stress levels and welfare in guinea pigs.

The aim of the present study was to assess the dynamics of changes in medial canthus surface temperature and behavioural responses in guinea pigs during a full-day public exhibition.

Materials and Methods

Animals and housing

The assessment of behavioural and physiological responses of guinea pigs during a public event was conducted at a public exhibition of domestic guinea pigs (*Cavia porcellus*) in June 2024. The study included 20 US Teddy guinea pigs (10 females, 10 males) aged 1–2 years. All individuals had previously participated in one exhibition. The exhibition hall was maintained at a constant temperature of 22 °C and relative humidity of 63%. During the exhibition, guinea pigs were housed individually in plastic enclosures (35 × 45 cm). The enclosures contained no environmental enrichment; animals had *ad libitum* access to hay. The arrangement and equipment of the enclosures reflected standard breeder practice and were not experimentally modified. Fresh water was provided at hourly intervals in accordance with breeder practice. Wood shavings were used as bedding. All animals were present at the exhibition from 7:30 h to 16:30 h.

Infrared thermography

Physiological responses were assessed using non-invasive infrared thermography. To minimise stress associated with novelty, all guinea pigs were habituated to the presence of the thermal camera for three consecutive days in their home environment. The camera was placed 50 cm from the cage for 4 h daily.

Surface temperature of the medial canthus was measured using a Testo 890-2 thermal imaging camera (Testo SE & Co. KGaA, Titisee-Neustadt, Germany) with emissivity set to 0.98 (Zheng et al. 2022) (temperature range −30 °C to 100 °C; sensitivity < 0.04 °C). Five measurements were performed at 2-h intervals during the exhibition. Animals remained in their exhibition enclosures during all measurements. Measurements were categorised as either preceded by interaction or without prior interaction. Interaction consisted of removing the animal from the enclosure, gentle handling and inspection (coat, ears, limbs), and returning it to the enclosure.

- Measurement 1 (M1) – 30 min after transport; after interaction with a familiar person (8:00–8:10 h)
- Measurement 2 (M2) – without prior interaction (10:00–10:10 h)
- Measurement 3 (M3) – after interaction with an unfamiliar person (12:00–12:10 h)
- Measurement 4 (M4) – without prior interaction (14:00–14:10 h)
- Measurement 5 (M5) – without prior interaction (16:00–16:10 h)

For each individual at each time point, five thermographic images of the left side of the head were obtained. The distance between the lens and the animal was maintained at 50 cm at approximately a 90° angle. Images were analysed using IRSoft software (Testo SE & Co. KGaA, Titisee-Neustadt, Germany). The medial canthus region was defined using a circular tool, from which mean temperature was extracted. The circle was positioned so that its upper and lower margins touched the upper and lower eyelids. The same region was used for all animals. Images with partially or completely closed or blurred eyes were excluded from analysis.

Behavioural analysis

Guinea pigs were video-recorded throughout the entire exhibition (9 h). From the total recording, five 15-min segments immediately following each thermographic measurement were selected (M1: 8:15–8:30 h; M2: 10:15–10:30 h; M3: 12:15–12:30 h; M4: 14:15–14:30 h; M5: 16:15–16:30 h) to assess behavioural and physiological responses within comparable time frames. The ethogram (Table 1) was developed based on previous studies (Gut et al. 2018; Namiki et al. 2024) and the authors' own observations.

Recordings were obtained using Sony AX53 HANDYCAM® 4K with CMOS Exmor R® sensor (Sony Corporation, Tokyo, Japan) and Niceboy Vega X Pro cameras (Niceboy s.r.o., Prague, Czech Republic). Cameras were installed prior to the animals' arrival to minimise disturbance. The distance between cameras and enclosures was 50 cm. Video recordings were analysed by two independent observers using Noldus Observer XT 17 software (Noldus Information Technology, Netherlands) with continuous sampling to obtain behavioural durations. Inter-observer reliability reached 97%.

Table 1. Observed behavioural patterns.

