Pinch-induced behavioural inhibition (clipthesia) as a restraint method for cats during veterinary examinations: preliminary results on cat susceptibility and welfare

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doi: 10.7120/09627286.25.1.115

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Abstract

Cats are often subjected to minimally painful or forced procedures during routine clinical practice, which can be poorly tolerated, leading veterinary surgeons to need to offer physical restraint, usually aided by an assistant. The aim of this study was to assess the effectiveness and ultimate welfare implications of using clipthesia as a method of restraint during veterinary examination. This was carried out in a real clinical setting and compared to manual scruffing. Twenty-seven cats were restrained, during a veterinary examination, using two stationery clips placed on the skin along the cervical dorsal midline, whilst a group of 13 cats were restrained through gentle manual scruffing. Susceptibility to clipthesia (ie a positive clip score) was observed in 81.5% of cats, while a complete response was found in 40.7% of subjects. The presence or absence of a disease/condition did not affect the susceptibility. Heart rate and the number of cats showing mydriasis (pupillary dilation) was statistically higher during manual scruffing, whilst plasma cortisol did not differ between the two groups. The more responsive the cats were to clipthesia, the more they displayed kneading and purring. These preliminary findings suggest that clipthesia is not more stressful than manual scruffing in restraining cats during a veterinary examination. However, not all cats were found to be susceptible to this method of restraint. Further research is needed to clarify whether clipthesia should be implementated as a matter of course in veterinary practice from the point of view of welfare and safety.

Keywords: *animal welfare, cat, clipnosis, clipthesia, scruffing, veterinary examination*

Introduction

In many vertebrate and invertebrate species, it is possible to trigger a state of profound immobility and relative unresponsiveness (usually called immobility reflex or animal hypnosis) through different types of sensory stimulation (Klemm 1971; Galup & Gordon 1974; Amir *et al* 1981). There seems to be four categories of conditions that facilitate such a state: repetitive stimulation; pressure on body parts; inversion; and restraint. This kind of immobilisation is associated with a lower reactivity to external stimuli, while the muscular tone is preserved. Flexor and extensor muscles can be contracted simultaneously, resulting in the maintenance of an awkward, immobile posture (Klemm 2001). The existence of a behaviour arrest system (BAS) has been hypothesised (Klemm 2001), which actively antagonises the onset of the movement and its maintenance. The neurophysiological basis of these so-called behavioural arrests has been identified via an inhibition of the dopaminergic system (Fleishmann & Urca 1988). In fact, the neurophysiological mechanism producing the motor inhibition and a reduction in the alert status is similar to that produced by some dopaminergic blocking drugs, such as antipsychotic drugs that induce ataraxia, ie reduced responsiveness to both innocuous and noxious external stimuli (Fleishmann & Urca 1988; Crowell-Davis & Murray 2006).

During routine clinical practice, cats are frequently subjected to minimal painful or forced procedures (such as blood withdrawal, nail clipping, measurement of rectal temperature, and lateral decubitus for x-rays). Such procedures are often poorly tolerated by cats, and consequently veterinary surgeons need to physically restrain the cat, usually with the help of an assistant. Manual scruffing prevents cats from escaping, but often leads to an increase in fear and aggression, and also to changes in physiological parameters, that make the examination less accurate.

In order to promote cat welfare and safety to people, the last few years have seen new methods of restraint proposed. For instance, Leedy *et al* (1983) suggested using a rubber band around the base of the cat's ears whenever a practitioner needed mild restraint, especially in the absence of any assistants. Much interest surrounded the idea of clipthesia — a behavioural inhibition induced by pinches placed on the dorsal neck skin (McCune 2010) — which has increasingly been used as an alternative method of restraint in cats. However, few studies have investigated the effectiveness of this method and its effect on cat welfare. Tarttelin (1993) pioneered the use of different clips applied to different areas of the body. Pozza *et al* (2008) found the greatest response by attaching the clips to the dorsal neck area. Analysing the behavioural and physiological parameters of 31 laboratory cats, the authors concluded that pinch-induced behavioural inhibition was not associated with fear or pain responses. Valente *et al* (2013) found no difference in the physiological and behavioural parameters of cats when comparing Clipnosis® to another restraining method.

The aim of this study was to assess the effectiveness and consequences for welfare of using clipthesia as a method of restraining cats during a veterinary examination. This was carried out in a real clinical setting and compared to gentle manual scruffing.

