Thoughtful or distant farmer: Exploring the influence of human-animal relationships on rabbit stress, behaviour, and emotional responses in two distinct living environments

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Abstract

Both the nature of the human-animal relationship (HAR) and housing conditions significantly impact the welfare of farmed animals. To evaluate the influence of HAR on the behaviour, emotions and stress of rabbits (*Oryctolagus cuniculus*) in two distinct outdoor living environments, we allocated 144 young rabbits to four groups (CPX-H, CPX-N, SPL-H, SPL-N) differing in the living environments (CPX for complex, and SPL for simple). The treatment by human (H) involved daily provision of additional food resources and stroking (thoughtful farmer). It commenced at 49 days of age and lasted for 16 days. N groups did not receive the treatment (distant farmer). The rabbits were observed between 48 and 73 days of age. Their behavioural responses to human presence were evaluated at 48 and 68 days using Qualitative Behaviour Assessment (QBA) and scan sampling. A set of tests was conducted at 68 days of age to assess their reactions to a novel object and human presence. Stress levels were measured by analysing corticosterone concentrations in their hair. Rabbits in the SPL environment spent significantly more time near the novel object than those in the CPX environment (24.7 vs 17.2%). Additionally, rabbits in the H treatment group spent more time near the human than those in the N treatment group (28.2 vs 17.1%) and accepted more strokes (90.2 vs 45.9%). Following the HAR treatment, rabbits in the H group were significantly more likely to be described as 'Affectionate/Interested' than those in the N treatment. Rabbits in the N treatment were described as 'Indifferent' significantly more in the SPL environment. However, there were no significant differences in hair corticosterone concentrations between the groups. These findings indicate that rabbits' responses are influenced by both their living environment and the quality of their relationship with humans. Encouraging positive interactions with animals may enhance their welfare and facilitate daily care from farmers.

Introduction

Animal welfare encompasses more than simply minimising negative emotions; it also involves fostering positive ones. For instance, Fetiveau *et al.* (2023) demonstrated that providing growing rabbits (*Oryctolagus cuniculus*) with outdoor access to pasture allowed them to engage in a variety of natural behaviours, with grazing being the most prominent — behaviours that indoor pens did not support. Promoting positive emotions can be achieved by enhancing the living environment with materials or natural enrichment, thus increasing its complexity (Wood-Gush & Vestergaard 1989). Newberry (1995) highlighted that offering a diverse range of food types and adding structural complexity to the environment can serve as valuable enrichment for farm animals. Outdoor access, for example, facilitates exploration and patrolling, enriching the animals' experience. Additionally, the presence of a farmer can also act as a form of enrichment, especially when the human-animal relationship is positive (Claxton 2011). Research has shown that a positive human-animal bond can yield immediate benefits by promoting positive emotions (Boissy *et al.* 2007) and long-term advantages by enhancing stress resilience (Rault *et al.* 2020). A positive perception of humans by animals can reduce stress during handling and husbandry. Conversely, a negative human-animal relationship, where animals perceive humans negatively, can impair growth, health, and welfare, complicating daily care for farmers (Pinillos *et al.* 2016).

In France, over 90% of meat rabbits are raised in wire cages in intensive production farms without outdoor access (France AgriMer 2024). In these systems, no positive relationship between the farmer and the animals is formed. Two main factors limit human-animal interactions: (i) high stock density, with 600 reproducing females per farmer (European Food Safety Authority [EFSA] 2020), and (ii) cage housing, which restricts physical contact between the farmer and the rabbits. Additionally, aversive physical interactions that animals experience throughout their lives, such as vaccination, tattooing, weighing, insemination, and transport, can reinforce their fear of humans. In organic rabbit production systems in which rabbits have access to outdoor grazing areas, positive physical interactions between farmers and animals can also be minimal. This is as a result of the size of the paddocks or the use of mobile cages which prevent farmers from entering the rabbits' living areas (Gidenne *et al.* 2024). Such low levels of interaction can lead to handling problems and a higher stress in response to humans, as has been shown in calves (Boivin *et al.* 1994).

Estep and Hetts (1992) showed that animals can perceive humans in one of five ways: as predators; as prey; as part of the environment with no social connections; as symbionts; or as conspecifics, depending on their familiarity with humans and the predictability of the relationship. Since rabbits are small prey animals their perceptions of humans can significantly impact their stress levels and emotional states. Compared to indoor housing, rabbits living outdoors are exposed to various stimuli or threats (visual, olfactory, and auditory), which can increase their stress level. However, a more complex living environment could reduce stress from certain stimuli by allowing the animals to express avoidance responses and hide. For instance, wild rabbits use and explore their environment, including shelters and shrubs, to express their natural anti-predator behaviour (Villafuerte & Moreno 1997). Fetiveau *et al.* (2021) demonstrated that rabbits with outdoor access exhibited lower reactivity (i.e. the capacity to respond to a stimulus) towards humans or a novel object compared to rabbits living exclusively indoors. This reduced reactivity may indicate diminished fear of humans or a lack of interest in humans, who may be perceived as a less significant part of the environment.

A transition towards farming systems that respect animal welfare is demanded by EU citizens (Delanoue *et al.* 2018). Pasture-based systems, which provide rabbits with access to outdoor spaces, combined with other food production systems such as agroforestry, can improve animal welfare and reduce the environmental impact of livestock production (Bonaudo *et al.* 2014). Agroforestry, which integrates trees and animals, enhances the complexity of the living environment for farm animals. However, depending on the orchard's architecture (e.g. the height of the lowest branches, the distance between trees, and the placement of pens around trees; see Savietto *et al.* 2023), it may, to some extent, increase ergonomic risk factors for farmers in the daily care of their animals. Thus, it is challenging for humans to stand under trees and catch small, fast animals. In this context, fostering a positive human-animal relationship may assist farmers in the daily care of their animals, as familiar rabbits are less likely to avoid humans. This relationship can also benefit the animals by improving their welfare, health, and growth.

This study aimed to describe the effect of a treatment involving the provision of an additional food resource and stroking by humans, compared to no treatment, on rabbits' behavioural and emotional responses to their breeder and their stress levels in two different outdoor environments (complex: pastures under an apple orchard; or simple: pastures without an orchard). We hypothesised that treatment with a human could be positive for rabbits. Specifically, rabbits provided with a novel food resource and exposed to stroking by humans could exhibit more positive emotional reactions towards humans and an attenuated stress response compared to those not receiving this treatment. Additionally, we hypothesised that outdoor living conditions could influence stress levels: rabbits in the complex environment (apple orchard) may have lower stress levels compared to those in the simple environment (grassland). We developed the Qualitative Behaviour Assessment (QBA) for rabbit species for the first time. Our aim being to describe the correlation between emotional states and quantitative behavioural responses. Additionally, we compared rabbits' hair corticosterone concentrations to assess stress levels.

Material and methods

Animals were handled in accordance with the recommendations of the European Union (Directive 2010/63/EU) and French legislation on the protection of animals used for scientific purposes (2013). All protocols were approved by the Ethics Committee No 115 of the French Ministry of National Education, Higher Education, and Research (authorisation number APAFIS #35391-2021091717004334 v6). The experimental farm was approved by the French Ministry of Agriculture (approval number A263131402).

A chronogram of the entire experiment is available in the Supplementary material (see Figure S10).

Study animals and housing conditions

A total of 144 crossbreed rabbits (½ PS119 × ⅛ Burgundy × ¼ INRA-1777 × ⅛ French-Lop) of both sexes (50% males and 50% females) were raised for 28 days starting from weaning (at 45 days of age; 17th October 2022) to 73 days of age (14th November 2022). Rabbits were housed outdoors in wooden shelters (100 × 52 × 92 cm; length × width × height) placed in an 18.75 m^2 paddock (7.5 × 2.5 m; length × width) in groups of six animals (three males and three females), giving a total of 24 housing units. Each shelter consisted of two distinct areas: a ground floor and a first floor. The ground floor had no features except a ramp-stair. The first floor (made of wood) was divided into two areas: (i) an entrance plus a feeding area, equipped with a feeder; and (ii) a resting area, covered with barley straw and untreated wood chips. The outdoor area was demarcated by eight rigid mesh fencing panels (2.5 × 0.83 m; width × height) lined with a triple torsion fence (2.5 × 2.5 × 0.5 m; length × width × height). This is a new housing system for rabbits, based on agroecology principles (low animal density and interspecific services between apple trees and rabbits) and designed by Savietto *et al.* (2023).