Observed behaviour	Definition
Lying in the open area	The animal lies with its ventral body surface in contact with the bedding; no part of the body is in contact with the walls of the box.
Lying along the enclosure wall	The animal lies with its ventral body surface in contact with the bedding, with at least part of the body touching the wall of the box.
Feeding behaviour	Active ingestion of food. The animal grasps and consumes food, typically accompanied by locomotor activity.
Comfort behaviour	Self-directed maintenance behaviours, including scratching with a hind limb, rapid body movements (e.g. head or body shaking), and nibbling or licking parts of the body surface.
Hiding behaviour	The animal attempts to conceal itself, typically by burrowing into the bedding.
Abnormal behaviour	Repetitive scratching at the walls of the box using the forelimbs and/or licking the walls of the box.
Freezing behaviour	Immobility characterised by cessation of all body movements for a duration longer than 1 second.

Statistical analysis

Statistical analyses were performed using Unistat 6.5 for Excel (Unistat Ltd., London, UK). To assess changes in surface temperature, the mean value from the thermographic images obtained at each measurement was calculated for each guinea pig. The recorded medial canthus temperatures for each individual were subsequently compared across the different measurement time points. Behavioural responses were evaluated based on the duration (in seconds, s) of the observed behavioural patterns. Differences in the duration of the recorded behaviours between measurement time points were analysed. Data were analysed using Friedman ANOVA followed by *post hoc* multiple comparisons (Tukey HSD). Statistical significance was set at $P < 0.05$.

Results

The results of surface temperature changes are presented in Fig. 1. Surface temperature measured at the medial canthus changed significantly during the exhibition ($P < 0.05$). Measurements performed immediately after interaction (M1 and M3) showed significantly higher temperatures ($P < 0.05$) compared to M2, M4, and M5. The median temperature in M1 was 37.56 °C, while in M3 it reached 38.50 °C, with a significant difference detected between these two measurements ($P < 0.05$). In contrast, measurements not

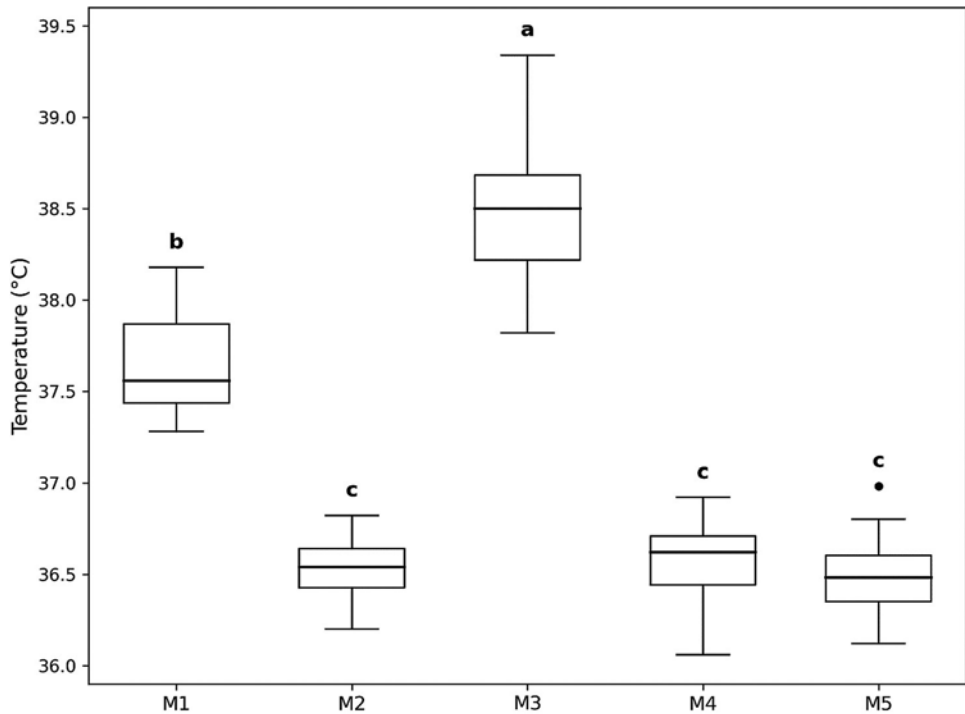


Fig. 1. Surface eye temperature of guinea pigs across measurements during the exhibition.

^{a, b, c, d} Different superscripts indicate significant differences ($P < 0.05$). M1–M5 denote Measurements 1–5.

preceded by interaction (M2, M4, and M5) consistently showed lower temperatures. A significant decrease was observed in M2 compared to M1 (median 36.54 °C; $P < 0.05$). Temperatures recorded in M4 and M5 were comparable to M2 (median 36.62 °C and 36.48 °C, respectively), with no significant differences among these three measurements ($P > 0.05$).