Materials and methods

Participants

The sample was made up of 40 cats undergoing routine veterinary procedures, consisting of auscultation and blood sampling, on the examination table of a veterinary clinic. All participating cats were pets, recruited from the various patients brought into a veterinary clinic in Tuscany, Italy. All cats were examined by the same female veterinarian and procedures were all carried out in the presence of owner(s) and an assistant.

Twenty-seven cats (16 males and eleven females; with mean [± SD] age of 5.5 [± 3.8] years) made up the CLIP group, where restraint was attempted using two stationery clips placed on the skin along the cervical dorsal midline. Another group of 13 cats (the SCRUFF group, including eleven males and two females; 8.0 [± 5.6] years old) were restrained using the traditional method, ie through gentle manual scruffing (see Table 1 for the characteristics of cats). Cats were put in the CLIP group if the owner gave their consent to the application of the clips, otherwise, the cat was included in the SCRUFF group. According to the veterinarians' previous experience with cats of both groups, this assignment was not biased by cats' manageability during the clinical examination.

Procedure of restraint

The procedure for the CLIP group was as follows. The cat was removed from the carrier and, following Pozza *et al* (2008), two 5-cm stationery clips (Staples®) were applied to the cervical area (the 'scruff'), one just behind the pinnae and the other right behind the first clip. Pressure was gradually applied. Clips were kept in place for the period of time required for the behavioural and physiological assessments to be carried out (lasting from 2 to 5 min).

The effectiveness of clipthesia as a method of restraint was evaluated using a Likert scale ranging from –3 to +3, modified by Pozza *et al* (2008). A description of the different ranks (or clip score; CS) was created in order to assign a score based on the cats' responses. Clip scores are described in Table 2.

Cats rated with a positive score (+1 to +3) were assessed as susceptible to clipthesia. Figure 1 shows a cat with total susceptibility, ie clip score +3.

The procedure for the SCRUFF group differed from that of the CLIP group only in terms of the type of restraint, ie gentle manual scruffing by an assistant.

Figura I: Spontaneous lateral decubitus, ventroflexion with the tail curled up
under the body, and relaxed limbs and tail (clip score: +3). Note the two stationery clips placed on the skin of the cervical area.

Evaluation of cat welfare

The welfare of both cat study groups was assessed via plasma cortisol concentration during restraint, and a comparison of changes in heart rate and pupil diameter before and during restraint. Behaviour was assessed in terms of kneading and purring displays during restraint. Additionally, the day following examination, owners of the CLIP group were telephoned by the veterinary assistant and asked whether their cat’s behaviour, on returning home, differed (similar, better or worse) from that observed following previous visits, ie when the cat had been restrained by manual scruffing.

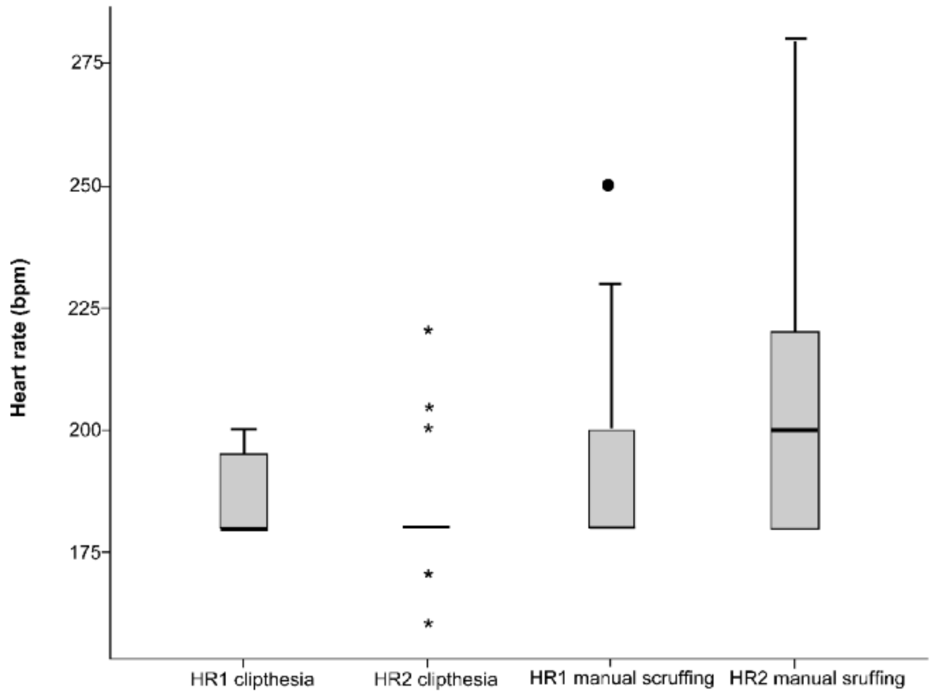

Figure II: Heart rate (bpm) in cats restrained by clipthesia and gentle manual scruffing before (HR1) and during (HR2) restraint. For each box, the bottom and top horizontal lines represent the lowest and highest values, the lowest and top edge of the tinted box represent the lower and upper quartile, the horizontal line within the tinted box represents the median, the small circles represent the outliers, and the stars represent the extreme outliers.