Rabbits were born indoors in wire cages. At weaning, rabbits of both sexes were evenly distributed into two experimental groups based on paddock location: a complex environment (CPX; n = 72 rabbits, 12 paddocks of six rabbits each; see Figure S1 in Supplementary material) located within a row of four apple trees in a pasture sown with *Festuca arundinacea*, *Dactylis glomerata*, *Lolium perenne*, *Onobrychis viciifolia*, *Lotus corniculatus* and *Trifolium repens*; and a simple environment (SPL; n = 72 rabbits, 12 paddocks of six rabbits each; see Figure S2 in Supplementary material) situated in a pasture area consisting solely of *D. glomerata*, without any trees.

Throughout the experiment, no artificial lighting was provided, and the rabbits had unrestricted access to water and grass. They were fed a pelleted diet free of coccidiostats (STABIGREEN, Terrya, Rignac, France) *ad libitum* which contained 15.4 MJ of gross energy per kg of dry matter (DM), 15.6% crude protein, 37.3% neutral detergent fibre, 20.1% acid detergent fibre, and 6.4% acid detergent lignin on a DM basis. The pellets were provided in a hopper feeder located in the first floor feeding area of the shelter. The feeder was always filled to ensure it never became empty. To allow the rabbits

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to graze continuously throughout the growing period, each paddock was moved to a new location once a week, maintaining a minimum distance of 3 m between paddocks (see Figures S1, S9, S10 in Supplementary material).

Treatment, living environment, and experimental groups

In each living environment (CPX or SPL), rabbits were exposed to two distinct types of human-animal relationships. Half of the rabbits (n = 72, 12 paddocks of six rabbits each, evenly divided between CPX and SPL) received a treatment by humans (H treatment), designed to enhance the human-animal relationship (HAR), while the other half received no treatment (N treatment) and served as a control. Consequently, there were four experimental groups: CPX-H, CPX-N, SPL-H and SPL-N.

In the no treatment (N) group (12 paddocks with six rabbits each), rabbits experienced minimal human contact. They were only observed for daily supervision (1 or 2 min twice a day to check on the rabbits without handling them or entering the paddock), food delivery, and water provision (5 min once a week). Litter-box cleaning and provision of a new paddock occurred weekly, during which the rabbits were temporarily confined in their shelter for 15 min while the paddock fences were moved. Weekly health assessments and weighing were also conducted; the rabbits were gently closed in the shelter and individually picked up for a brief veterinary examination and weighing before being returned to the paddock. The shelter was reopened 15 min after the examination of all six rabbits was completed. During weighing, the rabbits were carefully grasped by the skin on their backs, with hands supporting their hind legs, and immediately placed in a weighing box, in accordance with standard handling techniques used for the safe handling of rabbits on commercial farms. In the human treatment (H) group (12 paddocks with six rabbits each), rabbits received the same husbandry as the no treatment group but were given additional human interaction. The experimenter spent approximately 10 min each day in each H paddock (5 min in the morning between 0700 and 0900h and 5 min in the early evening between 1600 and 1800h). During this time, the experimenter sat in the enclosure close to the shelter and offered each rabbit an additional food resource (Cuni Adult, Versele-Laga, Kapellestraat, Belgium). As the rabbits became more familiar and approached, they were individually stroked while eating the provided food. All six rabbits in an H paddock systematically received the treatment (H). The rabbits were spoken to in a soft voice and any necessary handling entailed rabbits being gently grasped with both hands with one hand supporting the chest and the other wrapped around the pelvis, avoiding any grasping by the back skin.

Whenever the rabbits from both groups (N or H) were subjected to potentially uncomfortable procedures (such as catching, handling, tattooing, vaccinating, or weighing), the humans involved wore white outfits to help the animals associate the colour with the subsequent activity. For all other interactions, the humans responsible wore blue tops and green trousers. The choice of clothing colour was based on the visual range of rabbits, which is capable of perceiving several shades of green, blue, and white (Gidenne 2015). The same four individuals were responsible for the rabbits' husbandry (feeding, weighing, cleaning, etc), with two of them also involved in the treatment by human. The treatment lasted 16 days, from 49 days of age (October 21, 2022) to 64 days of age (November 5, 2022) (see Figure S10; Supplementary material).

Tests

Two tests were conducted to assess the rabbits' behaviour and their relationship with humans. Each test consisted of two stages (adapted from Waiblinger *et al.* 2006 and Graml *et al.* 2008): (i) the proximity stage; and (ii) the touch stage. The first test evaluated the rabbits' response to a human (human test or 'HT'), while the second assessed their response to a novel object (novel object test or 'NOT'). All paddocks (n = 24, including 6 CPX-H, 6 CPX-N, 6 SPL-H, and 6 SPL-N) underwent the same tests on the same date. The rabbits were familiar with the testers, as the two tests were performed by the same individuals responsible for the treatment by human and husbandry. Each rabbit had a visible ear tattoo and could also be identified by the colour and patterns of their fur. The person responsible for video recording (tester 1) had a broad field of vision and assisted tester 2 in selecting the next rabbit for the tests.

The tests were conducted in the rabbits' paddocks during the day. When some rabbits were resting inside their shelter before the test, they were gently placed outside their shelter and the door was closed to facilitate testing of the entire group (all six rabbits). Before starting the test, tester 2 closed the shelter and ensured that the rabbits were not too scattered across the paddock area. If the rabbits were dispersed, tester 2 gently herded them closer to the centre of the paddock. Tester 2 waited approximately 2 min after entering each paddock before beginning the test. During the test, no food was provided to the rabbits.

Human Test (HT), proximity stage

The HT proximity stage was conducted when the rabbits were 68 days old (see Figure S10; Supplementary material). Tester 2 stood in the centre of each paddock in front of the hut and filmed her feet for 2 min. A GoPro Hero 7 camera (GoPro, San Mateo, USA) was mounted on tester 2's chest using a chest harness, while tester 1 recorded the entire scene from outside the paddocks. At 10-s intervals, the number of rabbits in very close contact with tester 2 (within < 10 cm) was counted. For each rabbit within this defined area, the latency (s) to approach (within < 10 cm of the test person's feet) or touch tester 2 for the first time was recorded. Additionally, the total time spent in close proximity (< 10 cm) to tester 2 was calculated for each rabbit.

Human Test (HT), touching stage

The touching stage of the HT was conducted immediately following the proximity stage (see Figure S10; Supplementary material). All rabbits were individually tested, except for two individuals (one from the SPL-N group and one from the CPX-N group), who had been nursed for several days in close contact with humans for health reasons. Tester 2 slowly approached each target rabbit from approximately 2 m away, moving at a rate of one step per second. Upon reaching a distance of roughly 30 cm from the animal, tester 2 knelt for approximately 10 s and attempted to stroke the rabbit on the back or flank, delivering one stroke per second up to a maximum of five strokes per animal. Three trials of five strokes each were conducted, with each attempt to approach a rabbit considered a trial, allowing for a maximum of 15 accepted strokes per rabbit. The number of successful strokes was recorded for each rabbit (ranging from a minimum of 0 to a maximum of 15 strokes). The test was video recorded by tester 1, using a semi-professional

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digital camera (Panasonic DMC-FZ300, Panasonic Corporation, Kadoma, Osaka, Japan) from outside the paddock.

