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The influence of human interaction on guinea pigs: Behavioral and thermographic changes during animal-assisted therapy

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ABSTRACT

Guinea pigs are often involved in animal-assisted therapy (AAT) but there is little knowledge about the effects of human contact on guinea pigs involved in AAT. The aim of this study was to investigate effects of availability of a retreat, presence of conspecifics, prior experience with AAT, and human interaction on indicators of welfare in guinea pigs involved in AAT. Guinea pigs of both sexes and different ages (n = 20) were assigned to a randomized, controlled within-subject trial with repeated measurements. Each guinea pig was tested in four settings: (I) therapy with retreat possibility with conspecifics, (II) therapy with retreat possibility without conspecifics, (III) therapy without retreat possibility, and (IV) setting without human interaction. We measured changes in eye temperature, as a proxy to infer stress levels, at 5-s intervals with a thermographic camera. All sessions were video recorded and the guinea pigs' behavior was coded using continuous recording and focal animal sampling. For the statistical analysis we used generalized linear mixed models, with therapy setting as a fixed effect and individual guinea pig as a random effect. We observed a temperature increase relative to baseline in settings (I) therapy with retreat with conspecifics present and (III) therapy without retreat. The percentage of time a guinea pig was petted was positively correlated with a rise in the eye temperature independent of the setting. Time spent eating was reduced in all therapy settings (I-III) compared to the setting without HAI (human animal interaction) (IV). In the setting with retreat (I), guinea pigs showed more active behaviors such as locomotive behavior or startling compared to the setting without retreat (III) and the setting without HAI (IV). When no retreat was available (III), they showed more passive behaviors, such as standing still or freezing compared to therapy with retreat (I). Based on our results we identified the behaviors "reduced eating", "increased startle" and "increased freezing" as indicators of an increased stress level. Petting the guinea pigs was correlated with a rise in the eye temperature and might be a factor which can cause stress. Our results support the suggestion that guinea pigs involved in AAT should have a retreat possibility, should have access to conspecifics, and should be given time to adapt to a new setting. In this way, stress might be reduced.

1. Introduction

Guinea pigs are part of various animal-assisted interventions, but there is a lack of knowledge regarding the effects of their involvement in such interventions or of human contact in general on their welfare, comprising physical and emotional state [1,2]. Animal-assisted therapy (AAT) is a form of animal-assisted interventions where an animal is involved in a therapeutic setting. This approach is often used for people who are difficult to reach using conventional therapeutic methods. AAT is planned and structured by trained professionals with the goal to improve emotional, social and physiological functioning of the patient [3]. For example, guinea pigs are introduced in occupational therapy in neurorehabilitation where patients train their fine motor and cognitive skills by planning what to feed the guinea pigs, cutting vegetables and hand-feeding the guinea pigs or by cleaning and arranging the guinea pigs living environment [4]. The presence of guinea pigs can motivate

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patients to interact and talk e.g. about childhood memories or it provides the possibility to discuss needs of others and of oneself and to identify with the different characters in the group which is used for example within psychotherapy or activation therapy. Recent research shows that involving animals in interventions has numerous positive effects on humans' social behavior, emotional states such as anxiety or depression, and physiological parameters such as blood pressure, heart rate or respiratory rate [5,6].

Guinea pigs (*Cavia aperea f. porcellus*) are a common species in animal-assisted interventions. Research shows that interacting with guinea pigs can have positive effects on patients' social behavior [7] and their ability to make contact and communicate [8]. Guinea pigs are social and curious animals [9]. They are easy to keep and handle, which makes them suitable for AAT. However, the guinea pig is a prey species and stress-prone [10]. In order to carry out AAT in an ethical manner and with respect to One Health, it is crucial to have adequate knowledge about behavior, needs and health as well as indicators and methods for regulation of stress of the involved animals [3]. It is an ethical obligation to scientifically examine the effects on the animals involved in AAT to avoid a tradeoff of human against animal welfare [11]. One Health recognizes the inseparable linkage between humans, animals and their environment and is defined as added value in human and animal health and welfare that is achieved by a closer cooperation of different disciplines [11–13]. To understand this link within AAT, research about effects on the involved animals is crucial. Knowledge of how negative effects on the animals can be reduced is needed as well as knowledge on how conditions should be designed so that both the patient and the animals can benefit.

Effects of AAT on the involved animals are increasingly scientifically investigated especially in dogs [14,15] and horses [16], with mixed results. However, only two studies have investigated guinea pigs in AAT so far [17,18]. Gut and colleagues [17] investigated the behavior of five female guinea pigs during AAT with and without retreat possibility, in comparison to a setting with retreat possibility and no human interaction. The study provided evidence that the possibility to retreat is a key factor to reduce stress in guinea pigs and should always be provided during AAT. The limiting factors of the study were the small sample size and that it remained unclear how the behavioral observations were related to physiological reactions of the guinea pigs. Therefore, we wanted to combine behavior with physiological data in the present study.

Physiological stress in guinea pigs has been studied noninvasively using saliva cortisol levels [19] or fecal glucocorticoid metabolite concentrations [20]. However, these methods have limitations, as described in previous studies on other animal species, e.g. regarding the correlation with blood cortisol levels [21]. To avoid these problems, we measured physiological stress via infrared thermography. Infrared thermography is a relatively new method of non-invasive stress quantification which measures body temperature from a distance [22]. Body temperature is a very sensitive stress parameter and allows for real-time information about physiological stress processes. Stress is associated with different autonomous, endocrinal and neurochemical changes as well as behavioral changes [1,23]. These prepare an organism for potential threats [24] leading to a rise of the internal body temperature (hyperthermia) within a short time [24] and a decrease of the outer body temperature in the extremities due to vasoconstriction [25]. A thermal imaging camera features infrared sensors which make radiometric measurements of the outside body temperature. This technology is very precise concerning temperature measurement and spatial resolution [26,27]. Numerous studies have shown that eye temperature of various mammalian species rises in stressful situations and is correlated with core body temperature (e.g. cattle [28], horses [29], dogs [30]). A study with chickens indicated that even the intensity of the stressor can be evaluated with a thermographic camera [25]. Furthermore, a thermographic video camera allows for video recording of animals in the absence of humans [31].