For each cat, pupil diameter and heart rate were evaluated when cats were still inside their carrier, before the clinical examination, and again during restraint prior to bloodtaking. Pupil diameter was assessed, in keeping with Reiner (1986), by both the veterinary assistant and the veterinary surgeon. Pupils were rated as being normal, in miosis

(contracted) or in mydriasis (dilated) twice. Comparing the second assessment (during restraint, prior to blood-taking) to the first (in the carrier), an evaluation was made as to whether the pupil diameter was stable, decreased or increased due to the clips. Such assessments resulted in a total inter-observer reliability (100% of agreement). Heart rate was measured via auscultation with a stethoscope.
Blood samples were collected during restraint, after auscultation, from the cephalic vein and using a butterfly needle.
Immediately following collection, the blood was centrifuged and frozen at –18°C for storage until analysis. The plasma cortisol was evaluated with an immunodiagnostic method (Diametra®, Milan, Italy). Taking a blood sample was not possible in five cats (two of the CLIP group and three of the SCRUFF group) due either to them being too difficult to handle or owners not willing to give their consent. Two other samples were unusable due to being lipaemic. A total of 13 blood samples from the CLIP group and ten from the SCRUFF were used for statistical analysis on plasma cortisol.

Statistical analysis

Data on plasma cortisol in the two groups were compared using the Mann-Whitney test ($P < 0.05$). The Wilcoxon test ($P < 0.05$) was used to compare heart rate before versus during restraint within CLIP and SCRUFF groups.
The Kruskal-Wallis test ($P < 0.05$) was used to investigate possible differences in plasma cortisol and heart rate depending on the clip score (+3, +2, +1 or other) within the CLIP group.
The Chi-squared, or Fisher's test when appropriate ($P < 0.05$), was used to compare the number of cats showing mydriasis, kneading and purring in the CLIP and SCRUFF groups. The same test was also used to assess whether the clip score affected physiological and behavioural parameters analysed, and to compare susceptibility to clipthesia between cats with and without a disease/condition.

Results

The results are summarised in Table 3.

Susceptibility to clipthesia (ie clip score: +1, +2 or +3) was observed in 81.5% of cats in the CLIP group, while a complete response (CS: +3) was found in 40.7% of subjects. Only one cat was designated as having a negative score, and in this case the clips were removed immediately after evaluating the heart rate and pupil diameter. The presence or absence of a disease/condition did not influence susceptibility to clipthesia (78.6 versus 84.6%; $\chi^2 = 0.008$; $P = 0.927$). All cats with a positive CS showed a behavioural inhibition a few seconds after application of the clips. When the clips were removed, all the cats in the CLIP group immediately recovered a normal quadrupedal posture and, spontaneously, returned to their carrier. Behavioural inhibition and CS remained unchanged throughout the duration of clip application. According to the reports of owners who had been regular visitors to the clinic, some of the cats were more relaxed and calm when back home compared to when they had been restrained in the traditional manner.

