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The effect of blindfolding and swaddling on the stress response to handling in domestic rabbits

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Abstract

*Stress during handling can negatively impact the welfare of an animal. Gradual habituation or systematic desensitisation can reduce this but is not always possible. Blindfolding has been shown to decrease stress indicators, including heart rate and struggling in cattle (*Bos taurus*), but has not been studied in domestic rabbits (*Oryctolagus cuniculus*). We surveyed 49 wildlife rescues, rehabilitators and veterinarians, and found that blindfolding and swaddling are widely used and believed to reduce stress and struggling in rabbits. However, these methods may simply preclude escape and result in sensitisation over repeated exposure. We next conducted a controlled trial investigating the effects of blindfolding and swaddling repeatedly over five days on behavioural and physiological indicators of stress in 40 domestic rabbits. Neither blindfolding nor swaddling had significant impacts on heart or respiratory rate, compared to a partial hood control treatment, which involved similar levels of manipulation, but without visual or movement restriction. Behavioural responses showed variable trends, eg rabbits were more likely to approach food after handling with swaddling. Baseline levels varied significantly between individuals, suggesting previous experience to be an important determinant. Heart rates were universally high, showing that individuals in this study were stressed by handling to such an extent, that overall, effects of both blindfolding and swaddling on physiological indicators of stress were minimal. We suggest that blindfolding and swaddling may be useful as means of limiting the procedure duration and risk of injury by reducing struggling, but this study provides no evidence that welfare is otherwise improved.*

Keywords: *animal welfare, blindfolding, handling, rabbit, stress, swaddling*

Introduction

There are many reasons why humans may wish to handle animals. Handling is often a necessary part of good husbandry, eg grooming, facilitation of veterinary care, rehabilitation. However, handling, even if aimed at improving animal welfare, can cause stress and thus negatively impact welfare. Stress has been linked with adverse physical and psychological consequences, such as reduced immunological responses and reduced reproductive success (eg Mitchell *et al* 1988; Maier *et al* 1994; Verga *et al* 2007); and potentially life-threatening blood parameter changes, including acidosis, for example, during capture and confinement of dusky sharks (*Curcharhinus obscurus*; Cliff & Thurman 1984). Effects are even observed in domestic stock during routine laboratory handling. New Zealand White rabbits (*Oryctolagus cuniculus*) were subjected to laboratory handling and procedures mimicking a five-dose, 59-day vaccine toxicology study (Sellers *et al* 2017). In the treatment groups exposed to additional handling and procedures, increased myocardial inflammatory foci were observed compared to the control group. A separate study of New Zealand White rabbits also identified a correlation between increased stress and high levels of intraoperative cardiorespiratory instability and apnoea (Krall *et al* 2019). This highlights the potential clinical significance of reduced stress in a veterinary setting. Similarly, long-term health may be impacted by stress. For example, in species that synthesise their own vitamin C, stress has been associated with reduced plasma vitamin C concentration, which can have a negative impact on biological processes (Mateos *et al* 2010).

The extent of the stress response can be affected by many factors, including novelty, past experience, intensity of the stressor and perceived danger. An individual may become particularly stressed by handling if they have not been gradually habituated or have previously experienced rough or inappropriate handling (Gray 1987; Grandin 1997).

Reducing stress can be beneficial for both the handler and the animal and low stress handling and restraint techniques should be adopted but these vary between species. Tranquilisers are used during the capture of a variety of deer

species and have been found to be effective at reducing short-term stress, although this may be predominantly by facilitating quick handling (Conner *et al* 1987; Diverio *et al* 1996; Mentaberre *et al* 2010). Pharmaceutical intervention is not always possible and can carry risks of adverse reactions or varying efficacy (eg Bataller & Keller 1999; Wilkinson 2005). The use of nutraceuticals (products derived from food sources and believed to have health or medical benefits) may help to reduce the stress response of an individual, although evidence for efficacy is limited (eg McDonnell *et al* 2013; Unwin *et al* 2019), and for charities the cost may be prohibitive. Alternatively, behaviour modification techniques can be used to habituate the animal to being handled, for example, via desensitisation and counter-conditioning (eg Miller 1960; Stephens & Toner 1975; Dantzer & Mormede 1983; Podberscek *et al* 1991; Jones & Waddington 1992; Broom 2007; Unwin *et al* 2019). These options may not be possible for wildlife or all domestic animals. In addition, specific handling techniques have been proposed, for example, Green (2003) advocates the use of visual and auditory restriction plus hobbling (strapping together an animal's legs) to pacify medium-sized deer. Covering the eyes or blindfolding has been claimed to reduce struggling and heart rate during handling of cattle (*Bos taurus*) (White 1990; Mitchell *et al* 2004). However, in horses (*Equus caballus*), blindfolding has been shown to increase heart rate and struggling (Parker *et al* 2004). Mantor *et al* (2014) reported a reduction in perceived stress, as well as decreased number of bites to the handler, when a gloved hand was used to cover the eyes of wild California ground squirrels (*Otospermophilus beecheyi*) during handling. However, the study focused primarily on injury to the squirrels during trapping and handling and may not necessarily reflect reduced stress levels. The effectiveness of a handling method may be due to making the handling less aversive for the animal or simply making handling quicker and safer for the operator. Hence, reduced struggling may result from reduced stress or, where escape is not possible, it could result from animals (eg rabbits) freezing in response to a stressor instead (Collewijn 1977). Evidence to show whether blindfolding and other restriction methods reduce stress is currently lacking for most species, and further studies need to consider other measures in addition to handleability.