The aim of this study was to investigate the effects of a retreat possibility, the presence of conspecifics, prior experience with AAT, and human interaction on stress and welfare in guinea pigs involved in AAT using video thermography and behavioral observations. Based on our previous study [17], we proposed two hypotheses. (1) Provision of a retreat and giving the animal free choice of interactions are associated with reduced physiological and behavioral stress indicators. (2) The presence of conspecifics leads to reduced physiological and behavioral stress indicators during AAT.

2. Materials and methods

This study was approved by the Veterinary Office of the canton Basel-Stadt, Switzerland (N° of approval: 2713). It was conducted in accordance with the Animals (Scientific Procedures) Act 1986, European Directive EU 2010/63, and the Guidelines for the Use of Animals in Research of the Association for the Study of Animal Behavior and the Animal Behavior Society. AAT was performed according to the IAHAIO guidelines [3]. Break-off criteria were defined as an excessive display of stress-associated behavior by the animal. No session was stopped early, and no adverse incidents occurred.

2.1. Subjects

Twenty guinea pigs (*Cavia aperea f. porcellus*) of mixed sources, breeds, sexes and different levels of experience with AAT were part of this study (Table 1). They were identifiable by natural markings. Six guinea pigs (five intact females, one castrated male), kept in two groups of three animals each (group 1 and 2), had been involved in AAT at REHAB Basel, a rehabilitation clinic in Switzerland, on a regular basis with up to two therapy sessions per day for at least one year. Fourteen guinea pigs (10 intact females, four castrated males) were provided by private households and had not been involved in AAT before. They were kept in six groups of two to three animals, respectively (groups 3 to 8).

One female guinea pig of group 7 died unexpectedly during the data collection phase without showing clinical signs of illness prior to death. A postmortem examination was carried out at the Institute of Veterinary Pathology in Zurich (case N° S19–0788). Sepsis due to *Streptococcus pneumoniae* was identified as the cause of death. The data of this animal were not included in the analysis. Data collection stopped immediately after the incident due to implementation of hygiene measures which led to some missing data from two other guinea pigs. This led to a final sample size of 19 investigated guinea pigs.

Table 1
Subject characteristics.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Group size	3	3	3	2	2	2	3	2
Gender	3 ♀	2 ♀, 1 ♂	3 ♀	2 ♀	2 ♀	2 ♂	2 ♀, 1 ♂	1 ♀, 1 ♂
Experience with AAT	Yes	Yes	No	No	No	No	No	No
From private households	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Ages (years)	6.5, 7.5, 7.5	1.5, 5.5, 5.5	2, 6, ?	6, ?	2, ?	5, 5	1.5, 3, 3	1.5, 1.5

♀: female, ♂: castrated male, ?: exact age unknown but older than one year

Fig. 1. Housing of the guinea pig groups in a multistory cage of 3 m^2.

2.2. Handling and housing

During the study, each group of guinea pigs was housed in a multistory cage (Fig. 1) of 3 m^2 in accordance with Swiss standards for animal welfare. The cage contained shelters, twigs, wood shavings, hay, straw and a bowl of water. The guinea pigs had access to hay, straw and water ad libitum. We fed them three times a day with fresh vegetables, herbs, grass and grains. For guinea pigs from private households, we collected the information about handling, housing and feeding prior to the study start using a questionnaire filled in by the animal owners. We made sure that the animals were used to the same food as provided at the study facility. All animal owners described their guinea pigs as trustful (3.63 ± 0.86 on a scale from 1 (not at all) to 6 (very much)). There was a certain variance in the statements whether the animals would like to be petted (2.13 ± 1.17 on a scale from 1 (not at all) to 6 (very much)), however, all animal owners stated that the guinea pigs would not like to be held or carried (1.38 ± 0.60 on a scale from 1 (not at all) to 6 (very much)). All owners stated that they had regular daily contact with their guinea pigs (several times a day) and that the animals were familiar to hand-feeding.

All animals were housed and handled under similar conditions, whether originating from private households or residing at the rehabilitation center. External animals arrived two weeks before the beginning of the study to give them an appropriate period of acclimatization prior to data collection. The timeframe for acclimatization was chosen according to results of Obernier and colleagues [32]. During the first week, the guinea pigs acclimatized in their cages to the new surroundings and the food. They were not handled otherwise. In the second week, they were transferred to and hand-fed in the table enclosure used for therapies (Fig. 2) daily to familiarize them with the study setting and the person conducting the study. During the third week, we collected the data.

2.3. Study design

The study followed a randomized controlled within-subject design with repeated measurements. We observed each guinea pig twice in four different settings.

(I) Therapy setting with retreat and access to conspecifics: animals had free choice of human interaction or retreat into their enclosure.
(II) Therapy setting with retreat without access to conspecifics: one single animal had free choice between human-interaction and retreat in its table enclosure.
(III) Therapy setting without retreat without access to conspecifics: one single animal was placed on a plush pet bed in the lap of the patient.
(IV) Setting without human interaction with access to conspecifics: animals were in the table enclosure without a patient or another human present.

A total of 147 observations were made, consisting of two observations per guinea pig per setting. Due to the previously described data loss, the final analysis for both physiology and behavior included: (I) Therapy with retreat n = 38, (II) therapy without conspecifics n = 36, (III) therapy without retreat n = 36, (IV) setting without HAI n = 37.

The experiments were carried out with one test subject rather than actual patients (hereafter referred to as "patient") in order to standardize the interactions as much as possible. Each guinea pig was tested in three settings per day in different setting orders. Each day, every guinea pig was the focal animal in one to two settings using a crossover design. This experimental setup simulated real AAT sessions as performed at REHAB Basel. The order of the settings and their distribution over the different trial days were predetermined in a randomized order. Carry-over effects were accounted for by taking breaks of 10 minutes between the three settings that were tested on the same day. Settings are described below. Settings I, III and IV were set up similar to the pilot study of Gut and colleagues [17].

All four settings took place in a room designated for AAT at REHAB Basel. Each session started with the transfer of the guinea pigs from their cage to the table enclosure followed by habituation of 30 minutes in the table enclosure. During this time, ambient noise and activity were kept to a minimum. Settings I, II and IV took place in a table enclosure specially designed for AAT with guinea pigs. A 1.2 m^2 table framed with a Plexiglas wall was set up in a standardized way with bedding, shelters (two hay tunnels and two wooden houses), hay, straw, twigs and a water bowl (Fig. 2), referred to as "enclosure part". Adjacent to the

Fig. 2. Table enclosure divided in the two areas: Enclosure part and board part. S1, S2: Wooden houses; S3, S4: Hay Tunnels; H: Hay; St: Straw; T: Twigs; W: Water Bowl; B: Bedding; P: Pet bed.

front was an attached wooden front table, hereafter referred to as "board part", on which there was a plush pet bed. Both areas, the enclosure part as well as the board part, were freely accessible to the guinea pigs and offered opportunity to interact with humans or retreat in a shelter. In setting III the guinea pig was placed in the plush pet bed on the lap of the patient.