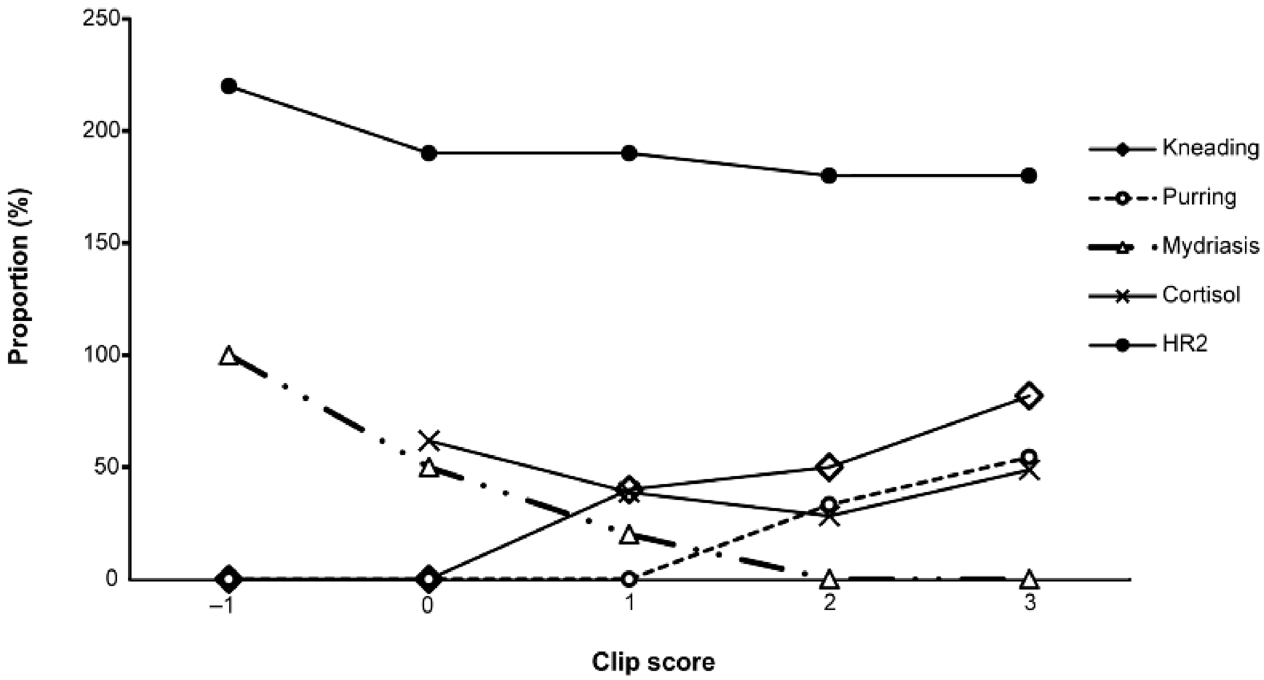

Figure III: Proportion (%) of cats showing kneading, purring and mydriasis, and values of plasma cortisol (ng ml–1) and heart rate during the application of clips (HR2 in bpm) according to the clip score.

Plasma cortisol did not differ statistically when the CLIP group was compared to the SCRUFF (median and minimum-maximum range: 46.3 versus 61.8, 12.8–72.1 versus 6.8–112.9; U = 75.50; P = 0.716).
Heart rate was not statistically different in CLIP and SCRUFF groups prior to restraint (Figure 2; U = 133.50; P = 0.228), however it was statistically higher in the SCRUFF group during restraint (U = 90.50; P = 0.013).
Mydriasis was assessed as a comparison of pupil diameter before and then during the restraint. It was statistically less frequent in the CLIP group than in the SCRUFF (14.8 versus 69.2%; $\chi 2$ = 9.494; P = 0.002).
Although cats in the CLIP group showed a greater tendency to display kneading (29.6 versus 7.7%; $\chi 2$ = 1.327; P = 0.249) and purring (51.9 versus 30.8%; $\chi 2$ = 0.839; P = 0.360) compared to those in the SCRUFF group, this difference did not reach a statistically significant level. All the cats that displayed kneading, also purred, but not *vice versa*.
Figure 3 shows the main findings regarding sub-groups of cats with differing clip scores.
No difference was found in the comparison of sub-groups of cats with different clip scores for plasma cortisol ($\chi 2$ = 1.123; P = 0.772), and heart rate before ($\chi 2$ = 2.422; P = 0.490) or during restraint ($\chi 2$ = 4.572; P = 0.206). However, mydriasis in the CLIP group was only observed in subjects with a clip score ranging from –1 to +1 (n = 4), whilst miosis was seen in subjects rated +2 or +3 (n = 3). The majority of cats belonging to the CLIP group did not show an increase or decrease in pupil diameter when comparing the two evaluations. Figure 3 appears to indicate that, with an increase in clip score (ie a higher response to clipthesia), there is a greater tendency for kneading and purring, a decrease in mydriasis and heart rate during restraint.

Discussion

The extent to which animals' welfare is compromised can be assessed using various parameters, with physiological and behavioural indicators being the most common for pets. To establish stress and subsequent welfare problems in dogs and cats, behavioural parameters are of special interest, because measurement is relatively easy and non-invasive (Mariti *et al* 2012). However, in this study, due to the inhibitory aspect of the restraint itself, the behavioural assessment was limited to observation of purring and kneading. Here, the welfare of cats was assessed mainly through autonomic responses that were easily assessable during a routine clinical examination (ie heart rate and pupil size), and biochemical parameters (plasma cortisol).