Novel Object Test (NOT), proximity, and touching stages

The Novel Object Test (NOT) was conducted when the rabbits were 69 days old (see Figure S10; Supplementary material). It was designed similarly to the Human Test and performed independently. The aim was to compare the rabbits' reactions to a human versus a novel object, confirming that the responses to humans were not solely attributable to the rabbits' temperament or reactivity but were a result of the H treatment. The novel object was a yellow tennis ball attached to a 150-cm long metallic broomstick, which was handled by tester 2 and placed in the centre of each paddock. Tester 2 remained outside the paddock during the test. In the proximity stage, the number of rabbits within a radius of < 10 cm around the ball was counted at 10-s intervals for 2 min. The latency to approach (< 10 cm from the ball) or touch the ball for the first time was recorded for each rabbit, along with the total time spent close to the ball (within < 10 cm). In the touching stage of the NOT, tester 2 placed the ball near each rabbit and attempted to stroke it repeatedly on one flank, delivering one stroke per second, with a maximum of five strokes per animal. A maximum of three trials (with each attempt to approach a rabbit counted as a trial) was allowed. The total number of strokes was recorded for each rabbit (ranging from a minimum of 0 to a maximum of 15 strokes across all three trials). The test was video-recorded by tester 1 using the same digital camera (Panasonic DMC-FZ300) as before from outside the paddock.

Behavioural evaluation

Quantitative behavioural evaluation

A quantitative assessment of the rabbits' behaviours was conducted when the animals were 48 days old, prior to the treatment, and again at 68 days of age during the first stage of the human test (see Figure S10; Supplementary material). The assessor, who was responsible for the daily husbandry of the animals, the HAR process, and the QBA, performed the evaluations. Each paddock was video-recorded by tester 1 for 2 min with the camera placed outside the paddock, while tester 2 remained in the centre of the paddock. The recorded videos were analysed by a single assessor (tester 2). Behaviours expressed by the group of rabbits were collected for 2 min using the scan-sampling method, in which the assessor observed a different rabbit approximatively every 2 s, scanning the entire group visually. The ethogram comprised nine distinct behaviours: immobility (the rabbit remains stationary in the same position); rearing towards the test person (the rabbit places its front legs on the person and stands on its back legs); approaching the test person (the rabbit moves towards the person); watching the test person (the rabbit clearly observes the person, with its face and eyes directed at the person's head); in contact with the test person (the rabbit makes physical contact with the person); sniffing or nibbling the test person's shoes (the rabbits interact with the person's feet using its nose or mouth); sniffing or nibbling the test person's clothes (the rabbits interact with the person's clothing and body using its nose or mouth); moving away from the test person (the rabbit moves in the opposite direction or runs away); and other activity not associated with the test person (includes behaviours such as grazing, grooming, resting, etc).

Qualitative behavioural evaluation

Designing a Qualitative Behaviour Assessment (QBA) for rabbits

A list of 21 QBA descriptors for rabbits was established based on the work of Wemelsfelder *et al.* (2000, 2001). Over three years, two observers evaluated rabbits in various contexts and spontaneously proposed qualitative descriptive terms to characterise the group atmosphere. They also incorporated some descriptors developed for other species (Souza *et al.* 2021). These descriptors were linked to the animals' body language and facial expressions. The objective was to develop at least sixteen descriptors (and potentially more) that spanned a range from negative to positive valence and from low to high intensity. The two individuals involved in the QBA assessment were trained in the method by expert scientists (see *Acknowledgments*), and the intra-class correlation coefficient (ICC) concordance score was measured during the training (see *Statistical analysis*).

Qualitative Behaviour Assessment (QBA)

The QBA was conducted concurrently with the quantitative assessment, utilising the same video recordings analysed by testers 1 and 2. Both individuals, trained in QBA, independently scored the animals on the 21 descriptors developed (see Table 1 and Table S1; Supplementary material, as well as *QBA design* and video clips) using a 125-mm visual analogue scale to reflect the intensity of expression for each descriptor by each group of rabbits. Each 125-mm visual analogue scale ranged from zero, indicating that the behavioural expression was completely absent in all observed animals, to 125 mm, signifying that the behavioural expression was dominant in all observed animals. To minimise bias, the observers were unaware of which group (H or N) of rabbits was being scored; however, the living conditions were easily recognisable in the videos due to the presence (or absence) of trees. The intra-class correlation coefficient (ICC) concordance score between the observers was calculated over 40% of the recorded time on the raw data scores (see *Statistical analysis*).

Hair corticosterone level

At 42 days of age (prior to weaning), a 5 × 5 cm patch of hair was shaved from the back of each rabbit (beneath the scapular region, around the thoracic vertebrae) using veterinary electric clippers (Super Trim, Wahl GmbH, Unterkirnach, Germany). The shaved hair was discarded. Then, at 73 days of age, approximately 250 mg of hair that had grown since the initial cut was collected from 72 rabbits (18 rabbits per experimental group, with 50% males and 50% females) in the same area that had been shaved previously. The samples were stored at –20°C until corticosterone extraction, following the method adapted from Davenport *et al.* (2006) as described by Fetiveau *et al.* (2023) and Fillon *et al.* (2023). In rabbits, the main glucocorticoid hormone is corticosterone (Heimbürge *et al.* 2019). Cortisol ($C_{21}H_{30}O_5$) and corticosterone ($C_{21}H_{30}O_4$) are structurally similar molecules and can be detected by the same antibodies. We used a competitive immunoassay kit designed for the quantitative measurement of salivary cortisol (Salimetrics® Cortisol Enzyme Immunoassay kit, Salimetrics, LLC, State College, USA), following the procedure developed by Salimetrics®. Briefly, corticosterone was extracted from 50 mg of hair cut into pieces less than 1 mm in size and resuspended in Salimetrics® buffer. Optical density was measured using

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Table 1. List and characterisation of 21 qualitative behaviours descriptors for rabbits

Descriptor	Description
Relaxed	Flabby, relaxed posture; eyes opened or half closed; not agitated; can be at rest both sitting and lying down without signs of stress; ears not directed.
At Ease	Unfazed; comfortable; relaxed attitude and/or state of mind.
Calm	Half asleep, half awake; relaxed; quiet; observant.
Active	Receptive and attentive to the environment; sudden actions and reactions to stimuli.
Vigilant	Tense; attentive; watching; ears raised; may be frozen; careful; on guard.
Alert	Tail may be raised; the animal may be raised and frozen; may stamp; nervous; muscle tension present.
Indifferent	Undisturbed by the environment and other animals; independent; self-sufficient; unshakeable; carries on without feeling disturbed.
Curious	Interested in the environment; explores, smells, looks, and interacts actively; lively and proactive attitude.
Joyful	Engaged in capering jumps or bursts of speed; very active; small jerks and head shakes; wiggling ears; cheerful face (eyes wide open, bright; relaxed cheeks)
Frustrated	Discontent; dissatisfied; may gnaw or scratch (incisively); insists on arranging elements of the environment.
Nervous	Agitated; excited; sudden or frantic jumping or running; may stamp; very reactive; possibly aggressive in response to approaching conspecifics.
Worried	Frequently alert; concerned about the environment; abnormal vigilance toward the group and stimuli; anxious; may stamp.
Scared	Appears frightened; flees or hides; does not dare to show itself; may stamp.
Attentive	Focused and observant.
Inhibited	Unable to act; prostrate; possibly dominated; refrains from acting; ears pinned back; animal pinned to the ground.
Affectionate	Seeks contact; engages in allogrooming; shows affection to humans or congenial companions.
Pleased	Needs are fulfilled; shows satisfaction (satisfied face); happy; delighted.
Unshakeable	Not easily disturbed or rattled; rarely troubled; undeterred; does not change plans in response to environmental stimuli.
Interested	Explores; engages with the environment; invested in the elements of space and their arrangement in a calm and peaceful manner.
Peaceful	Does not disturb other animals; happy; relaxed posture; friendly to others; enjoys the environment; conveys a sense of calm to the group.
Confident	Does not express fear; moves into open spaces without hesitation; reacts decisively; acts according to desires without inhibition from others; independent.

a Glomax spectrophotometer (Promega, Madison, WI, USA) at wavelengths of 450 and 490 nm. The concentration of corticosterone in each sample was determined by interpolation with a four-parameter non-linear regression curve fit using Myassays software. The results were then converted to pg mg^{-1}, considering the dilution factors.