2.3.1. Setting I: therapy setting with retreat possibility and access to conspecifics

In the therapy setting with retreat possibility, the grouping of guinea pigs in the table enclosure was always according to their original social group. The setting duration was 30 minutes. The patient tried to encourage the animals to approach with fresh succulent food (60 g of vegetables, i.e. carrots, celery stalks, lettuce, grass and dandelions, per animal). The guinea pigs were encouraged to gather pieces of vegetable from wooden pet puzzle toys, branches with holes or a wooden board with holes. The patient fed and petted the animals on the board part, while they had the possibility to retreat into the shelters at any time during therapy.

2.3.2. Setting II: therapy setting with retreat possibility without access to conspecifics

In the therapy setting without interaction with conspecifics, the setting was identical to the therapy setting with retreat except that the animal had no contact with their social group. One guinea pig was in the table enclosure for five minutes without its social partners, but had visual, olfactory and auditory contact with its group in the same room. The patient tried to attract the animal with fresh succulent food (20 g of vegetables, e.g. carrots, celery stalks, lettuce, grass and dandelions per animal). The guinea pig was encouraged to gather vegetable pieces from wooden pet puzzle toys, branches with holes or a wooden board with holes. The patient fed and petted the animal on the board part, while it had the possibility to retreat into shelters at any time during therapy.

2.3.3. Setting III: therapy setting without retreat possibility without access to conspecifics

In the therapy setting without retreat possibility, one guinea pig was placed in the pet bed on the lap of the patient for five minutes while having visual, olfactory and auditory contact with its social partners in the same room. The patient fed the guinea pig with fresh succulent food (20 g of vegetables e.g. carrots, celery stalks and lettuce, grass and dandelions per animal) and petted the animal. The guinea pig did not have the possibility to leave the lap to retreat into shelter. Break-off criteria were defined as an excessive display of stress-associated behavior by the animal (e.g. piloerection, repeated eye-closing or an attempt to jump out of the pet bed on the patient's lap).

2.3.4. Setting IV: setting without human interaction with access to conspecifics

In the setting without HAI, the guinea pigs were in the table enclosure for 30 minutes in their social group. There was no patient present and no human-animal interaction. The guinea pigs had free access to the enclosure part and the board part. They could approach fresh succulent food (60 g of vegetables, e.g. carrots, celery stalks and lettuce, grass and dandelions per animal) distributed on the board part and the front enclosure part, which were the areas that would also be accessible by humans during therapy with retreat (see setting I).

2.4. Data collection

2.4.1. Physiological measurements

Eye temperature was measured using the thermography video camera FLIR T530 with a wide angle (41°) objective. The camera was calibrated for reflecting temperature, consisting of living (e.g. animals, humans) and non-living (e.g. heating, solar irradiation) components in the surroundings of the measured animal before every setting.

Temperature data were collected using continuous video recording at a distance of approx. one meter between the objective and the focal animal at an angle of approx. 90° The image analysis software FLIR Tools (Version 5.13.18031.2002) was used to measure the maximum temperature (°C) within an oval area traced around the eye, including the eyeball and approx. 0.5 cm around the outside of the eyelids. For analysis, the thermographic videos were divided into five-second intervals and the absolute maximum value for each interval was exported. On this basis, a mean temperature value per condition was calculated. The temperature change relative to the baseline temperature was used for the data analyses. The baseline temperature of each animal was determined by a mean of measured values during the last 15 minutes of the habituation phase in the table enclosure before the start of the settings.

2.4.2. Behavior observations

All sessions were filmed using a video camera (Sony™ Camcorder). For coding, the video recordings were trimmed to contain one setting each. In that way, the video coder was blinded with regard to setting order. It was not possible to blind for the type of setting, because this was visibly obvious to the coder.

Animal behavior was analyzed using continuous recording and focal sampling [33] by coding the videos with Noldus Observer XT 12.5 according to the ethogram designed by Gut and colleagues [17]. All videos were coded by one trained coder. Intra-rater reliability ranged between 0.84 and 0.99 and inter-rater reliability with the observer of our previous study [17] was between 0.88 and 0.93, as measured by Cohen's kappa [34]. Tables of the intra-rater and inter-rater reliabilities are available in the additional material (see supplement, Table 5 and 6).

The following behaviors were observed according to our previously developed ethogram (see supplement, Table 7) [17] and served as dependent variables:

a) Individual behavior: ingestive behavior, locomotive behavior, comfort behavior
b) Interactions with the environment: explorative behavior, non-explorative behavior
c) Social behavior towards conspecifics: sociopositive behavior, general socionegative behavior
d) Active human animal interaction (HAI): sociopositive HAI, general socionegative HAI
e) Passive human animal interaction: being petted
f) Vocalization
g) Other groups: visibility (guinea pig is in view), unexpected behavior (i.e. a sound from outside), observation on-going (start to end of the setting without pre-/postprocessing)

Frequencies of short countable behaviors were calculated as n/60 s. Longer enduring state behaviors were calculated as percentages of the observed time. For most behaviors, the denominator “visible and on-going” was used. This ensured that the reference time (100%) only counted when the therapy was on-going and the animal was visible in the camera. For “vocalization,” “hiding,” “on board part,” and “in enclosure unsheltered,” only the denominator “on-going” was used because these behaviors also occurred when the animal was not visible.

2.5. Data processing and statistical analysis

We used IBM SPSS Statistics, Version 23.0, for all analyses and considered P values < 0.05 as statistically significant.

2.5.1. Physiological measurements

Thermography data were calculated as the temperature change relative to the baseline temperature in °C. A generalized linear mixed model with setting (I, II, III or IV) as fixed effects and individual guinea pig as random effect were used. As effect size, the linear coefficient (b) was calculated, i.e., the difference in the temperature changes. Influence of additional factors (day of data collection, sex, room temperature, previous AAT experience, percentage of time being petted) was tested by including them as covariates into the model. Model diagnostics included visual checks for normality of residuals. All residuals were approximately normally distributed. To check for a possible confounding effect of the different setting lengths, we fitted an additional model equivalent to the previous one but including only the first five minutes of each setting.