The results of this study showed that clipthesia was less likely to induce an increase in heart rate and pupil diameter compared to manual scruffing. This is in accordance with previous findings, in which cats acted as their own control. In fact, using telemetry devices, Pozza *et al* (2008) found that the application of clips did not affect heart rate, blood pressure and body temperature, but induced miosis. In addition, Valente *et al* (2013) found that blood pressure, heart rate and cortisolaemia did not differ statistically when comparing Clipnosis® to another restraining method. The data in the present study also suggest that an increase in the clip score corresponded to a decrease in mydriasis. This is in agreement with Reiner (1986), who found that cats responsive to the scruff immobility reflex (induced by grasping cats firmly by the nape of the neck and lifting them off the floor of the cage) were more likely to display miosis. In addition, the more responsive the cats were to clipthesia, the more they displayed kneading and purring. These behaviours are usually classified as infantile, even when displayed by adult cats, but little is known about them. Purring is considered to be a form of vocal communication, directed towards people especially (Bradshaw *et al* 2012). It is traditionally interpreted as indicating pleasure, emitted in a relaxed cat (see the stress score presented by Casey & Bradshaw 2007), however, in actual fact, purring is seen in a wide variety of circumstances (Bradshaw & Cameron-Beaumont 2000). For instance, although cats may purr when asking for food (McComb *et al* 2009) or when being stroked (Von Muggenthaler & Wright 2003), it will also be observed when they are slightly anxious (Overall 2013; p 347), in pain, or seriously ill (Rochlitz 2009). Von Muggenthaler and Wright (2003) explained the latter in terms of natural selection, having demonstrated that the frequencies of cat purring improve healing time, bone strength and mobility. Beaver (2003; p 101) hypothesised that purring in cats just before death and following a chronic disease may reflect a state of euphoria, perhaps resulting from an endorphin release, similar to that experienced by terminally ill humans. However, the acoustic structure of purring changes according to the context, and probably to the function (McComb *et al* 2009). It is therefore hard to give an unequivocal interpretation of purring.

In this study, purring and kneading were slightly more frequent in cats that were highly responsive to clipthesia, and kneading was associated with purring. This seems to suggest that all these behaviours, including the pinchinduced inhibition, may be related to the infantile stage and maintained in adult cats.

As regards behaviour, cat responses to clipthesia appeared to differ from that of freezing observed in fearful animals, including cats. In subjects scoring +3, the behavioural response resembled that of kittens picked up by the skin of the neck by the queen to be moved from one nest site to another (Hart 1978). In fact, cats that were highly responsive to clipthesia showed a ventroflexion with the tail curled up under the body, in addition to a minimal response to tactile stimuli. Similar observations were also reported by Reiner (1986) and Pozza *et al* (2008).

In the present study, an immediate resumption of normal mentation and quadrupedal standing was observed in all cats after clip removal. In addition, according to the owners, some cats appeared more relaxed and calm when back home after clipthesia than when traditionally restrained (similar to findings by Pozza *et al* 2008). This point requires further investigation, as it may represent a significant benefit for cats, especially those needing regular veterinary care or that find examinations very stressful.

The observation of cat behaviour after clips were removed, revealed that none of the cats, regardless of the score, showed signs of pain or discomfort due to application. This is entirely plausible as a pressure of 300 mm Hg applied for prolonged periods, ie more than three hours, is required to cause ischaemic skin necrosis (Tsuji *et al* 2005). Instead, the clips used in this study, were identical to those described by Pozza *et al* (2008). It can thus be assumed that their compressive strength ranged between 140 and 160 mm Hg, not exceeding the average blood pressure of the cat (Pozza *et al* 2008).

The combination of findings regarding the physiological and behavioural parameters supports the hypothesis that clipthesia is less stressful for cats than manual scruffing during a veterinary examination. The increase in heart rate and pupil diameter observed more often in manual scruffing than when using the clips suggests that scruffing provides greater stimulation of the orthosympathetic system of cats, indicative of a state of alert and warning. As this study was carried out in a real clinical setting, it is likely that many cats were already distressed prior to being restrained. However, the findings suggest that manual scruffing induced a further activation of the sympathetic system (mydriasis and increase in heart rate), whilst such changes were not observable in cats restrained by clipthesia (heart rate and pupil diameter did not vary after the application of clips). Reiner (1986) found that, during the scruff immobility reflex, the activity of the noradrenergic neurons PS-off cells within the locus coeruleus complex fell silent, as occurs during paradoxical sleep (PS). However, as heart rate and pupil diameter are not solely under sympathetic control, the findings could be due to a decrease in the sympathetic tone as well as an increase in the parasympathetic tone (Reiner 1986).