Domestic rabbits are common pets and hence important to study. Throughout domestication, they have maintained many of the behavioural traits of their wild counterparts and therefore provide a good estimation of the responses of wild rabbits to handling, rehabilitation and translocation (Stodart & Myers 1964; Trocino & Xiccato 2006). As a prey species, they have developed behaviours that aid escape from predators, including defensive behaviours, such as biting and kicking, vocalisations and postures as well as freezing (McBride 2014). Rabbits are highly motivated by social interaction, and many seek conspecific contact and recover more quickly from stressors if housed with other rabbits (Burn & Shields 2020).

A recent survey of rabbit owners in the UK and the Republic of Ireland showed that different handling methods are commonly used for different purposes (Oxley *et al* 2018). The most frequent reason for handling was to move the rabbit and the most common method was holding the rabbit close to the handler's body whilst supporting the hind legs. Scruffing was more frequently used by owners with laboratory experience, as was holding the rabbit with the head tucked between the arm and the body, which could be considered a form of visual restriction. This handling method was also described by Vennen and Mitchell (2009), as possibly reducing stress. Lying the rabbit on its back is thought to be helpful for some clinical procedures (eg Wilczynska *et al* 2021) and consequently is mostly used for health checks (Oxley *et al* 2018). This method, however, is potentially problematic as it can cause tonic immobility, which is thought to be indicative of a stressed state (Buseth & Saunders 2015; Oxley *et al* 2018). While Oxley *et al* (2018) reported that some owners disapproved of the technique, the use of tonic immobility, as well as ear scruffing, may be more common in specific disciplines (eg breeding or showing), or by inexperienced handlers who may be unaware of the negative impact of certain handling techniques or be unable to recognise negative responses, potentially misjudging signs of stress as endearing (eg Buseth & Saunders 2015; Oxley *et al* 2018). It is often suggested that swaddling helps to reduce the stress and struggle response of rabbits to handling (Harcourt-Brown & Whitwell 2003; Fisher 2010; Buseth & Saunders 2015). Swaddling in this case refers to the act of wrapping the rabbit securely in a cloth (usually a towel) with the head exposed and the body and limbs enclosed, also known as 'bunny burrito' (Vennen & Mitchell 2009; Cope & Hawe 2018). There has however been little research into the physiological and behavioural effects of swaddling, nor blindfolding as handling techniques for rabbits.

This paper investigates the use and effect of two different techniques on stress during repeated rabbit handling. Blindfolding was investigated, as this is a technique used in a wide variety of species. Swaddling was investigated as this is advocated as a positive handling method in domestic rabbits (eg Fisher 2010; Richardson & Keeble 2014; Varga 2014; DeCubellis 2016). We first carried out a survey to explore the frequency of use, and perceived value, of blindfolding and equivalent immobilising techniques in the rescue, rehoming and veterinary environment. Next, we conducted an experimental study, with experimental tests adapted from studies by Rooney *et al* (2014) and Unwin *et al* (2019). Although blindfolding is often carried out simply by covering the rabbit's eyes with the human hand, to standardise treatment here we used a hood which covered the eyes, and to elucidate whether any effect was due to lack of vision (rather than hood placement), we compared this to application of a hood with eye holes.