2.5.2. Behavior observations

Data analysis was performed analogously to Gut and colleagues [17].

We analyzed countable behavior data using a generalized linear mixed model with Poisson distribution. In case of overdispersion, observed via deviance (DF), we fitted a model using the negative binomial distribution. Setting was used as a fixed effect, and individual guinea pigs were used as random effect. The logarithmized duration of the session was specified as an offset variable to correct the video length so that each video had the same impact regardless of its length. As effect size, the incidence rate ratio (IRR) was calculated, that is, the relative change in the rates of the observed event. To analyze the data of state behaviors, the arcsine transformed percentage of the analyzed time in total was used. A generalized linear mixed model with setting (I, II, III or IV) as fixed effects and individual guinea pig as random effect was used. As effect size, the linear coefficient (b) was calculated, that is, the difference in the proportions but estimated on arcsine scale. Influence of additional factors (day of data collection, sex, room temperature, previous AAT experience) was tested by including them as covariates into the models. The following behaviors were shown too rarely to be analyzed: Resting, jumping, drinking, sociopositive social behavior, socionegative social behavior, sociopositive HAI.

Model diagnostics included visual checks for normality of residuals. All residuals were approximately normally distributed. We fitted a second model for both countable and state bahaviors equivalent to the previous ones but including only the first five minutes of each setting to check for a possible confounding effect of the different setting lengths.

3. Results

3.1. Physiology: eye temperature compared within and between different settings

3.1.1. Temperature changes relative to baseline temperature within each setting

Eye temperature changes relative to the baseline temperature led to the following results: The therapy setting with retreat (I) and without retreat (III) resulted in an increase of the mean eye temperature of 0.36 ± 0.40 °C and 0.20 ± 0.39 °C, respectively, relative to the baseline temperature. No change in the mean eye temperature compared to baseline was found during therapy without conspecifics (II) and the setting without HAI (IV).

3.1.2. Comparison of relative eye temperatures between settings

Comparing the relative eye temperatures between the different therapy settings led to the following results: In the therapy setting with retreat (I), there was a greater increase in mean eye temperature

Table 2
Relative changes in eye temperature (difference to baseline) compared between different settings.

Setting	N	NoR	D	M	Coef	95% CI	P value	
(I) Therapy with retreat	38	*157.58 ± 53.33*	30	0.36 ± 0.40	*Ref*			
(II) Therapy without conspecifics	29	33.72 ± 12.90	5	-0.01 ± 0.41	-0.38	-0.52 to -0.23	< 0.001	***
(III) Therapy without retreat	36	38.23 ± 14.22	5	0.20 ± 0.39	-0.16	-0.28 to -0.04	0.012	*
(IV) Setting without HAI	36	123.84 ± 52.68	30	0.00 ± 0.40	-0.36	-0.48 to -0.25	< 0.001	***

N, number of sessions; NoR, Number of readings per session ± standard deviation; D, Duration of setting in minutes; M, mean relative to baseline temperature in degree Celsius ± standard deviation; Coef, coefficient (effect size); CI, confidence interval.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistically significant compared to setting I (Therapy with retreat).

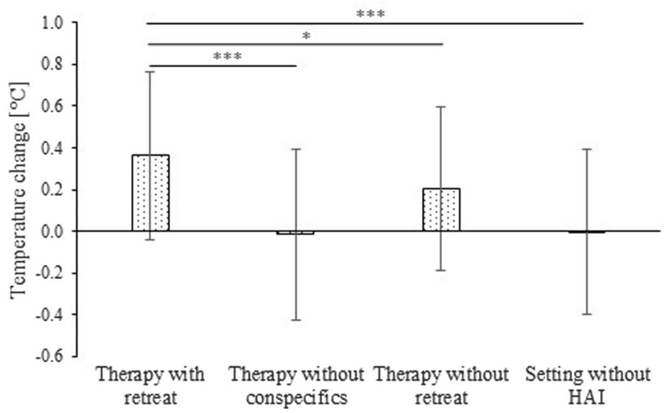

Fig. 3. Mean eye temperature changes relative to the baseline for the different settings. Error bars denote one standard deviation of the mean, * $p < 0.05$, *** $p < 0.001$ statistically significant compared to setting I (therapy with retreat).

relative to baseline than in all other settings (II – IV) (Table 2, Fig. 3).

The day of data collection (one to six) had an influence on thermography values. As data collection for each guinea pig group progressed, the measured mean relative eye temperature dropped continuously from collection day one to collection day six (b = -0.05, CI = -0.09 to - 0.03 $P = 0.035$). For this reason, the models were also run with the data collection day as covariate. The results did not differ significantly from the results presented above and the significance levels of the *P*-values remained the same. The analysis of the first five minutes of all settings did result in the same findings as the analysis of the whole data (see supplement, Table 11)

3.2. State behaviors compared between different settings

3.2.1. Location

The percentage of time spent hidden in a shelter did not differ between the settings (Table 3). The guinea pigs more often came to the board part of the enclosure in the setting with retreat and conspecifics (I) compared to the therapy with retreat but without conspecifics (II) (b = -19.30, CI = -26.65 to -11.95, $P < 0.001$) and the setting without HAI by trend (IV) (b = -9.19, CI = -18.37 to -0.01, $P = 0.050$). They spent more time in the enclosure part but outside of shelters in the therapy without conspecifics but with retreat (II) (b = 11.48, CI = 3.84 to 19.83, $P = 0.004$) and in the setting without HAI (IV) (b = 15.16, CI = 8.27 to 22.05, $P < 0.001$) compared to therapy with conspecifics and retreat (I).

3.2.2. Locomotive behavior

The guinea pigs spent more time moving around in the therapy with retreat (I) than in the therapy without retreat (III) (b = -6.27, CI = -9.46 to -3.08, $P < 0.001$) and the setting without HAI (IV) (b = -6.52, CI = -8.36 to -4.68, $P < 0.001$) (Table 3). On the other hand they spent less time standing still in the therapy with retreat (I) compared to the setting without HAI (IV) (b = 5.48, CI = 2.70 to 8.26, $P < 0.001$). In the therapy without retreat (III) the percentage of time spent freezing was much higher than in the therapy with retreat (I) (b = 17.59, CI = 12.11 to 23.07, $P < 0.001$).