Statistical analysis

All analyses were conducted using R statistical software version 4.2.1 (R Core Team 2022).

Data from the proximity and touching stages of both the human and novel object tests were not normally distributed and followed a negative binomial distribution, as confirmed by over-dispersion σ (evaluated using a Chi-squared test). These data were analysed by fitting a negative binomial generalised linear mixed model (R package glmm, version 1.4.4), with living environment (two levels: CPX or SPL) and treatment by human (two levels: H or N) as fixed effects, and paddock as a random effect. Quantitative behavioural scores were analysed using a generalised linear mixed model with living environment (two levels: CPX or SPL) and treatment by human (two levels: H or N) as fixed effects and paddock as a random effect. To assess intra-observer reliability for the qualitative behavioural assessment design, the Intra-class Correlation Coefficient (ICC) was calculated using the R package irr (version 0.84.1) on raw scoring data. To avoid bias, the principal component analysis (PCA) was then based on the scores of a single assessor. For each living environment, QBA scores before and after the treatment by human period were analysed using PCA with a correlation matrix and no rotation (R package FactoMineR, version 2.9). The suitability for PCA-KMO was tested using the kmooptimal-solution function. Variables with individual KMO values < 0.5 were removed from the dataset. The treatment effect on PCA dimensions 1 and 2 was analysed using the Kruskal-Wallis test (similar to the Mann-Whitney *U* test, allowing comparison of more than two groups) for each living environment. To investigate the relationship between quantitative and qualitative measures of rabbit behaviour, a Spearman Rank Correlation Coefficient was calculated to correlate the original ethogram-based quantitative behaviour scores for each behaviour with the individual qualitative rabbit scores on each QBA dimension produced during the previous PCA analysis.

Corticosterone data were analysed using a linear mixed model (R package lme4, version 1.1–35.1), with living environment (two levels: CPX or SPL) and treatment by human (two levels: H or N) as fixed effects, and paddock as a random effect.

Results

Reaction to a human

Proximity test

Rabbits in the H and N treatment groups exhibited similar mean (± SEM) latencies to contact the human of 16.1 (± 2.5) and 19.8 (± 3.3) s, respectively ($P = 0.35$; data not shown). Although not statistically significant, the latency to contact the human was lower in the CPX compared to the SPL environment (on average, 14.7 [± 2.4] and 21.7 [± 3.4] s, respectively; $P = 0.08$). There was no interaction between treatment by human and living environment regarding the latency to touch the human for the first time ($P = 0.83$). Approximately two rabbits per paddock approached the human every 10 s in the H group, while about one rabbit per paddock approached in the N group ($P < 0.05$; see Figure S3; Supplementary material), with no effect of living environment ($P = 0.85$). The interaction between treatment by human and living environment was not significant ($P = 0.06$). Rabbits spent more time near the human in the H compared to the N treatment, averaging 28.2 (± 3.4)

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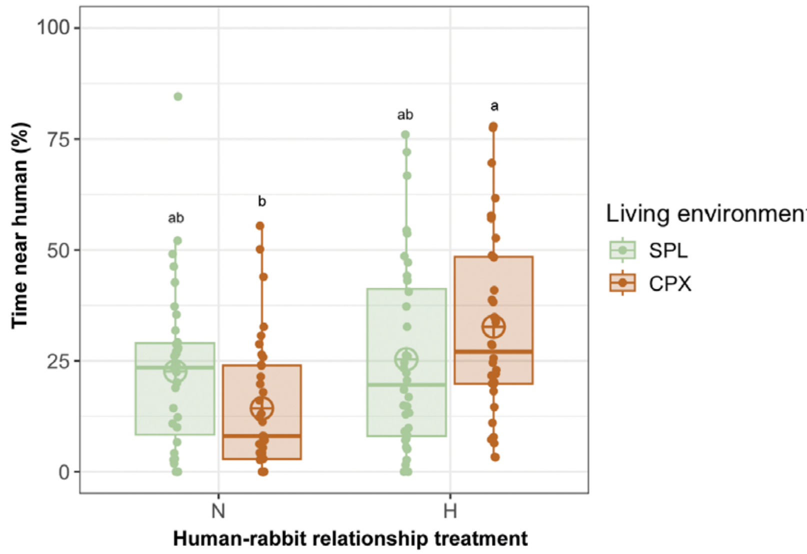

Figure 1. Percentage of time rabbits spent near the human (< 10 cm) during the proximity test according to the human-rabbit relationship treatment (N for no treatment by human and H for treatment by human) and the living environment (SPL for simple and CPX for complex). Different superscripts indicate significant differences at $P < 0.05$. The target symbol represents the mean.

versus 17.1 (± 2.1)% of the time, respectively ($P < 0.001$; see Figure 1), with no effect of living environment ($P = 0.75$). The interaction between treatment by human and living environment was not significant ($P = 0.06$). Rabbits in the CPX-H group spent significantly more time near the human compared to those in the CPX-N group (32.1 [± 5.7] vs 14.1 [± 2.5]% of the time, respectively; $P < 0.05$). In contrast, for the SPL-H and SPL-N groups, there was no difference in the proportion of time rabbits spent near the human (24.7 [± 4.5] vs 20.7% [± 3.6]% of the time, respectively; $P = 0.89$).

Touch test

Rabbits in the H treatment accepted significantly more strokes than those in the N treatment, averaging 90.2 (± 8.6) vs 45.9 (± 4.6)% of accepted strokes, respectively ($P < 0.001$; Figure 2). The effect of the living environment was not significant ($P = 0.06$), nor was the interaction between treatment by human and living environment ($P = 0.11$).

Reaction to a novel object

Proximity test

Rabbits exhibited similar latencies to approach the novel object in both the H and N treatments, averaging 25.9 (± 4.0) vs 31.7 (± 4.5) s, respectively ($P = 0.33$; data not shown). Similarly, latencies were comparable between the CPX and SPL environments, with averages of 29.6 (± 4.4) and 27.7 (± 4.0) s, respectively ($P = 0.74$). There was no interaction between treatment by human and living environment

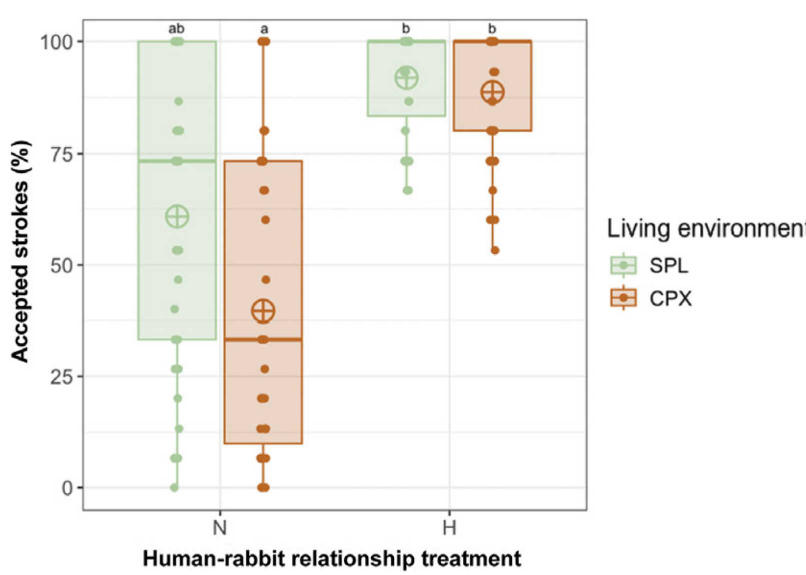

Figure 2. Percentage of strokes rabbits accepted from the human during the touch test according to the human-rabbit relationship treatment (N for no treatment by human and H for treatment by human) and the living environment (SPL for simple and CPX for complex). Different superscripts indicate significant differences at $P < 0.05$. The target symbol represents the mean.