Previous studies have suggested that exposure to a potential stressor, such as handling, can lead to reduced (habituation) or increased stress (sensitisation) upon subsequent exposure depending upon the perceived positivity of the previous exposure (Grandin 1997; Broom 2007). In order to investi-

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gate differences in stress responses, whilst correcting for any baseline differences, forty rabbits were handled repeatedly once a day for five days. Each rabbit was handled using the same handling technique on all five occasions: blindfolding; swaddling; or as a partially hooded control treatment, held on the floor between the examiner's legs with the examiner's arms either side of the rabbit's body and the rabbit's head facing away from the examiner. Measures of stress recorded included general behaviour (in a novel environment and in response to a person), level of struggling, and a subjective measure of calmness during handling. Physiological measures included heart and respiratory rate, and saliva was collected to analyse cortisol concentration. Measures were taken during a health check and compared on days one and five.

Several hypotheses were tested:

• Blindfolding during handling reduces *physiological* measures of stress (respiratory and heart rates) in domestic rabbits on subsequent exposure;

• Blindfolding during handling reduces *behavioural* indicators of stress, such as aversion (eg freeze, struggle, bite, kick or thump) and avoidance of handler in domestic rabbits on subsequent exposure;

• Swaddling during handling reduces *physiological* measures of stress in domestic rabbits on subsequent exposure; and

• Swaddling during handling reduces *behavioural* indicators of stress, such as aversion (eg freeze, struggle, bite, kick or thump) and avoidance of handler in domestic rabbits on subsequent exposure.

Ethical approval

Prior to data collection, ethical approval was obtained from the University of Bristol Animal Welfare and Ethical Review Board (AWER: BUB/16/024). Approval was obtained through the Faculty of Health Sciences Research Ethics Committee for the survey portion of the study.

Section 1: Survey

Materials and methods

An online survey created using Googleforms™, was piloted and then distributed to managers of Royal Society for the Prevention of Cruelty to Animals (RSPCA) centres (rehabilitation and rehoming) and by Rabbit Welfare Association and Fund (RWAF) to all rabbit rehoming organisations in the UK. There was no incentive for participation and responses were anonymous. The survey consisted of 17 questions regarding the use of blindfolding and other methods for reducing stress (Table 1; see supplementary material to papers published in *Animal Welfare*: https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). The survey was opened on 12th July 2016 and closed, with responses downloaded, on 22nd August 2016. Survey results were used to identify the number of rehabilitation and rehoming centres using blindfolding as well as the reasons for use and the prevalence of any other handling techniques.

Results

Of the 49 responses, 29 came from rehoming centres, six from rehabilitation centres and 14 from other individuals (including four veterinary practices and four private owners). In total, 41 dealt with domestic animals exclusively, seven with both wildlife and companion animals and one exclusively wildlife. Rabbits were currently being treated by 48 of the 49 respondents; 60.4% (n = 29) reported to always use methods to reduce stress during the handling of rabbits, 12.5% (n = 6) reported always using a towel or hood (blindfolding) during handling and 18.5% (n = 9) reported always using blindfolding during transport. Only 27.1% (n = 13) of respondents reported that they would never use a towel or hood during handling of rabbits. Other methods reportedly used during rabbit handling include nutraceuticals (n = 4), a covered or enclosed carrier (n = 11) and transporting a bonded pair together (n = 9). One respondent described using swaddling specifically, while seven responses included variations in the use of towels as a means of covering the body or head of the rabbit or merely for comfort. Two respondents mentioned using specialised methods of handling the rabbit in order to limit struggling but were unclear regarding specific methodology.

Overall, 64.5% of respondents that treated species other than rabbits (n = 31) used visual restriction during transport or handling of a variety of species. 'Other methods', such as covering the carrier, using blankets with familiar scent and nutraceuticals were used by 87.1% of the respondents. These respondents identified cats (*Felis silvestris catus*) as the species for which they would most often use blindfolding (n = 16) or 'other methods' to reduce stress (n = 18). The most common reason reported for using blindfolding was as a means of reducing stress in the animal (71.4% of 49 responses), whilst the most frequent reason for not using a blindfold was that it was deemed unnecessary (Table 1; https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material).

Discussion

The survey highlighted that blindfolding is used during the handling of rabbits and other species. The majority of individuals who reported using blindfolding, do so with the intention of reducing the animals' stress response. Other methods are also employed, such as aromatherapy sprays and, particularly in the case of rabbits, transporting and handling with a known conspecific or swaddling. Nevertheless, when asked why blindfolding was not used, 14.6% of people who did not use it, thought that it was ineffective. Two individuals said they would never use blindfolding, whilst four said that they would sometimes use blindfolding to reduce stress, implying that they consider each case individually and that blindfolding is not necessarily considered a universal preventative measure against

stress. Despite being advocated as a positive handling method (eg Fisher 2010; Richardson & Keeble 2014; Varga 2014; De Cubellis 2016), swaddling was only specifically mentioned by one respondent, although two referred to using towels to wrap or cover rabbits. This suggests variation between respondents in their opinion of the value of using swaddling. This survey focused on the handling of rabbits in veterinary, rehoming and rescue environments. It would be useful to also explore other settings in which rabbits are kept and handled, for example, laboratories where large numbers of rabbits are housed and other forms of handling and restraint may be common, as well as investigating the different types of blindfolds that may be used.