3.2.3. Ingestion

The guinea pigs spent the most time eating in the setting without HAI (IV). Compared to that, time spent eating was significantly reduced in the therapy setting with retreat (I) (b = 7.15, CI = 3.56 to 10.74, $P < 0.001$) (Fig. 4). Moreover, the guinea pigs spent less time eating in the therapy setting without retreat (III) compared to the therapy with retreat (I) (b = -18.28, CI = -26.76 to – 9.80, $P < 0.001$). No significant differences in eating behavior were observed between the therapy with retreat (I) and the therapy without conspecifics (II).

3.2.4. Passive HAI

The guinea pigs were petted (b = 26.93, CI = 21.60 to 32.26, $P < 0.001$) and held (b = 14.20, CI = 11.26 to 17.13, $P < 0.001$) longer during therapy without retreat (III) compared to therapy with retreat (I). They allowed being petted longer during therapy with conspecifics (I) compared to therapy without conspecifics (III) (b = -2.65, CI = -4.08 to -1.21, $P < 0.001$).

3.2.5. Vocalization

Guinea pigs spent less time vocalizing in the therapy without conspecifics (II) in comparison to the therapy with conspecifics (I) (b = -2.43, CI = -4.25 to -0.61, $P = 0.009$).

3.3. Count behaviors compared between different settings

3.3.1. Hiding

Guinea pigs retreated more frequently into shelters in the therapy with retreat (I) (b = 0.53, CI = 0.40 to 0.69, $P < 0.001$) compared to the setting without HAI (IV) (Table 4).

3.3.2. Startling

The guinea pigs startled more often in the setting with retreat (I) compared to the setting without HAI (IV) (b = 0.25, CI = 0.14 to 0.44, $P < 0.001$) and compared to the setting without retreat (III) (b = 0.25, CI = 0.07 to 0.87, $P = 0.03$) (Fig. 5).

3.3.3. Freezing

Freezing occurred more often when guinea pigs were on the lap and thus had no retreat available (III) (b = 12.02, CI = 8.40 to 17.20, $P < 0.001$) compared to the therapy with retreat (I) (Fig. 6).

3.3.4. Explorative and comfort behavior

No significant differences in frequencies of explorative or comfort behaviors were found between the settings I to IV. There was a tendency that explorative behavior was shown more often in therapy with retreat (I) compared to the setting without HAI (IV) (b = 0.41, CI = 0.16 to 1.07, $P = 0.07$) (Table 4).

3.3.5. Socionegative active HAI

Guinea pigs showed more socionegative interactions towards humans in the therapy without retreat (III) (b = 6.98, CI = 3.69 to 12.30, $P < 0.001$) compared to therapy with retreat (I). They also showed more socionegative interactions towards humans in the setting with conspecifics (I) compared to the setting without conspecifics (II) (b = 0.21, CI = 0.06 to 0.79, $P = 0.02$).

3.4. Other factors

The longer the guinea pigs were petted during the settings, the higher the increase in eye temperature was relative to baseline (b = 0.57, CI = 0.14 to 1.00, $P = 0.010$). The day of data collection had a significant influence on eye temperature during setting and was, therefore, used in the models (see Section 3.1). The following factors did not have a significant influence on thermography results: sex, absolute room temperature and experience in AAT prior to this study (see Supplement, Table 8).

The absolute room temperature had an effect on behavior. The mean room temperature over all the sessions was 18.43 ± 1.47 °C. In therapy with retreat (I) the room temperature was 18.37 ± 1.46 °C, in therapy without conspecifics (II) it was 18.46 ± 1.44 °C, in therapy without retreat (III) it was 18.41 ± 1.55 and in setting without HAI (IV) it was 18.47 ± 1.44 °C. Higher room temperatures at the beginning of each data collection correlated with more frequent hiding (b = 1.17, CI = 1.09 to 1.26, $P < 0.001$) and less time on the board part with the

Table 3
State behaviors in percentage of the observed time compared between different settings.

	Behavior	Setting	N	M	SD	Coef	95% CI	*P* value	
Location	Hiding	(I) Therapy with retreat	38	26.67	24.37	*Ref*			
		(II) Therapy without conspecifics	36	28.87	31.29	1.42	-6.11 to 8.95	0.71	
		(IV) Setting without HAI	37	18.28	15.03	-6.00	-13.01 to 1.01	0.09	
	In enclosure unsheltered	(I) Therapy with retreat	38	37.56	25.46	*Ref*			
		(II) Therapy without conspecifics	36	55.27	32.39	11.84	3.84 to 19.83	0.004	**
		(IV) Setting without HAI	37	59.47	21.37	15.16	8.27 to 22.05	< 0.001	***
	On board part	(I) Therapy with retreat	38	35.77	30.56	Ref			
		(II) Therapy without conspecifics	36	15.87	25.75	-19.30	-26.65 to -11.95	< 0.001	***
		(IV) Setting without HAI	37	22.25	19.69	-9.19	-18.37 to -0.01	0.050	
Locomotion	Moving	(I) Therapy with retreat	38	12.26	4.96	*Ref*			
		(II) Therapy without conspecifics	36	10.74	6.63	-2.67	-6.18 to 0.84	0.14	
		(III) Therapy without retreat	36	7.34	7.48	-6.27	-9.46 to -3.08	< 0.001	***
		(IV) Setting without HAI	37	5.79	2.59	-6.52	-8.36 to -4.68	< 0.001	***
	Standing Still	(I) Therapy with retreat	38	85.27	6.71	*Ref*			
		(II) Therapy without conspecifics	36	81.69	21.19	-2.26	-7.96 to 3.45	0.44	
		(III) Therapy without retreat	36	75.45	19.47	-5.44	-10.48 to -0.39	0.035	*
		(IV) Setting without HAI	37	90.96	7.64	5.48	2.70 to 8.26	< 0.001	***
	Freezing	(I) Therapy with retreat	38	0.68	0.72	*Ref*			
		(II) Therapy without conspecifics	36	1.68	4.68	0.17	-1.63 to 1.97	0.85	
		(III) Therapy without retreat	36	17.17	17.79	17.59	12.11 to 23.07	< 0.001	***
		(IV) Setting without HAI	37	1.51	2.40	1.71	0.52 to 2.89	0.005	**
Ingestion	Feeding time	(I) Therapy with retreat	38	78.10	10.76	*Ref*			
		(II) Therapy without conspecifics	35	69.73	30.49	-6.21	-15.10 to 2.68	0.17	
		(III) Therapy without retreat	36	51.33	31.45	-18.28	-26.76 to -9.80	< 0.001	***
		(IV) Setting without HAI	37	87.22	9.13	7.15	3.56 to 10.74	< 0.001	***
Passive HAI	Petted	(I) Therapy with retreat	38	0.79	1.16	*Ref*			
		(II) Therapy without conspecifics	35	0.18	0.65	-2.65	-4.08 to -1.21	< 0.001	***
		(III) Therapy without retreat	36	28.22	20.53	26.93	21.60 to 32.26	< 0.001	***
	Held	(I) Therapy with retreat	38	0.00	0.00	*Ref*			
		(II) Therapy without conspecifics	35	0.00	0.00	0.00	0.00 to 0.00	–	
		(III) Therapy without retreat	36	7.56	8.68	14.20	11.26 to 17.13	< 0.001	***
Vocalization		(I) Therapy with retreat	38	0.97	1.45	*Ref*			
		(II) Therapy without conspecifics	36	0.32	0.73	-2.43	-4.25 to -0.61	0.009	**
		(III) Therapy without retreat	36	2.13	5.95	0.85	-2.76 to 4.45	0.65	
		(IV) Setting without HAI	37	0.80	1.56	-0.28	-2.08 to 1.53	0.77	