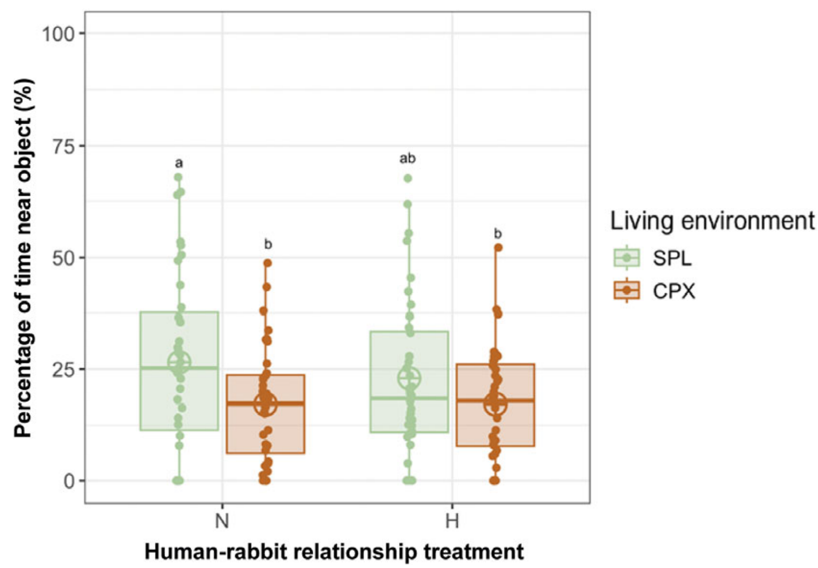

Figure 3. Percentage of time rabbits spent near the novel object (< 10 cm) during the proximity test according to the human-rabbit relationship treatment (N for no treatment by human and H for treatment by human) and the living environment (SPL for simple and CPX for complex). Different superscripts indicate significant differences at $P < 0.05$. The target symbol represents the mean.

regarding latency to touch the novel object ($P = 0.29$). Approximately one rabbit per paddock approached the novel object every 10 s in both the H and N treatments ($P = 0.58$). In the CPX group, approximately one rabbit per paddock approached while, in the SPL environment, approximately two rabbits per paddock approached ($P < 0.05$). Rabbits spent less time near the novel object in the CPX environment compared to the SPL environment, averaging 17.2 (± 2.1) versus 24.7 (± 2.9)% of the time, respectively ($P < 0.05$; see Figure 3). There was no effect of treatment by human ($P = 0.67$) or interaction between treatment by human and living environment ($P = 0.67$).

Touch test

Rabbits in the SPL-H group accepted a similar number of strokes from the novel object compared to rabbits in the SPL-N group, averaging 70.9 (± 10.4) versus 85.1 (± 12.8)%, respectively ($P = 0.82$). In contrast, rabbits in the CPX-H group accepted a significantly higher number of strokes than those in the CPX-N group, with averages of 62.9 (± 9.1) versus 31.7 (± 4.9)%, respectively ($P < 0.05$). The interaction between treatment by human and living environment significantly influenced the number of strokes rabbits accepted from the novel object ($P < 0.05$; see Figure 4).

Behavioural evaluation

Quantitative evaluation

Following the treatment by humans, rabbits in the H treatment were observed to be 'approaching' and 'in contact with the human' more frequently than those in the N treatment. Specifically, the averages were 11.1 (± 1.0) versus 6.2 (± 0.8)% for 'approaching' and

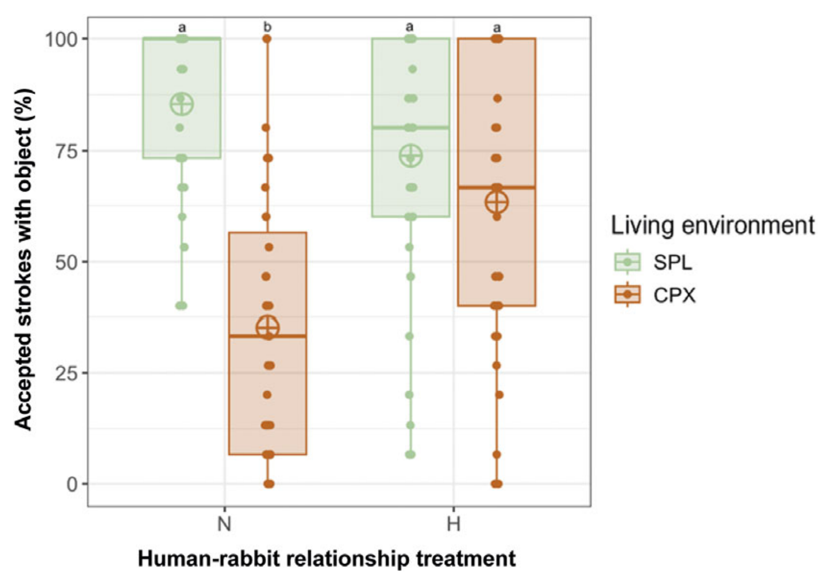

Figure 4. Percentage of strokes rabbits accepted from the novel object during the touch test according to the human-rabbit relationship treatment (N for no treatment by human and H for treatment by human) and the living environment (SPL for simple and CPX for complex). Different superscripts indicate significant differences at $P < 0.05$. The target symbol represents the mean.

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Table 2. Mean (± SD) percentages of behavioural occurrences in rabbits by human-animal relationship treatment (HAR: H for treatment by human and N for no treatment by human) and living environment (E: SPL for simple and CPX for complex) before and after treatment. *P*-values were obtained using Generalised linear mixed models (ANOVA test) on raw data. Means with different superscripts in the same row differ significantly at an alpha value of 0.05

Living environment (E)	SPL		CPX		*P*-values		
Human-animal relationship (HAR)	H	N	H	N	HAR	E	HAR × E
Before HAR treatment (age 48 days)							
Rearing toward human	0.4 (± 0.5)	0.1 (± 0.3)	0.0 (± 0.0)	0.6 (± 1.0)	0.44	0.35	0.99
Approaching human	9.7 (± 3.6)	7.7 (± 1.5)	8.2 (± 3.4)	7.3 (± 2.0)	0.17	0.40	0.65
Watching human	14.0 (± 5.3)	12.1 (± 3.5)	10.8 (± 3.8)	8.0 (± 3.9)	0.58	0.41	0.82
In contact with human	2.2 (± 2.3)	3.1 (± 2.0)	0.8 (± 0.9)	2.1 (± 1.9)	0.23	0.22	0.60
Nibbling, sniffing shoes	0.4 (± 0.6)	0.2 (± 0.6)	1.4 (± 3.3)	2.3 (± 0.7)	0.99	0.99	0.99
Nibbling, sniffing clothes	7.4 (± 5.3)	11.9 (± 4.7)	8.5 (± 6.2)	7.4 (± 4.4)	0.53	0.55	0.48
Moving away from human	3.2 (± 1.7)	3.2 (± 0.9)	3.4 (± 1.8)	3.9 (± 1.1)	0.80	0.77	0.87
Other activity	62.3 (± 11.6)	61.6 (± 4.9)	66.9 (± 10.2)	68.3 (± 6.8)	0.94	0.79	0.93
After HAR treatment (age 68 days)							
Rearing toward human	1.4 (± 1.6)	0.1 (± 0.3)	0.5 (± 0.8)	0.0 (± 0.0)	0.99	0.99	0.99
Approaching human*	10.4 (± 5.7)^{ac}	6.4 (± 1.3)^{b}	11.9 (± 3.3)^{a}	6.1 (± 1.6)^{bc}	< 0.05	0.51	0.71
Watching human	4.4 (± 3.1)	9.1 (± 3.3)	6.4 (± 3.5)	17.6 (± 14.5)	0.09	0.60	0.91
In contact with human*	5.5 (± 5.2)^{a}	0.5 (± 0.9)^{a}	18.1 (± 14.5)^{b}	0.9 (± 1.6)^{a}	< 0.05	< 0.05	0.44
Nibbling, sniffing shoes	3.0 (± 4.2)	10.5 (± 4.7)	3.6 (± 3.3)	3.3 (± 3.9)	0.06	0.16	0.13
Nibbling, sniffing clothes	10.5 (± 7.8)	10.2 (± 4.6)	8.1 (± 6.9)	5.0 (± 2.4)	0.97	0.41	0.81
Moving away from human	0.1 (± 0.3)	1.3 (± 1.3)	0.6 (± 0.6)	2.3 (± 1.7)	0.14	0.46	0.99
Other activity	64.7 (± 13.1)	61.7 (± 8.7)	50.8 (± 17.9)	64.7 (± 18.3)	0.81	0.76	0.74

11.7 (± 2.3) versus 0.7 (± 0.3)% for 'in contact' ($P < 0.05$; Table 2). Additionally, rabbits in the CPX environment demonstrated a higher frequency of being 'in contact with the human' compared to those in the SPL environment, with averages of 10.2 (± 1.3) versus 2.9 (± 0.6)% ($P < 0.05$).