The results of the behavioural analysis are summarised in Table 2. The proportion of behavioural categories differed significantly between measurements ($P < 0.05$). Throughout the exhibition, guinea pigs devoted the least time to feeding behaviour. In measurements immediately following interaction (M1 and M3), the proportion of time spent feeding was significantly lower compared to measurements without prior interaction ($P < 0.05$). Comfort behaviour was also less represented in M1 and M3 than in the remaining measurements ($P < 0.05$). Hiding behaviour was most frequent during M3 compared to all other measurements ($P < 0.05$), whereas the least time spent hiding was observed in M2 and M5, with no difference between these two measurements ($P > 0.05$). Abnormal behaviour was most prevalent in M1, followed by a significant decline ($P < 0.05$). The lowest occurrence of abnormal behaviour was recorded in M3, M4, and M5, with no significant differences among these measurements ($P > 0.05$). Freezing behaviour was highest in measurements following interaction ($P < 0.05$), whereas the lowest values were observed in the final measurement. In all five measurements, lying along the enclosure wall was significantly more frequent than lying in the open area of the enclosure ($P < 0.05$), and this difference was consistent across all time points.

Table 2. Duration of behaviours expressed as a percentage of the total observation time (15 min) across individual measurements during the exhibition.

Behaviour	Measurement 1	Measurement 2	Measurement 3	Measurement 4	Measurement 5
Lying in the open area	2.3 ^{ab}	3.23 ^a	1.27 ^b	2.76 ^a	2.78 ^a
Lying along the enclosure wall	59.8 ^c	53.33 ^d	65.72 ^a	61.04 ^b	60.88 ^b
Feeding behaviour	0.02 ^b	0.58 ^a	0.01 ^b	1.16 ^a	1.95 ^a
Comfort behaviour	1.15 ^b	1.82 ^a	0.57 ^c	1.65 ^a	1.69 ^a
Hiding behaviour	25.46 ^b	21.71 ^c	43.7 ^a	24.24 ^b	21.45 ^c
Abnormal behaviour	29.28 ^a	20.87 ^b	9.06 ^c	7.26 ^c	7.15 ^c
Freezing behaviour	6.41 ^a	4.27 ^b	6.67 ^a	4.17 ^b	2.36 ^c

a, b, c, d Different superscripts within a row indicate significant differences ($P < 0.05$).

Discussion

Changes in ocular surface temperature throughout the exhibition day indicate that guinea pigs' responses were not constant over time. The highest temperatures were recorded immediately after interaction with unfamiliar people. The higher temperature observed after handling performed during the exhibition, compared to handling at its beginning, may suggest that disturbance of an already stabilised state elicited a stronger response. Elevated values may also have been influenced by the fact that the handling was performed by an unfamiliar person, representing an additional stressor. Based on available literature, the observed increase in surface temperature may reflect an acute stress response (Stewart et al. 2007; Yarnell et al. 2013; Jerem et al. 2018; Boileau et al. 2019). However, temperature changes may also result from general arousal (Polgár et al. 2019) and are not necessarily associated exclusively with negative experiences (Proctor and Carder 2015). Although handling may affect thermographic results due to residual heat transfer at the contact site (Vacuškova et al. 2025), the measured region (medial canthus) was not directly touched. The absence of cumulative temperature increases in measurements without prior interaction suggests that mere presence at the exhibition did not lead to progressive stress accumulation. Similar temperature increases during human interaction have been reported by Wirth et al. (2020) and Namiki et al. (2024).

Behavioural analysis in our study demonstrated that guinea pigs exhibited changes in the distribution of individual behavioural categories during the exhibition. Recognised behavioural indicators of stress in guinea pigs include a reduction in comfort and feeding behaviour, together with an increased occurrence of freezing (Hennessy et al. 2004; Wirth et al. 2020), as well as hiding behaviour and the appearance of stereotypic behaviours such as bar biting (Brandão and Mayer 2011). The observed increase in hiding and freezing, accompanied by a reduction in comfort and feeding behaviour following prior handling during the exhibition, may therefore reflect stress-induced changes. Similar findings were reported by Gut et al. (2018). In contrast, Kase et al. (2021) did not detect differences in feeding, comfort behaviour, or hiding in guinea pigs after contact with humans. The divergent findings in our study may be explained by differences in sample size ($n = 10$ in Kase et al. 2021), but also by the fact that, in our case, the animals were observed in an unfamiliar and noisy environment, which may have imposed a greater stress load. Another contributing factor may have been the individual housing of guinea pigs during the exhibition, despite the species being inherently social (Sachser et al. 1998). The absence of conspecifics may have intensified the stress response observed in our animals.