HAI, human animal interaction; N, number of sessions; M, mean in % of observed time; SD, standard deviation; Coef, coefficient (effect size), CI, confidence interval. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistically significant compared to setting I (therapy with retreat).

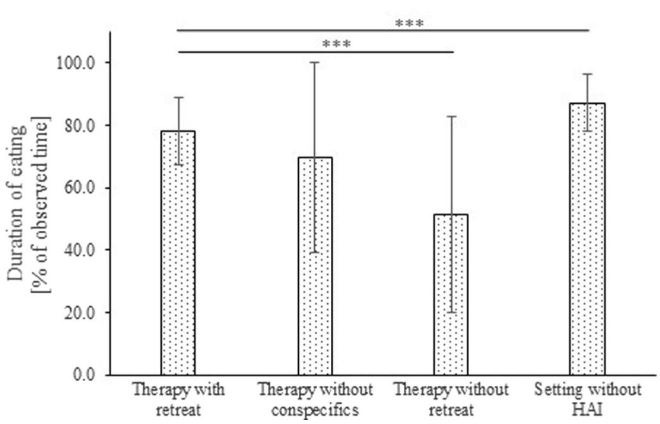

Fig. 4. Duration of eating during the different settings. Error bars denote one standard deviation of the mean, *** p < 0.001 statistically significant compared to setting I (therapy with retreat).

pet bed (b = - 3.33, CI = - 5.90 to - 0.76, P = 0.011). Higher room temperatures correlated with longer time spent freezing (b = 0.88, CI = 0.02 to 1.73, P = 0.44) as well as the tendency for more frequent freezing (b = 1.12, CI = 0.99 to 1.27, P = 0.074). The animals spent less time eating (b = - 2.04, CI = - 4.03 to - 0.055, P = 0.044) and less time vocalizing (b = - 0.71, CI = - 1.24 to - 0.17, P = 0.01) at higher temperatures.

The guinea pigs who already had experience in AAT prior to the study hid (b = 0.59, CI = 0.39 to 0.90, P = 0.013), startled (b = 0.28, CI = 0.13 to 0.59, $P < 0.001$) and froze (b = 0.40, CI = 0.26 to 0.59, $P < 0.001$) less frequently than animals who had not been previously involved in AAT. Furthermore "AAT-experienced" guinea pigs spent more time standing still (b = 7.84, CI = 4.15 to 11.53, $P < 0.001$) and eating (b = 10.71, CI = 3.70 to 17.72, P = 0.003) than the guinea pigs who had no prior experience with AAT.

Female guinea pigs were petted longer than male guinea pigs (b = 3.31, CI = 0.20 to 6.42, P = 0.037). Day of data collection did not have a significant influence on behavioral results (see Supplement, Tables 9 and 10).

There were only minor changes in the results when only data of the first five minutes of all settings were analyzed (see supplement, Tables 12 and 13).

4. Discussion

We identified the availability of a retreat, the presence of conspecifics, previous experience with AAT, and human contact as important factors for changes in behavior and eye temperature in guinea pigs involved in AAT. The influence of these factors are considered consecutively.

4.1. Availability of a retreat and its influence on welfare of guinea pigs

According to our hypothesis that provision of a retreat and giving the animal free choice of interactions are associated with reduced physiological and behavioral stress indicators, we expected a higher physiological stress level and therefore a larger rise in eye temperature in the setting without retreat (III) compared to the setting with retreat (I). However, we could not confirm this hypothesis. During both

Table 4
Frequencies count behaviors compared between different settings.

Behavior	Setting	N	M	SD	RR	95% CI	*P* value	
Hiding	(I) Therapy with retreat	38	0.28	0.21	*Ref*			
	(II) Therapy without conspecifics	36	0.30	0.26	1.06	0.75 to 1.50	0.73	
	(IV) Setting without HAI	37	0.15	0.11	0.53	0.40 to 0.69	< 0.001	***
Startling	(I) Therapy with retreat	38	0.16	0.17	*Ref*			
	(II) Therapy without conspecifics	36	0.10	0.23	0.56	0.26 to 1.18	0.13	
	(III) Therapy without retreat	36	0.03	0.13	0.25	0.07 to 0.87	0.03	*
	(IV) Setting without HAI	37	0.03	0.05	0.25	0.14 to 0.44	< 0.001	***
Freezing	(I) Therapy with retreat	38	0.14	0.14	*Ref*			
	(II) Therapy without conspecifics	36	0.23	0.40	1.74	0.99 to 3.08	0.06	
	(III) Therapy without retreat	36	1.41	0.96	12.02	8.40 to 17.20	< 0.001	***
	(IV) Setting without HAI	37	0.15	0.12	1.22	0.94 to 1.57	0.13	
Explorative behavior	(I) Therapy with retreat	38	0.03	0.06	*Ref*			
	(II) Therapy without conspecifics	36	0.03	0.16	0.79	0.23 to 2.64	0.70	
	(III) Therapy without retreat	36	0.01	0.03	0.23	0.03 to 1.60	0.14	
	(IV) Setting without HAI	37	0.01	0.03	0.41	0.16 to 1.07	0.07	
Comfort behavior	(I) Therapy with retreat	38	0.14	0.09	*Ref*			
	(II) Therapy without conspecifics	36	0.07	0.16	0.48	0.22 to 1.04	0.06	
	(III) Therapy without retreat	36	0.07	0.19	0.76	0.30 to 1.94	0.57	
	(IV) Setting without HAI	37	0.12	0.10	0.97	0.70 to 1.35	0.86	
Socionegative active HAI	(I) Therapy with retreat	38	0.06	0.07	*Ref*			
	(II) Therapy without conspecifics	36	0.02	0.09	0.21	0.06 to 1.79	0.02	*
	(III) Therapy without retreat	36	0.41	0.68	6.98	3.96 to 12.30	< 0.001	***