Qualitative Behavioural Assessment (QBA)

QBA design

We successfully developed 21 descriptors that encompass a range from negative to positive valence and from low to high intensity (see Figure S8; Supplementary material). This exceeds our initial goal of 16 descriptors. Each descriptor is accompanied by a brief definition to facilitate its interpretation (see Table 1, Table S1 [Supplementary material] as well as video clips). The two assessors demonstrated a high level of agreement, achieving an intra-class correlation coefficient (ICC) of 0.96 ($P < 0.001$) for all descriptors during the cross-check training.

Dimensions of rabbit behavioural expression

The dimensions of rabbit behavioural expression are detailed in the Supplementary material (see Table S2).

Before the treatment period, Dimension 1 of the SPL assessment accounted for 31.4% of the total variation, while Dimension 2 explained 29.1%, resulting in a cumulative explanation of 60.5% of the total variation. Dimension 1 ranges from 'At ease/Unshakeable' to 'Nervous/Frustrated', whereas Dimension 2 spans from 'Indifferent/Calm' to 'Interested/Affectionate.' For the CPX assessment, Dimension 1 explained 31.8% of the variation, and Dimension 2 accounted for 29.1%, together summing to 60.9% of the total variation explained. Dimension 1 ranges from 'Confident/Curious' to 'Indifferent/Worried', while Dimension 2 extends from 'Nervous/Vigilant' to 'At ease/Relaxed'.

After the treatment period, Dimension 1 of the SPL environment accounted for 37.9% of the variation, while Dimension 2 explained 26.2%, resulting in a cumulative total of 64.1% of the variation explained. Dimension 1 ranges from 'Peaceful/At ease' to 'Frustrated/Nervous', and Dimension 2 spans from 'Interested/Affectionate' to 'Indifferent'. In the CPX environment, Dimension 1 explained 55.6% of the variation, with Dimension 2 accounting for 15.9%, yielding a total of 71.5% of the variation explained. Dimension 1 ranges from 'Confident/At ease' to 'Indifferent/Worried', while Dimension 2 is characterised as 'Scared/Alert' (with no negative co-ordinate).

Qualitative behavioural assessment treatment effects

Before the treatment by human, there were no significant differences between the H and N treatments in either Dimension 1 or Dimension 2 for rabbits in the SPL environment, nor for animals in the CPX environment (see Figures 5[a] and [b]). After the treatment by human, rabbits in the H treatment were rated as significantly more 'Affectionate/Interested' compared to those in the N treatment. Additionally, rabbits in the N treatment were described as significantly more 'Indifferent' in the SPL groups ($P < 0.05$; see Figure 5[c]). No significant differences were detected between H and N treatments in the first two dimensions for rabbits in the CPX environment (see Figure 5[d]). When combining both SPL and CPX environments, there were no significant differences between H

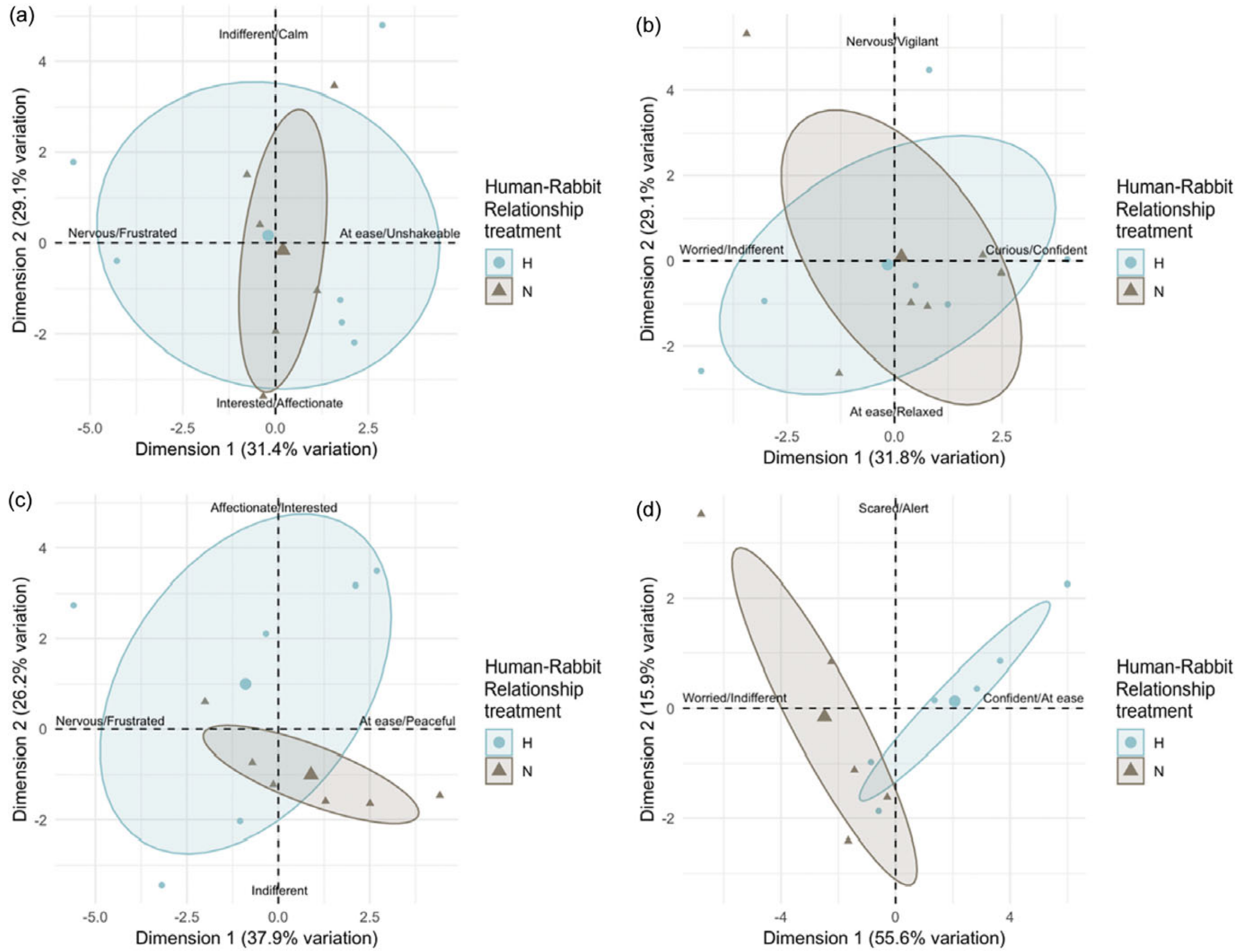

Figure 5. Showing the distribution of the paddocks (six rabbits per paddock) along the first and second principal components dimensions according to the human-animal treatment (N for no treatment by human and H for treatment by human) for (a) before treatment (at 48 days of age) in the simple (SPL) environment, (b) before treatment (at 48 days of age) in the complex (CPX) environment, (c) after treatment (at 68 days of age) in the SPL environment and (d) after treatment (at 68 days of age) in the CPX environment.

and N groups in either Dimension 1 or Dimension 2 before (see Figure S5A; Supplementary material) or after (Figure S5B) the treatment by human. Furthermore, no differences were found between the CPX and SPL environments in either dimension before or after the treatment (see Figures S6C and D; Supplementary material).