The lack of a statistical difference between both groups as regards plasma cortisol requires careful consideration. Cortisolaemia in cats reaches maximal levels approximately 15 min after the stressor event (Iki *et al* 2011), and a decline has been demonstrated 30 min subsequent to that (Genaro *et al* 2007). The cats participating in this study had already experienced a stressful event (being placed in the carrier, transported by car, staying in the waiting room, etc), and therefore it is not possible to attribute a unequivocal relationship between the plasma cortisol values and the state of welfare during restraint.

In contrast to the results of this study, McBride *et al* (2006) found that in rabbits, trancing led to an increase in heart rate, respiratory rate, plasma cortisol, as well as an increase in fearful behaviour after restraint. Such differences between cats and rabbits suggest that, although many people refer to this behavioural inhibition using generalised terminology, eg animal hypnosis, it is likely that such states are caused by different underlying systems. In the case of freezing, the basolater amygdala is involved (Power & McGaugh 2002), whilst in cats it is likely that the behavioural inhibition originates in the forebrain (Pozza *et al* 2008). According to Klemm (2001), the tactile stimulation of particular areas of the skin, instead of stimulating the alert system, can activate the BAS and provoke a state of hypomotricity and hyporeactivity. Electrophysiological studies have demonstrated that there is an interaction between the somatosensory region of the skin of the neck and the vestibular system (Ezure & Wilson 1984). Intense stimulation of these somatosensory areas can consequently inhibit the ability to maintain quadrupedal standing, as observed in cats scoring +2 or +3.

The use of clips during routine veterinary examinations is not condoned by a number of veterinary surgeons and behaviourists, while others have concerns regarding the ethics of its use (Rodan *et al* 2011). However, some veterinarians and behaviourists also condone the use of manual scruffing (Rodan *et al* 2011), which is the established method of restraining cats during examinations. The current study compared the two methods in a real clinical setting and suggests that manual scruffing is more stressful than clipthesia. In fact, all the previous literature agrees on the impact it has on cat behaviour and physiology. However, more research is needed in order to fully comprehend its impact on cat welfare, before we can consider it a safe and ethical way of restraining cats during veterinary examinations.

The Feline Advisory Bureau (2010) recommends handling cats using minimal restraint. It is possible that the use of clips, allowing the veterinary surgeon to restrain the cat with minimum physical contact and with fewer people around to help, is less intrusive for cats than manual scruffing. This may have important implications for cat welfare and management, as the use of clipthesia may enable cats to have a better experience when being examined. This, in turn, could make cats more tolerant to subsequent clinical examinations. According to Klemm (2001), during behavioural arrest, sensory stimuli are not only recorded, but also processed and stored. Therefore, it is important that, as in the present study, cats are handled calmly and gently during restraint, in order to avoid any negative associations with the clinical examination or with the application of the clips.

It must be highlighted that not all cats are susceptible to clipthesia. Tarttelin (1993) found that 67% of cats were susceptible to the use of clips, however the lack of detail provided did not lead to a direct comparison with the findings of the present study. Reiner (1986) reports that nine out of 17 adult cats showed a profound scruff immobility reflex induced by grasping and lifting cats by the nape of the neck. Pozza *et al* (2008) reported a positive score in 92% of subjects. This study found that almost half of the cats showed a complete response, which meant that the veterinarian could perform the auscultation and blood sampling from the fore limb unassisted. Similarly, out-with the study period, other minor veterinary procedures (eg measurement of rectal temperature, inspection of eyes and ears, x-ray and cutting of the nails) were all able to be performed by the veterinarian herself. In many other cases, cats were partially inhibited by the clips, and minimal assistance was required in order to handle the cat during the examination.

Future research is needed in order to understand which factors affect a cat's susceptibility to clipthesia. For instance, illness did not affect susceptibility, either in this study or in Pozza *et al* (2008), whilst the repeated use of clips may affect susceptibility in terms of the number of cats responding positively and the clip score (Pozza *et al* 2008). In addition, Pozza *et al* (2008) reported that if cats were already aroused, they were less likely to respond positively to the application of clips, which has also been reported anecdotally in clinical use. The unresponsiveness to clipthesia observed in those cats already aroused could be attributed to activation of the thalamocortical alert system prior to the activation of the BAS.