Section 2: Experimental study

Materials and methods

Forty domestic rabbits of varying but unknown ages, from four separate sites, were included (Table 2; see supplementary material to papers published in *Animal Welfare*: https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). Cohorts came from: a privately owned group of rehomed rabbits in Taunton (cohort 1; n = 14); Rabbit Welfare Association and Fund (RWAF), a rabbit charity in Taunton (cohort 2; n = 6); Little Valley RSPCA rehoming centre in Exeter (cohort 3; n = 6); and Windwhistle Warren rehoming centre in Gloucester (cohort 4; n = 14). Individuals that were undergoing any veterinary treatment were not included in the study, so all subjects were deemed healthy and free from medical conditions that may influence the study. Very nervous or aggressive individuals, as identified by the rehoming centre staff, were also excluded. We excluded individuals that had been moved into new housing or bonded with a conspecific within a week of commencement of the study. Individual rabbits underwent five tests over five consecutive days, with the exception of rabbits from cohort one, for which there was a delay of one week between the first test and the four succeeding tests, due to experimenter illness. Each test lasted between 10 and 15 min and involved exposure to a novel environment and handling.

Rabbits were housed in a variety of enclosures including hutches, sheds, runs and pens (Table 2; https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). At each site, the rabbit enclosures were numbered and randomly allocated to one of the three groups (blindfold, swaddling, control) by using the random number function in Microsoft Excel®. Rabbits were housed individually (n = 10), in pairs (n = 23), or groups of three (n = 7). Those housed in groups or pairs were allocated to the same test group, to avoid any social contagion effects on conspecifics. The experimenter alternated between testing the lightest or darkest coloured individuals first to avoid any unconscious bias. Rabbits were tested sequentially, and in the same order, on each of the five days of testing. Individuals housed together were never used sequentially, to limit any cumulative stress caused by the experimenter's repeated approaches to the enclosure. All rabbits remained at their respective centre after completion of the study. If any rabbit showed any extreme negative behaviours at any point during a test (eg open-mouth breathing), the test would be stopped immediately, and the rabbit returned to its home enclosure. This never occurred.

Although the survey revealed that, in practice, blindfolding was achieved in numerous ways (eg towels, hands and hood), for standardisation we used novel fabric hoods. Five hoods were made using black nylon fabric and a collar fastening (Figure 1[a]). Hoods were designed with a breathing hole over the nose and could be adjusted to fit rabbits of varying size and breed. Swaddling involved sitting the rabbit in the centre of a towel. The towel was first folded over the rump and then wrapped around the body so that only the rabbit's head was exposed (Richardson & Keeble 2014; Figure 1[b]). Rabbits in the control group were fitted with a hood identical to that used for blindfolding, but with the eyes exposed (Figure 1[c]), to test whether any differences observed with blindfolding were due to visual restriction and not the act of fitting a hood.

A wooden arena (approximately 4 m^2 in area and 1 m high) was assembled at each site in a quiet area for use as the novel environment. The base of the arena was equally divided into nine squares to allow quantification of movement (based on Unwin *et al* 2019 with modifications). Tests that took place within the novel arena were recorded using a Canon® Legria FS200 video camera (Canon Ltd, Uxbridge, UK) (Figure 1[d]). Time taken capturing and transporting the rabbit to the novel arena was also recorded.

Throughout the study, the experimenter wore a long-sleeved white lab coat and carried a stopwatch around her neck. To facilitate calm handling, Pet Remedy™ (a Valerian-based nutraceutical manufactured by Unex Designs Ltd, Torquay, UK) was applied to one sleeve of the lab coat at the start of each day of testing for all cohorts, and half-way through each day of testing at the larger cohort sites 1 and 4. The lab coat was washed between cohorts and towels were washed between use to remove scents of unfamiliar rabbits and as a precaution against the potential effects of a build-up of Pet Remedy™ over the course of the study.

The following protocol was used on each of the five days.