The relationship between qualitative and quantitative measures of rabbits' behaviour

Prior to the treatment by human, PCA Dimension 1 ('Unshakeable/At ease' to 'Nervous/Frustrated') for rabbits in the SPL environment correlated positively with 'Other activity' ($R_s = 0.62$; $P < 0.05$; see Table 3). For rabbits in the CPX environment, PCA Dimension 1 ('Confident/Curious' to 'Indifferent/Worried') positively correlated with both 'Nibbling/Sniffing clothes' ($R_s = 0.73$; $P < 0.05$) and 'Nibbling/Sniffing shoes' ($R_s = 0.82$; $P < 0.05$). Additionally, PCA Dimension 2 ('Nervous/Vigilant' to 'At ease/Relaxed') negatively correlated with 'Approaching human' ($R_s = -0.69$; $P < 0.05$) and 'Moving away from human' ($R_s = -0.70$; $P < 0.05$). No significant correlations were found between PCA Dimension 2 and the behaviours of rabbits in the SPL environment.

After the HAR treatment, PCA Dimension 1 ('At ease/Peaceful' to 'Frustrated/Nervous') correlated positively with 'Sniffing or Nibbling shoes' ($R_s = 0.62$; $P < 0.05$) for rabbits in the SPL environment. Furthermore, PCA Dimension 2 ('Affectionate/Interested' to 'Indifferent') positively correlated with 'In contact with human' ($R_s = 0.63$; $P < 0.05$) for rabbits in the SPL environment. In contrast, PCA Dimension 1 ('Confident/At ease' to 'Indifferent/Worried') negatively correlated with 'Rearing toward human' ($R_s = -0.71$; $P < 0.05$) and 'In contact with human' ($R_s = -0.82$; $P < 0.05$) for rabbits in the CPX environment. No significant correlations were found between PCA Dimension 2 and behaviours for rabbits in the CPX environment.

Hair corticosterone level

While the interaction between the treatment by human and the living environment was significant regarding hair corticosterone levels ($P = 0.03$), no differences in corticosterone levels were observed between the SPL-H and SPL-N groups (on average, 2.08 [± 0.2] vs 2.22 [± 0.2] pg mg^{-1} of hair, respectively; $P = 0.92$; see Figure 6) or between the CPX-H and CPX-N groups (on average, 2.39 [± 0.2] vs 1.79 [± 0.1] pg mg^{-1} of hair, respectively; $P = 0.10$).

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Table 3. Spearman rank correlation coefficients (R_s) between qualitative and quantitative behavioural measures in rabbits by living environment (SPL for simple and CPX for complex). Results computed before and after human-animal relationship (HAR) treatment

	Qualitative expressive scores							
	Dimension 1				Dimension 2			
	SPL		CPX		SPL		CPX	
Quantitative behaviour measures	R_s	*P*-value	R_s	*P*-value	R_s	*P*-value	R_s	*P* value
Before HAR treatment (age 48 days)								
Rearing toward human	0.12	0.72	–0.10	0.75	0.08	0.81	0.38	0.23
Approaching human	–0.47	0.12	0.55	0.06	–0.54	0.07	–0.69*	< 0.05
Watching human	–0.19	0.56	0.32	0.32	–0.28	0.38	– 0.01	0.97
In contact with human	0.33	0.29	0.26	0.41	–0.44	0.15	0.11	0.74
Nibbling, sniffing shoes	–0.34	0.28	0.82*	< 0.05	–0.33	0.29	–0.24	0.45
Nibbling, sniffing clothes	–0.17	0.59	0.73*	< 0.05	–0.41	0.18	–0.46	0.13
Moving away from human	0.01	1.00	0.47	0.13	–0.38	0.22	–0.70*	< 0.05
Other activity	0.62*	<0.05	0.12	0.72	–0.03	0.92	–0.08	0.81
After treatment (age 68 days)								
Rearing toward human	0.10	0.75	–0.71*	< 0.05	0.46	0.13	–0.45	0.16
Approaching human	0.24	0.45	–0.37	0.27	0.08	0.80	0.18	0.60
Watching human	0.39	0.21	0.23	0.49	–0.45	0.15	0.07	0.83
In contact with human	–0.24	0.45	–0.82*	< 0.05	0.63*	< 0.05	–0.38	0.24
Nibbling, sniffing shoes	0.62*	<0.05	0.04	0.90	–0.12	0.70	0.03	0.92
Nibbling, sniffing clothes	0.38	0.22	–0.46	0.15	–0.45	0.14	–0.03	0.94
Moving away from human	0.24	0.46	0.43	0.19	–0.27	0.40	–0.01	0.98
Other activity	0.14	0.66	0.58	0.06	–0.24	0.45	0.00	1.00

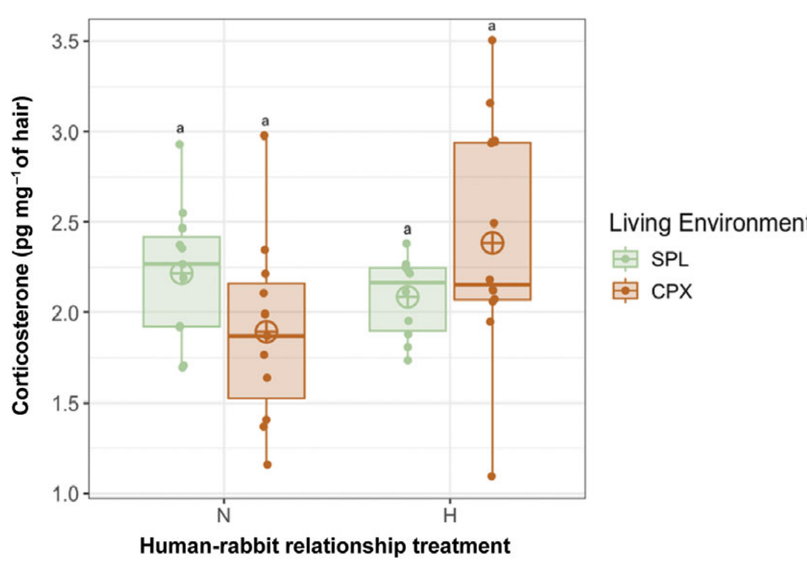

Figure 6. Concentration of corticosterone (pg mg^{-1} of hair) accumulated in the rabbits' hair (between 42 and 73 days of age) according to the human-rabbit relationship treatment (N for no treatment by human and H for treatment by human) and the living environment (SPL for simple and CPX for complex).

Discussion

The aim of this study was to evaluate the influence of treatment by human on the expressed behaviours, emotions, and stress response to humans in rabbits. Additionally, the study examined the effects of housing in either a complex or simple outdoor environment on these behaviours and emotional states. It is important to note that some rabbits were temporarily removed from their shelters to conduct tests on the entire group, which may have influenced individual rabbits depending on their temperament. However,

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the animals were accustomed to being handled and to not accessing their shelters during the day for routine activities such as cleaning, feeding, weighing, treatment, and daily inspections. In addition, to minimise stress, we allowed a 2-min period for the rabbits to recover their spontaneous activity before starting the tests. The tests were conducted in the rabbits' paddocks rather than a controlled test arena, which helped to limit disturbances. Additionally, the behaviour assessors could not be fully blinded to the conditions, as the same individuals were responsible for the husbandry, testing, and assessment. They could recognise the living conditions (such as the presence of trees or grassy areas) and some individual rabbits in the videos. Despite these technical and practical limitations, we believe that the results of our study remain valid.