HAI, human animal interaction; N, number of sessions; M, mean per 60 seconds; SD, standard deviation; RR, rate ratio (effect size); CI, confidence interval.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistically significant compared to setting I (therapy with retreat).

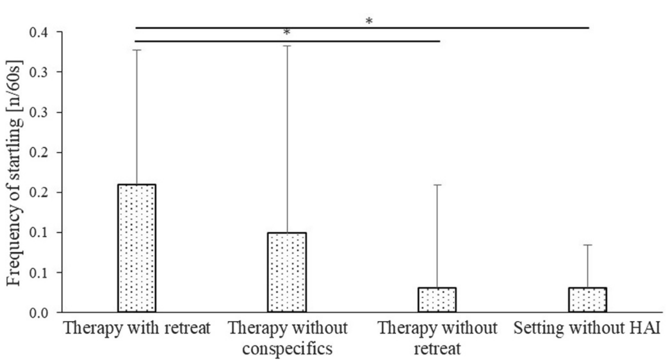

Fig. 5. Frequency of startling behavior in the different conditions. Error bars denote one standard deviation of the mean, * $p < 0.05$, *** $p < 0.001$ statistically significant compared to setting I (therapy with retreat).

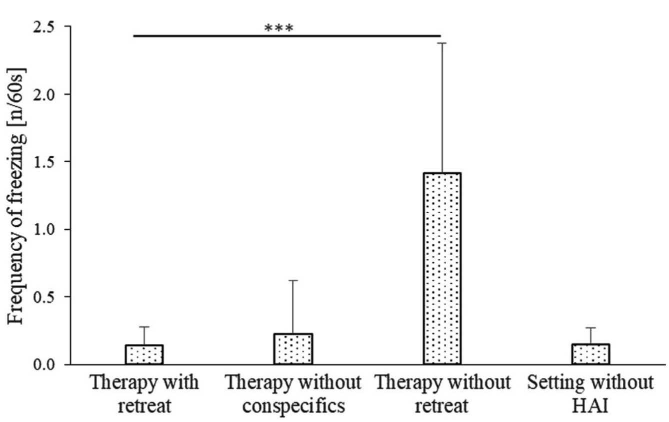

Fig. 6. Frequency of freezing behavior in the different conditions. Error bars denote one standard deviation of the mean, *** $p < 0.001$ statistically significant compared to setting I (therapy with retreat).

settings an increase in eye temperature was measured, and the increase in the setting with retreat (I) was even statistically significantly higher than in the setting without retreat (III).

In contrast to this, the behavior results indicate that the availability of a retreat has a significant effect on the welfare of the guinea pigs. In the setting without retreat possibility, the guinea pigs spent significantly less time eating compared to the setting with retreat possibility. Also, the guinea pigs showed more active behaviors (e.g. more movement, more frequent startling) when they had the possibility to retreat, whereas they showed more passive behaviors (e.g. more standing still, longer and more frequent freezing) with no possibility to retreat. Guinea pigs showed more frequent socionegative human-animal interactions (e.g. head-up and sudden locomotion away from the human) without retreat possibility. All these behavioral results are in line with our hypothesis and with our previous study [17], leading to the conclusion that the lack of retreat can lead to more stress and thus a reduced welfare in guinea pigs. "Reduced eating", "increased startling" and "increased freezing" were identified as behaviors that indicate increasing stress levels in guinea pigs.

The physiological and behavioral results differ. While according to behavioral data the guinea pigs seem to show more stress-associated behaviors when no retreat is available, the physiological arousal is even higher when retreat is possible. This poses a challenge in interpreting our results. However, it must be considered that the measured physiological arousal might be caused either by negative stress, but also by positive excitement or physiological exercise [35,36]. It is possible that the higher active coping in the therapy setting with retreat possibility versus the more passive coping in the therapy setting without retreat possibility might be correlated with a higher physical arousal when retreat is available.

4.2. *Presence of conspecifics and its influence on welfare of guinea pigs*

We hypothesized that the presence of conspecifics leads to reduced physiological and behavioral stress indicators during AAT. To test this hypothesis, we compared the therapy setting with retreat with access to conspecifics (I) to the therapy setting with retreat without access to conspecifics (II). Contrary to our hypothesis, we found that guinea pigs had a significantly higher eye temperature in the presence of conspecifics compared to their absence. Regarding behavioral changes, we found that the guinea pigs came less often to the board part and spent more time in the table enclosure when conspecifics were absent. Previous research showed that the presence of bonding partners could reduce physiological and behavioral reactions in guinea pigs in stressful situations [9,37]. Sachser and colleagues [9] call this phenomenon 'security-giving and arousal-reducing structure'. Our findings only partly support this, since we measured higher physiological arousal in the presence of conspecifics. But as noted, whether the increased eye temperature was caused by negative stress, increased physical activity, positive arousal or a combination of different factors remains unclear and requires further research.

Interestingly, the guinea pigs in our study vocalized less often when alone compared to being with their social group. This result indicates that vocalizing in our study was mainly communication between conspecifics in the same enclosure rather than calling for conspecifics when isolated or communication with the patient.