Home enclosure approach and transfer

A top-opening wire crate (46 × 29 × 29 cm; length × width × height) was used to transfer rabbits to the novel arena. A clean towel was placed within the crate for each rabbit. Another towel was used to cover the crate during transport to the novel arena. The experimenter approached each enclosure quietly whilst holding the crate. The crate was then placed outside the enclosure, hidden from the rabbit, if possible. The experimenter held her hand against the enclosure for 30 s and the rabbit's behaviour was recorded. If the rabbit was housed in an enclosure with no visibility (eg in a shed), then the door was opened before the experimenter held out her hand. The experimenter then entered the enclosure with the crate, or opened the hutch door, and started the stopwatch before attempting to capture the rabbit. Once the rabbit was captured, it was placed

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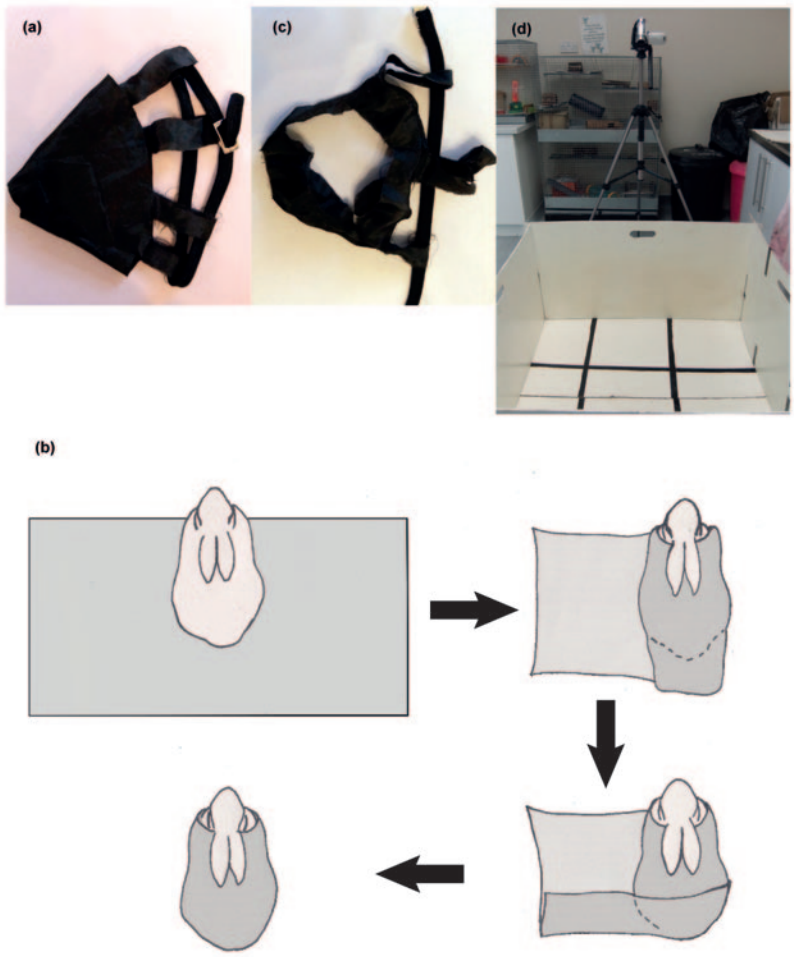
Figure 1

Showing (a) blindfold, (b) method for swaddling, (c) hood for control group and (d) novel arena.



inside the crate and the time taken to capture recorded (Table 3; see supplementary material to papers published in *Animal Welfare*: https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). During capture any signs of avoidance, such as kicking, grunting or thumping, were noted. A towel was placed over the crate and the rabbit was transferred to the novel arena. The time from the moment the crate was closed, until it was placed within the novel arena was recorded (Table 3; https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material).

Novel arena

Once within the novel arena, the towel was removed from the crate and the video recording started. The crate was then opened, the rabbit was removed and placed in the centre of the arena. The crate was removed from the arena and the rabbit's behaviour was observed for 1 min. Behavioural signs of fear or anxiety were noted, as well as exploratory behaviours, such as sniffing and an estimation of overall movement within the arena made, based on the number of quadrants moved into. The experimenter then held a carrot (a food item known to motivate all rabbits in the study) at rabbit height in one corner of the arena. The rabbit's proximity to the carrot was recorded. If the rabbit was in one corner of the arena, then the carrot was held in the opposite corner. After 30 s, the carrot was removed, and the experimenter entered the arena with a clipboard and sat down for a further 30 s recording the rabbit's behaviour throughout. Initial responses to the experimenter entering the arena were observed. The experimenter remained in the arena and placed the appropriate treatment (blindfold, towel or control) in the centre of the arena, allowing the rabbit opportunity to approach before applying this. The treatment was applied with the rabbit placed between the experimenter's legs, whilst she was in a kneeling position. The time taken to apply the treatment, as well as the rabbit's behavioural response was recorded. Once the treatment was securely fitted, the experimenter began to gently handle the rabbit (Days two to four) or perform a health check (Days one and five) for 2 min and heart and respiratory rate were recorded.