This study is the first to present a QBA of emotion in European rabbits. The two assessors demonstrated a high agreement score (ICC = 0.96), indicating that the descriptors used are clear and unambiguous for trained observers. Thus, the QBA method appears to be reliable for this species; however, it would benefit from further validation in various contexts and with different assessors (see, for example, Brscic *et al.* 2019; Muri & Stubsjøen 2017). We successfully assessed the behaviour and emotional state of rabbits in the presence of a test person after a brief treatment period. Although rabbits are often perceived as fearful animals, they appear to readily adapt to human interactions. Showing similarity with findings observed in horses *(Equus caballus)* (Minero *et al.* 2018) and giraffes *(Giraffa camelopardalis)* (Patel *et al.* 2019), our study demonstrated that rabbits in the H group exhibited behaviours indicative of positive emotions and displayed reduced avoidance of humans after being fed and stroked. However, while rabbits became more affectionate/interested, horses were noted to be relaxed/at ease, and giraffes were described as calm/confident. These variations could be attributed to interspecific differences. The treatment involving the provision of additional food resources and stroking may have positively influenced the emotional state of the rabbits, fostering increased interest and affection. Additionally, the introduction of novel food sources could have encouraged the rabbits to approach humans. It is also worth noting that the young age of the rabbits (post-weaning) may have played a role in their responses. Finally, the discrepancies in emotional expressions might stem from the use of different descriptors in the referenced studies.

The nature of interactions between humans and rabbits significantly influenced the rabbits' responses during the human test. Animals that received treatment from humans spent more time near the tester and were more likely to contact and approach the human compared to those who did not receive such treatment. Markowitz *et al.* (1998) suggested that the time spent near the stockperson can be viewed as a measure of affinity, defined as a desire for human contact. The lack of difference in the latency to contact the human among the experimental groups (CPX-N, CPX-H, SPL-N, and SPL-H) indicates that rabbits in the N treatment group did not exhibit greater avoidance of humans than those in the treatment group. The handling of rabbits in the N treatment group during weekly weigh-ins, combined with daily human interactions for food delivery and litter cleaning, may have facilitated some degree of habituation to the presence of a human (Csatádi *et al.* 2005). Additionally, rabbits exposed to treatment by humans accepted twice as many strokes compared to those who did not receive such treatment. This indicates that the animals likely found the feeding and strokes appealing or that they had become accustomed to human interactions. The strokes received during the treatment period may have fostered a more positive perception of humans among the rabbits. Although there is limited literature on how rabbits perceive strokes, previous studies have suggested a beneficial effect of human strokes on species that engage in allogrooming (Coulon *et al.* 2015). Rabbits naturally groom one another, particularly in areas such as the head, ears, neck, and back (Divincenti & Rehrig 2016). They typically enjoy being stroked in these regions. In our tests, we stroked the rabbits from their heads to their backs to maximise the duration of contact. Moreover, we observed differences in the rabbits' responses to stroking. Those in the N treatment displayed more submissive behaviours when stroked, such as having wide-open eyes and a rigid body posture. In contrast, rabbits in the treatment by human group appeared more relaxed and seemed to enjoy the stroking, exhibiting behaviours such as mid-closed eyes, a loose body, and dental vibrations (M Fetiveau, V Fillon, personal observation 2022). Additionally, rabbits in the treatment by human group were given a novel food resource during the intervention, making it challenging to attribute their motivation to interact solely to the human and strokes. It is likely that their positive responses were influenced by both stimuli: food and stroking. Future studies could benefit from further analysis of the qualitative aspects of these interactions.

The rabbits' responses to the human tests varied according to their living environment. For instance, those in the complex environment contacted the human more frequently than those in the simple environment. During the tests, the rabbits' shelters were locked in each pen, which caused a degree of disturbance, particularly in the simple environment. Several rabbits gathered at the shelter door, attempting to enter. In the wild, rabbits tend to remain close to their burrows when the environment lacks adequate refuges, such as shrubs or trees, as this decreases their protection against predators (Villafuerte & Moreno 1997). In our study, blocking access to the only refuge in the simple environment caused the rabbits to lose interest in the human, while this restriction did not affect the group living in the complex environment, where trees appeared to provide a sense of protection. Furthermore, the rabbits' responses to the treatment by humans were more pronounced in the complex environment than in the simple one, particularly during the proximity and touch phases of the human test. One possible explanation for this is that the rabbits in the SPL-N group were less shielded from humans than those in the CPX-N group. In the simple environment, all rabbits (both N and H groups) were readily able to see and hear the human during the treatment period, as it was an open area without any trees (see Figure S2; Supplementary material). In contrast, rabbits in the complex environment (see Figure S1; Supplementary material) may have experienced a more significant impact from human contact.

Rabbits' emotional responses varied between those exposed to treatment by human and those not exposed, but this difference was observed only in the simple environment. In the SPL-H group, rabbits appeared more 'Affectionate/Interested,' while individuals in the SPL-N group seemed more 'Indifferent.' Corticosterone levels did not differ between the groups. However, Handlin *et al.* (2011) showed that positive interactions between humans and dogs increased blood cortisol levels, potentially reflecting arousal. The rich array of stimuli in the complex environment may have led to increased distance from humans in the N group, while those in the H group appeared to recognise the benefits of interacting with humans, such as receiving strokes and food rewards during the treatment.

The responses of rabbits to the novel object test did not differ between the treated groups (H or N). However, their reactions

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varied significantly across both stages of the test depending on their living environment. Rabbits in the simple environment spent more time near the novel object compared to those in the complex environment. The presence of a diverse array of elements for interaction in the complex environment (such as leaves, apples, tree trunks, and branches) may have diminished their interest in the novel object. This finding aligns with the study by Fetiveau *et al.* (2021), which reported rabbits with outdoor access to be less inclined to approach a novel object compared to those without such access. Stolba and Wood-Gush (1981) showed that animals living in impoverished conditions showed greater interest in a novel object compared to those in enriched environments. While the simple environment in this study is not poor when compared to indoor-caged rabbits, it is possible that the variation in physical complexity (simple versus complex) influenced the rabbits' behaviour in a similar manner. Rabbits in the CPX-N group accepted half as many strokes with the novel object as those in the CPX-H group. In contrast, rabbits in both groups (treatment by human and no treatment) accepted a similar number of strokes with the novel object in the simple environment. Furthermore, handling the broomstick in the complex environment was more challenging due to tree branches that often caught on the stick, which could have frightened the CPX-N rabbits. Moreover, we observed that the rabbits' responses to object stroking were similar across all groups. They all appeared very surprised and gazed at the novel object during the interaction (M Fetiveau, V Fillon, personal observation, 2022; see examples in Figure S5; Supplementary material).

Animal welfare implications

The reactions of rabbits are significantly influenced by the quality of their relationship with humans and the type of environment within which they reside. Animals that received positive interactions with humans in physically complex environments expressed more positive emotions. Conversely, those that did not have increased interactions with humans were less inclined to engage in physical contact. These findings could guide farmers in adopting relationships that enhance daily care practices and improve the welfare of rabbits.

Conclusion

The present experiment suggests that the behaviour of rabbits can be influenced by both their relationship with humans and their living environment. Specifically, rabbits spent more time near humans and accepted more strokes when they had increased contact with them. Additionally, rabbits in the human treatment group were rated as more 'Affectionate/Interested' compared to those that received no treatment by human were described as significantly more 'Indifferent'. These findings could encourage farmers to maintain positive interactions with their animals, facilitating management tasks such as handling for health checks and treatments, while also enhancing animal welfare.

This study is the first to employ Qualitative Behaviour Assessment (QBA) in rabbits, demonstrating that their emotional expressions varied based on the nature of their interactions with humans, particularly in complex environments. The greater the environmental enrichment provided for rabbits, the greater the variety of stimuli available, which may lead them to be less responsive to unfamiliar humans. Future research could benefit from applying QBA on commercial farms with various housing systems to correlate farmers' attitudes with rabbit welfare in contrasting and less-enriched environments.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/awf.2024.54.

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Competing interest. None.

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