Nigrostriatal dopaminergic projections produce an increase in the cerebral cortex activity via excitatory effects through a direct circuit (thalamocortical excitatory pathway) and an inhibition of it through an indirect circuit (thalamocortical inhibitory pathway) (Gilman & Newman 2003). The dopaminergic system controls locomotion (nigrostriatal pathway), wakefulness, attention, cognition, and memory (mesocortical pathway), mood, emotions, and motivations (mesolimbic pathway), hunger, thirst, and blood pressure (tuberoinfundibular pathway) (Albanese *et al* 1986).

Assuming that the BAS has inhibitory effects on the nigrostriatal dopaminergic system (Klemm 2001), it might be hypothesised that, in aroused cats, the nigrostriatal projections activate the D1 and D2 receptors on neurons of the striatum, and hence the BAS-induced inhibition of the dopaminergic system is no longer effective.

This study may have a number of limitations in relation to the lack of standardisation, eg cats showing different diseases/conditions or the presence of a previous state of stress. However, the study was carried out in real-life situations, with cats assessed in a real clinical setting, which is the situation where clipthesia is likely and intended to be used. In addition, the cats were not laboratory animals and not deliberately exposed to potentially frightening or painful stimuli (the owners were bringing their cats for a check-up, not to take part in our study). Such characteristics make this study particularly important for its clinical implications and make it a good example of how welfare assessment in companion animals could be monitored and therefore improved. However, the interpretation of physiological data needs to be validated with a controlled study, in which the inclusion criteria and standardisation would allow a more reliable comparison of physiological indicators. A real situation study, as the one here proposed, can provide an interesting basis with which to build experimental studies.

In summary, the current study found that:

- Clipthesia can be considered as a method of restraint that is not more stressful than other methods (scruffing) regularly used in veterinary clinical practice;
- In a high percentage of cats, clipthesia can facilitate minor, non-painful procedures performed by one person alone;
- Clipthesia does not cause detectable skin damage where the clips are applied;
- Clipthesia can be effective in cats of any age;
- Pathological conditions do not seem to affect its effectiveness as a procedure of restraint; and
- Clipthesia does not appear as effective if cats are already aroused.

Animal welfare implications and conclusion

These preliminary findings suggest that clipthesia is not more stressful than manual scruffing in restraining cats during veterinary examination. Of the various methods of cat restraint often required for minor clinical procedures, which are not painful but poorly tolerated by cats, the application of the clips appears to be practicable and non-harmful to cats, as well as being useful when an assistant is not available or when physical contact with people increases cat distress. However, not all cats were found to be susceptible to this method of restraint. Further research is needed to clarify whether its customary use is advisable in veterinary practice from a welfare and safety point of view.

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Tab. 1: Characteristics of cats included in the sample.

Cat	Breed	Gender	Age (years)	Type of restraint	Disease
1	Birman	NM	12	clipthesia	hyperthyroidism
2	DSH	EF	0.5	clipthesia	bronchitis
3	Persian	NM	1.5	clipthesia	acute gastroenteritis
4	DSH	NM	11	clipthesia	skin tumor
5	DSH	NF	4	clipthesia	chronic renal failure; FeLV; FIP
6	DSH	NF	12	clipthesia	dermatitis
7	DSH	NF	2	clipthesia	no disease
8	DSH	NM	5	clipthesia	skin abscess
9	DSH	NM	2	clipthesia	hind limb trauma
10	DSH	NM	4	clipthesia	no disease
11	DSH	NF	5	clipthesia	no disease
12	Siamese	NF	2	clipthesia	no disease
13	DSH	NF	3	clipthesia	no disease
14	DSH	NF	3	clipthesia	chronic conjunctivitis
15	DSH	NM	3	clipthesia	no disease
16	DSH	NF	9	clipthesia	acute anemia; FeLV
17	DSH	NM	4	clipthesia	acute cystitis
18	DSH	NM	10	clipthesia	chronic renal failure
19	DSH	NM	5	clipthesia	no disease
20	DSH	NM	3	clipthesia	chronic conjunctivitis
21	DSH	NM	5	clipthesia	no disease
22	DSH	NM	5	clipthesia	no disease
23	DSH	NF	4	clipthesia	no disease
24	DSH	NM	11	clipthesia	no disease
25	DSH	NF	7	clipthesia	no disease