4.3. Experience with AAT prior to the study and habituation and its influence on welfare of guinea pigs

The guinea pigs' prior experience in AAT did not have an influence on eye temperature. However, AAT-experienced guinea pigs showed significantly less stress-associated behaviors such as hiding, startling and freezing compared to inexperienced animals. It remains unclear whether the experienced guinea pigs showed less stress-associated behavior because of specific character traits and were selected for involvement in AAT for this reason, or whether these behaviors were reduced over the duration of their therapy involvement due to habituation, which would be in line with Miller et al [38] and also seen in other species [39]. This question should be investigated in future studies.

Additionally to these long-term effects, the mean eye temperature relative to baseline decreased over the course of the test days in all guinea pigs, regardless of previous experience in AAT, indicating a habituation to this certain specific test setting. This indicates that the proposed acclimatization time in literature [32] might be insufficient. In future studies, items like the cameras should be introduced earlier. A certain degree of predictability of events, as well as contact with a permanent caregiver and a consistent arrangement of the therapy room and the enclosure could be reasons for the habituation seen in our study. These factors might therefore be important to consider when involving guinea pigs in AAT.

4.4. Human-animal interaction and its influence on welfare of guinea pigs

The presence of a human led to an increase of the eye temperature compared to the setting without HAI. Whether this result is caused by negative stress [22,24,25,28–30], by increased physical activity [35], as they spent significantly more time in locomotion when a human was present, by positive arousal [36], e.g. due to a particularly tasty food offer (although the same food was offered in each experiment) or by a combination of different factors remains unclear and requires further research. Still, we found a significant relationship between the length of time a guinea pig was petted and the rise of the eye temperature independent of the setting. Guinea pigs are highly social animals living in a group with strong social bonds. Nevertheless, they show very little close body contact with their conspecifics [10,40]. This could be a reason why physical contact from humans, such as petting, might cause stress in guinea pigs.

Behavior results revealed that guinea pigs in the setting without HAI spent most of their time in the table enclosure standing still (but not freezing), whereas they showed significantly more locomotive behavior and came to the board part much more frequently in the therapy setting with retreat, even though the same amount and type of food was available in both conditions. However, we also found decreased time spent eating, increased frequency of hiding and increased startling in the guinea pigs exposed to human contact.

We conclude that interaction with humans might lead to a certain degree of stress but no extreme stress-associated behaviors (e.g., eye-closing, piloerection, or attempts to flee like jumping out of the pet bed on the patient's lap) were observed in any therapy setting.

4.5. Influence of sex

We did not find an influence of sex on either eye temperature or behavioral outcomes. We controlled for influences of the sexual cycle of intact females and the differences between sexes by analyzing changes in the eye temperature relative to a baseline. We did not focus on cycle-dependent behaviors in females in this study, but we only included intact females and spayed males, which should not display typical male sexual-associated behaviors.

4.6. Limitations

The behavioral coding in this study could not be blinded. However, as in the previous study [17], the person coding the videos was not involved with AAT before the study and we used the same detailed coding scheme. Moreover, intra-rater and inter-rater reliability was high. The problem of multiple testing was carefully examined [41]. Since it was impossible to avoid multiple testing completely, the focus was set on reducing the number of specific questions. However, this study must be viewed as explorative, and results must be interpreted with caution. The low number of investigated animals and the relatively high variability of the values led to a relatively large standard deviation in the physiology results.

For ethical reasons, the duration of the therapy setting without retreat (III) and the therapy setting with retreat without access to conspecifics (II) was only five minutes whereas we chose a duration of 30 minutes for the therapy setting with retreat and conspecifics (I) and the setting without HAI (IV) to maximize external validity for these settings. Since the different length of the four settings might be a confounding factor, a habituation phase of 30 minutes before the start of each setting was implemented. Moreover, the results from the thermography and the behavioral analyses of only the first five minutes of all settings mostly stayed constant and led to the same conclusions as discussed above.

We obtained contradictory results regarding behavior and eye temperature, making interpretation difficult. But it shows the value of concurrent methods of stress measurement, as signs of stress might be missed when relying only on a single method. Observed behavior must also be interpreted with caution. Guinea pigs have very complex communication and behavior patterns [9,40]. In this study, the clear and distinct behaviors were coded with the ethogram (see Table 7). However, it is possible that there are much more subtle behaviors, which are also associated with stress, that were not perceptible in the video recordings. It is unclear to what extent our interpretation of the guinea pig observed behaviors correspond with their perception, health or longevity outcome, although we do have a simultaneous physiological measurement. As a prey species, they are likely adapted to frequent, but short, stressful situations. We did not measure the duration of the stress levels due to AAT. Further research on the long-term implications on guinea pig health and longevity should be done in the future.

4.7. Strengths

This is the first study investigating physiological as well as behavioral effects in guinea pigs assisting in AAT. We replicated findings of our previous study [17] in a larger population of guinea pigs and extended the design. In this study, the rise in eye temperature as an estimate of core body temperature served as an indicator of physiological stress. To our knowledge, this is the first study to use eye temperature of guinea pigs to assess stress levels. Moreover, we investigated the effect of the factors retreat possibility, presence of conspecifics, previous experience with AAT, and human contact, by comparing different types of settings. The randomized assignment of the order of the different settings for each individual guinea pig ensured that habituation effects influencing the results of the study were minimized. We also carefully controlled for confounding factors to ensure high internal validity. To reduce variance and to standardize interactions as much as possible, we did not work with real patients. This ensured that the guinea pigs were handled in the same way in each setting. However, we designed the interactions in a very similar way to real situations to reflect actual practice. The person acting as patient observed several actual therapy sessions before the study start. This ensures high external validity.

5. Conclusions

Our results suggest that availability of retreat, presence of conspecifics, experience with AAT and human contact can have important influences on the welfare in guinea pigs involved in AAT. Petting was identified as a key factor leading to stress in guinea pigs because it was associated with an increase in eye temperature. Based on our results, we identified the behaviors “reduced eating”, “increased startling” and “increased freezing” as indicators of increasing stress level and reduced welfare. This study supports our previous hypotheses that a “good practice” for guinea pig-assisted interventions includes retreat possibilities for the animals whenever possible to ensure free choice of human interaction, a certain degree of predictability of events (e.g. a consistent arrangement of the therapy room) and presence of conspecifics. Moreover, it is important that the involved animals have time to adapt and are carefully accustomed to new settings. In this way, stress can be reduced. Considering our observations, we can plan AAT in a way to minimize the tradeoff of human health benefits against reduced animal welfare, within the paradigm of One Health, seeking incremental benefits from a closer collaboration of human and animal health.

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Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2020.113076.

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