Baseline and post-treatment health checks

Health checks involved checking both ears with an otoscope, checking the nails, fur and body condition, as well as recording heart and respiratory rate. Heart rate was measured via a stethoscope and respiratory rate was calcu-

lated by counting breaths for 30 s. During the health check, the experimenter sat on the floor with the rabbit between her legs. The check took 2 min and the rabbit's behaviour throughout was rated. Any struggle response was noted including kicking and escape attempts (Table 3; https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). The blindfold or towel was then removed before obtaining a saliva sample for cortisol analysis. A Salimetrics® infant swab was held in the rabbit's mouth for between 60 s and 2 min or until the swab felt moist. Swabs were stored in Salimetrics® swab storage tubes and transferred to a –20°C freezer after completion of the day's tests. Saliva sampling occurred last and after the majority of testing, and as cortisol is believed to take 2–3 min to reach saliva in mammals (Bozovi *et al* 2013), this timing meant the sample reflected the stress associated with the test.

Treatment sessions

On Days two to four, the rabbits were not health checked, but were handled for 2 min with the treatment in place; the experimenter sat on the floor, with the rabbit between her legs, whilst she stroked the rabbit on the back and head. General behaviour observations were noted. Respiratory and heart rate were recorded after 30 s of handling.

Post treatment

The treatment was removed, the experimenter remained in the arena and held out a carrot for 30 s, recording the rabbit's closest proximity to the carrot. The video recording was then stopped, and the rabbit returned to the carrier, which was covered by a towel and transferred back to the home enclosure. The arena was cleaned between each test using odourless Dettol™ antibacterial spray.

Cortisol analysis was performed using a Salimetrics® Salivary Immunoassay Kit following advised protocol (Salimetrics 2014). Results were analysed using GENESIS® software for Windows. Twenty-two of the eighty samples achieved the 27 µl required for analysis however only two samples provided final readings of cortisol concentration (0.007 and 0.016 µg dl^{-1}) and hence results were not analysed further.

Behaviour

Eighteen behavioural variables were recorded in real time observation and/or extracted from the video recordings using the ethogram described in Table 3 (https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material), plus a subjective assessment made of the rabbit's overall calmness giving a total of 19 variables. All data recording was carried out by a single observer (KM), using scorings she had previously shown to be reliable via interobserver checks (Unwin *et al* 2019). There were 35 individual behaviours recorded in multiple test components that were used to derive number of different negative (eg freezing, withdraw, vocalise and struggle) and number of different positive behaviours (eg rear, lie stretch out, approach experimenter). This categorisation was based on that of Unwin *et al* (2019), and the exact variables possible differed with sub-test (eg approach experimenter was impossible in experimenter absent sub-test).

Statistical analysis

All data were analysed using IBM SPSS™ statistics 24 software for windows. Physiological and behavioural data were visually inspected for trends over the five days. Statistical comparisons were made between Days one and five using a repeated measure general linear model, with treatment group as a between-subjects factor and study day as a within-subjects factor. Weight was included as a covariate, as this may have had an effect on variables, particularly heart and respiratory rate. A parametric approach was tried, but the residuals from the models failed to conform to the assumptions required for the analysis and so all analysis was non-parametric. Kruskal-Wallis were used to test for significant differences in behaviour between the three handling groups, both in absolute levels on Days one and five and in changes from Day one to Day five. If a significant difference was identified, a Mann-Whitney *U* test was used to identify the specific groups that differed significantly.

Results

Treatment group did not significantly impact respiratory and heart rate (Table 4; see supplementary material to papers published in *Animal Welfare*: https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). Upon visual inspection of the data, a decrease in heart rate was observed in both the blindfold and swaddling groups from Day one to three, followed by an increase from Days three to five. In the control group, there was a slight increase on Day two followed by a gradual decrease to Day five. There was also no significant difference in latency to capture between treatment groups (Table 4; https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material).

There was a statistically significant difference between groups in terms of the number of squares moved into during the novel arena test on Day one ($P = 0.022$; Table 4; https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). Rabbits in the control group moved into significantly more squares on Day one than rabbits in the swaddling group. The decreased movement observed from Day one to five was also significantly different between groups ($P = 0.037$), with differences between swaddling and control groups reaching significance. There was a greater decrease in number of squares moved into on Day five, compared to Day one by rabbits in the control group than rabbits in the swaddling group.