The preference of guinea pigs for lying along the enclosure wall rather than in the open area may be associated with anxiety (Seibenhener and Wooten 2015; La-Vu et al. 2020).

According to Seibenhener and Wooten (2015), thigmotaxis (i.e., the tendency to remain close to walls) is observed in most rodents and is linked to their natural inclination to avoid large open spaces, which they perceive as potentially dangerous. For this reason, Olsson et al. (2003) recommended providing guinea pigs with shelters to protect them from exposure to open areas. Lee et al. (2023) demonstrated that in mice housed in confined spaces, stress levels can be reduced through the provision of environmental enrichment, adequate ventilation, and appropriate social housing conditions. These findings may also be applied to exhibition enclosures for guinea pigs, where the provision of shelters is particularly important, as it allows animals to avoid certain stressors and enhances their sense of security (Gut et al. 2018). Although the guinea pigs observed in our study were not provided with shelters in their exhibition enclosures, we recorded attempts to hide by burrowing into the bedding material. This behaviour was most frequent following interaction with unfamiliar people. These findings further support the importance of providing guinea pigs with opportunities for concealment. As a prey species, guinea pigs naturally rely on hiding behaviour. In the wild, their wild relative (*Cavia aperea*) uses dense vegetation as shelter from predators (Asher et al. 2004). The importance of shelter provision in guinea pigs has also been confirmed by Walters et al. (2012), Gut et al. (2018), and Wirth et al. (2020).

Abnormal behaviour observed primarily at the beginning of the monitoring period may be explained by a combination of stressors, including a novel environment, short-term transport, individual housing, and the absence of opportunities to escape or hide. According to Mason (1991), stereotypes in rodents frequently emerge in unfamiliar environments and are widely regarded as indicators of impaired welfare (Novak et al. 2016). The gradual decline in abnormal behaviour observed during the study may therefore be associated with habituation to the exhibition environment.

The observed response to handling may reflect a negative association of some individuals with human contact or insufficient habituation to handling. This may be related to prior negative experiences with humans or to limited previous exposure to human interaction. As reported by Fétiqueau et al. (2024), low levels of human–animal interaction can result in handling difficulties and increased stress responses during human contact. Similarly, Mellor et al. (2020) note that human–animal interactions may negatively affect animal welfare, particularly when animals are not accustomed to contact with people. Guinea pigs are generally considered a stress-sensitive species, which may further amplify their response to handling. However, findings from other species suggest that habituation to human contact can be achieved (Fétiqueau et al. 2024). Zucca et al. (2012) demonstrated that early-life handling in rabbits reduces fear of humans. Although this concerns a different species, it may be assumed that a similar habituation effect could occur in guinea pigs. Gradual habituation to handling and the establishment of positive human–animal interactions may therefore contribute to reducing stress during contact at exhibitions. Increased stress markers following handling have also been documented in other species, including elevated corticosterone concentrations in mice (Rasmussen et al. 2011) and parrots (Parks et al. 2023).

The results indicate that both physiological and behavioural responses of guinea pigs during the full-day exhibition showed dynamic changes. Handling during the exhibition was associated with responses indicative of an acute stress reaction. In contrast, the course of the exhibition day itself, in the absence of additional handling, was not accompanied by an increase in stress indicators. On the contrary, some behavioural indicators showed a gradual decline over time, which may suggest a certain degree of adaptation to the exhibition environment. The differing dynamics of physiological and behavioural responses further emphasise the importance of assessing multiple indicators when evaluating animal welfare. However, interpretation of the results is limited by the absence of comparison with

the home environment, which would allow for a more precise determination of baseline levels of the monitored indices. Given the limited scope of the study, further research is warranted. Future studies should include a larger sample size and focus in greater detail on specific environmental and organisational factors that may positively influence guinea pig welfare at exhibitions, such as the provision of shelters, gradual habituation to handling and transport, and modifications to exhibition enclosure design.

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