26	DSH	NM	1	clipthesia	no disease
27	DSH	NM	14	clipthesia	hyperthyroidism
28	Siamese	NM	2	scruffing	no disease
29	DSH	NM	3	scruffing	lymphoadenomegaly
30	DSH	NM	14	scruffing	hyperthyroidism
31	DSH	NM	18	scruffing	chronic renal failure
32	DSH	NM	10	scruffing	no disease
33	DSH	NM	5	scruffing	acute cystitis
34	Persian	NM	8	scruffing	acute cystitis
35	DSH	NF	2	scruffing	ataxia; FeLV
36	DSH	NM	3	scruffing	pododermatitis; FeLV
37	DSH	NM	2	scruffing	acute gastroenteritis
38	DSH	NM	9	scruffing	bronchitis, stomatitis, FIV
39	DSH	NF	17	scruffing	hypertrophic cardiomyopathy
40	DSH	NM	11	scruffing	acute cystitis

Legend: DSH=domestic short-hair cat; NM=neutered male; NF=neutered female; EF=entire female; FeLV=feline leukemia virus; FIV=feline immunodeficiency virus; FIP=feline infectious peritonitis.

Tab. 2: Description of clip scores.

Clip score	Description
+3	behavioural inhibition with lateral decubitus spontaneously maintained; tail and paws are relaxed; possible kneading and purring
+2	spontaneous sternal decubitus with acceptance of lateral decubitus; when not gently, manually restrained, the cat returns to sternal decubitus. Limbs are relaxed and, if extended, are slowly retracted
+1	spontaneous sternal decubitus with acceptance of lateral decubitus; when not gently, manually restrained, the cat quickly returns to sternal decubitus. Limbs are quickly retracted if extended
0	no response
-1	spontaneous sternal decubitus and no acceptance of the lateral decubitus. If not gently restrained, the cat attempts to get away
-2	sternal decubitus not spontaneously maintained and no acceptance of the lateral decubitus; the cat shows annoyance, shakes the head and/or ears, and tries to get away if not manually restrained
-3	arousal, vocalizations, and attempts to remove the clips

Tab. 3: Physiological and behavioural parameters of cats during the two kinds of restraint. Pupil size was assessed as the difference between the size before and during the restraint.

Cats	Type of restraint	Clip Score	Pupil size	HR1 (bpm)	HR2 (bpm)	Cortisol (ng/ml)	Kneading	Purring
1	clipthesia	3	stable	195	160	70.3	--	--
2	clipthesia	3	miosis	180	160	12.8	--	+
3	clipthesia	0	stable	180	204	72.1	--	--
4	clipthesia	1	stable	190	170	15.0	--	--
5	clipthesia	3	stable	180	180	15.6	--	+
6	clipthesia	3	stable	180	180	--	+	+
7	clipthesia	3	stable	200	200	--	+	+
8	clipthesia	2	stable	200	180	--	--	+
9	clipthesia	-1	mydriasis	200	220	--	--	--
10	clipthesia	2	stable	180	180	46.3	--	--
11	clipthesia	0	stable	180	180	61.8	--	--
12	clipthesia	3	miosis	180	180	--	--	+
13	clipthesia	3	stable	180	180	70.9	+	+
14	clipthesia	3	stable	180	200	65.7	+	+
15	clipthesia	3	stable	160	160	48.8	+	+
16	clipthesia	3	stable	220	220	30.2	--	--
17	clipthesia	2	stable	180	180	28.2	+	+
18	clipthesia	0	mydriasis	200	200	22.2	--	--
19	clipthesia	1	stable	180	190	--	--	--
20	clipthesia	1	mydriasis	200	200	--	--	+
21	clipthesia	0	mydriasis	180	180	--	--	--
22	clipthesia	1	stable	180	180	--	--	+
23	clipthesia	3	stable	150	110	--	+	+
24	clipthesia	2	stable	185	185	--	--	--
25	clipthesia	2	stable	120	120	--	--	--
26	clipthesia	2	miosis	180	180	27.7	+	+
27	clipthesia	1	stable	220	220	62.5	--	--
28	scruffing	--	mydriasis	180	200	--	--	--
29	scruffing	--	stable	180	220	61.8	--	+
30	scruffing	--	mydriasis	230	280	112.9	--	--
31	scruffing	--	mydriasis	200	200	23.8	--	--
32	scruffing	--	stable	180	180	68.6	+	+
33	scruffing	--	mydriasis	200	220	6.8	--	--
34	scruffing	--	mydriasis	200	200	14.3	--	--
35	scruffing	--	mydriasis	220	220	--	--	--
36	scruffing	--	stable	180	180	17.0	--	--
37	scruffing	--	mydriasis	180	180	75.8	--	+
38	scruffing	--	mydriasis	180	200	23.0	--	--
39	scruffing	--	mydriasis	250	250	64.8	--	+
40	scruffing	--	stable	180	180	87.6	--	--

Legend: HR1=heart rate before the restraint; HR2=heart rate during the restraint