The number of positive behaviours observed before the experimenter entered the novel arena was significantly different between groups on Day one ($P = 0.043$; Table 4; https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). A significant difference was observed between

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the swaddling and control group (P = 0.047) and the swaddling and blindfold group (P = 0.029). The swaddling group showed fewer positive behaviours than both the blindfold and control groups.

A significant relationship was observed between group and post-treatment proximity to the carrot on Day five (P = 0.020), with rabbits in the blindfold and control groups more frequently eating or contacting the carrot than rabbits in the swaddling group. The difference was significant only between the control and the swaddling group (P = 0.005). This relationship was not present after treatment on Day one or before treatment (Table 4; https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). When analysing the change from Day one to five in response to the carrot after treatment, a significant change in behaviour was identified (P = 0.031). This was evident between the blindfold and the swaddling group (P = 0.008). Individuals in the swaddling group were more likely to maintain a further distance from the carrot on Day five than Day one, whereas those in the blindfold group were observed to be closer to the carrot on Day five compared to Day one.

The positivity of the response to handling on Day five was significantly different between groups (P = 0.037; Table 4; https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material), with significant differences between the swaddling and control groups (P = 0.033) and the blindfold and swaddling groups (P = 0.022). There was an overall mean (± SEM) increase in positivity in the swaddling group of 0.31 (± 0.308). Both the blindfold (–0.50 [± 0.337]) and control (–0.36 [± 0.310]) groups were found to respond more negatively to handling over time. A significant difference was observed between groups in the number of escape attempts on Day one (P = 0.024), with significantly fewer escape attempts in the blindfold group than in the control (P = 0.015).

Discussion

No statistically significant differences in physiological indicators of stress were found between handling methods. Therefore, we have no evidence to suggest that the use of blindfolding or swaddling reduced physiological stress responses induced through handling, when compared to application of a partial hood. Previous research found that visual restriction led to decreases in heart rate in cattle, but increases in horses (Mitchell *et al* 2004; Parker *et al* 2004). Here, no significant difference was found with visual restriction in rabbits. It is possible that techniques such as blindfolding and swaddling have limited impact once a high level of stress is reached. A clinical examination including an otoscope may be especially aversive for an unhabituated rabbit and so the intensity of handling may have been too severe for either swaddling or blindfolding to significantly impact the measured stress response. Average heart rates for all groups were relatively high compared to normal physiological parameters, although they were within the normal range (154–300 bpm; Varga 2014). Respiratory rates, on average, exceeded the normal resting range of 30 to 60 breaths per min, in all groups, suggesting that stress levels were universally high (Varga 2014). We saw a decrease in heart and respiratory rate after Day two in the control group, but no other signs of habituation or desensitisation over the five-day period. With the absence of accurate history for many of the rabbits in this study, we cannot be sure if there had been previous exposure to positive handling or rough treatment. This may have led to universally elevated physiological measures in response to handling, that precluded any measurable benefit from either of the treatments. As capture time varied greatly between individuals, it is possible that physical activity prior to the treatment led to elevated heart and respiratory rate in some individuals. Similarly, as there were slight differences in transfer time to the novel arena, between individuals and cohorts, this may have had an effect on heart and respiratory rate (although cohorts were balanced between treatment groups). It is also possible that the challenge of counting fast heart rates meant that subtle differences between groups were not detected.

Although there were no significant differences between groups in physiological indicators of stress, there were some observed behavioural differences. Significant differences were observed in the number of escape attempts, but only on Day one, suggesting this variable was not affected by repeated handling and the groups were imbalanced at the outset. Response to the carrot after treatment was found to become significantly less positive in the swaddling group over the study, compared to the control (partial hood) group. This is perhaps not surprising given the lower levels of exploratory behaviour at baseline in this group. They may have required a longer recovery time from handling than the control group, due to aversion to the tester. However, due to baseline differences in this group, this does not conclusively show that the use of swaddling in itself contributed to increased stress. The blindfolded group, in contrast, decreased their distance from the carrot. Although, it is possible that this was due to the rabbit freezing when the blindfold was removed, and hence neither moving away from the examiner nor actively approaching the carrot.

The observed higher positivity score in response to swaddled handling, may have been impacted by restraint limiting struggling (Rooney *et al* 2014; Unwin *et al* 2019). Struggle response may also have been physically inhibited by the swaddling, giving an impression of increased positivity, although physiological responses suggest that stress levels remain unchanged.

The significant difference observed between treatment groups in the number of squares moved into and the number of positive behaviours prior to handling on Day one, suggests initially unbalanced groups, especially as these variables were measured prior to the application of the treatment. On Day one, swaddling group rabbits moved into significantly fewer squares than those in the control group. Similarly, individuals in the swaddling group were shown to exhibit significantly fewer positive behaviours than the control group or the blindfold group. Differences in previous handling experience, as well as personality could have contributed to these significant differences. The number of squares moved into decreased significantly more over time in the control group (change in

number of squares moved into) than in the swaddling group, however the potential for decreased exploration from baseline was lower in the swaddling group and a reduction in stress response was not reflected in the number of positive behaviours recorded in this group.

Swaddling was associated with higher positivity scores in response to handling when compared to the other two treatments, however this may have been a consequence of the physical restriction caused by being swaddled. Variables, such as response to carrot and number of positive behaviours, did not reflect a trend in increased positivity in the swaddling group and, in response to the carrot, the blindfold group appeared to show a more positive change. Hence, the different variables do not give a single consistent trend with treatment type. It is likely that neither treatment had the same effect on all rabbits, as extraneous factors such as history and temperament have a major effect.

Here, we used a diverse population within rehoming centres, this increased variability is challenging and may have precluded detection of more significant changes, however we believe this approach increases external validity since the sample is an accurate reflection of the general rabbit population in terms of variability. We also omitted the most nervous individuals to protect animal welfare, however, were they to have been included the differences seen may have been more pronounced.

In this study, rabbits housed together were allocated to the same treatment groups to avoid social contagion effects, they were tested separately and without sight of each other, but we treated them as independent subjects in the analysis. Building in the potential nesting effect, or only utilising one rabbit per enclosure would have been preferable were a bigger sample size available. We also analysed a large number of variables so the possibility of Type I errors, required consideration.

It is important to note that this study compares blindfolded rabbits to those that also had a partial hood, without their eyes being covered. This was selected to be able to elucidate the effect of covering the eyes, however we did not have a control treatment with no swaddling or head covering. It is likely that the act of placing the hood was stressful, and hence to fully understand the effect of handling it would be valuable to repeat the study with a control group of just holding the rabbit without any hood or swaddle. A further limitation is the lack of blinding of the experimenter, they were aware of the treatment for each rabbit and hence it is not impossible that there may have been unconscious bias in the results.

Results from the survey indicate that blindfolding and swaddling are routinely used in many species, including rabbits, principally with the intention of reducing acute stress. Our experimental study, however, suggests there is little benefit in terms of stress reduction from the use of either covering the eyes or swaddling, although we did not compare this to handling without any intervention. There were a small number of behavioural differences between the groups, but no significant physiological differences. Therefore, we cannot reject the null hypothesis that blindfolding or swaddling methods have no measurable effect on the stress response to handling in domestic rabbits. It was notable that many of the rabbits showed extreme physiological responses and behavioural aversion to the experimenter, suggesting that handling is generally aversive, and restraining is ineffective at reducing this. In this study, all handling was performed on the floor which is generally advised and so it is likely that for many rabbits in the general population, which are picked up, handling stress may be even greater (eg Lehmann 1991; Bradbury & Dickens 2016). However, here, we did catch and place the rabbit in a carrying crate which likely was stressful. It is likely that some variables, particularly heart and respiratory rate may have been elevated due to the examiner having to capture the rabbit prior to placement in the novel arena. In future studies of handling effects, limiting exposure to the examiner outside the treatment, for example, by having owners bring their rabbits to the examiner whilst extending the length of exposure to the treatment method, may help to identify subtle physiological differences between treatments. Since capture is likely stressful (yet a routine part of rabbit management), in the pet-owning population methods to train rabbits to enter of their own volition may be beneficial (McBride 2014; Bradbury & Dickens 2016).

Animal welfare implications

This study suggests that for domestic rabbits the covering of the eyes was insufficient in reducing stress caused by handling. Although lacking a full control group, the study provides no evidence that swaddling noticeably reduces stress. However, there may be a welfare benefit, if blindfolding or swaddling reduce procedure length or injury risk, but this study was unable to identify significant benefits. Use of swaddling or blindfolding may be suitable as a means of improving the safety of the rabbit or examiner or to shorten the length of necessary stressful procedures. However, both methods should be utilised with consideration that the rabbit may still experience great stress and alternative treatments, such as behaviour modification, aromatherapy, eg Pet Remedy™ (see Unwin *et al* 2019) and low stress-handling techniques may be necessary to ensure stress levels are minimised. It is also possible that, for some rabbits, use of either blindfolding or swaddling may increase stress. Further research is required to determine effective means of reducing stress during handling of domestic rabbits and, in turn, improving welfare.

Declaration of interest

None.

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