UNIVERSITI PUTRA MALAYSIA

BEHAVIOURAL AND ELECTROENCEPHALOGRAM RESPONSES OF CATS WHEN EXPOSED TO UNFAMILIAR PERSONS

NOR RAIHANAH BINTI RASHID

BEHAVIOURAL AND ELECTROENCEPHALOGRAM RESPONSES OF CATS WHEN EXPOSED TO UNFAMILIAR PERSONS

NOR RAIHANAH BINTI RASHID

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Behavioural and Electroencephalogram Responses of Cats When Exposed to Unfamiliar Persons" by Nor Raihanah binti Rashid and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

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PROFESSOR Ts DR GOH YONG MENG

RLATG, DVM (UPM), Ph.D (UPM)

Deputy Director of Research Grant Division,

Research Management Centre,

Universiti Putra Malaysia

(Supervisor)

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DR UBEDULLAH KAKA

DVM (Pak), M.Sc. (Pak), Ph.D (UPM)

Senior Lecturer

Faculty of Veterinary Medicine,

Universiti Putra Malaysia

(Co-supervisor)

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan VPD 4999 – Projek Ilmiah Tahun Akhir.

GERAK BALAS TINGKAH LAKU DAN ELEKTROENSEFALOGRAM KUCING APABILA DIDEDAHKAN KEPADA ORANG YANG TIDAK DIKENALI

Oleh

NOR RAIHANAH BINTI RASHID

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Penyelia: Prof. Ts. Dr. Goh Yong Meng

Penyelia Bersama: Dr. Ubedullah Kaka

Ketidakbiasaan kepada individu dan persekitaran menyebabkan tegasan yang ketara pada kucing. Suai temu dengan orang yang tidak dikenali, sama ada kakitangan veterinar atau sesiapa sahaja, boleh menyebabkan tegasan kepada kucing. Antara sebab mengapa sesetengah kucing lebih terkesan oleh tegasan selepas bersua dengan individu yag tidak dikenali, termasuklah tahap sosialisasi yang rendah semasa perkembangan anak kucing, serta faktor genetik dan perwarisan. Tegasan boleh menjejaskan fisiologi kucing, yang seterusnya mengganggu diagnosis veterinar. Selain itu, tegasan boleh menyebabkan kucing menjadi agresif, yang seterusnya meningkatkan risiko kecederaan dan pekerjaan kepada kakitangan veterinar. Oleh itu, adalah penting untuk menentukan tahap tegasan yang dialami kucing apabila didekati dan dikendalikan oleh orang yang tidak dikenali, supaya gerak balas tegasan dapat dikurangkan. Kajian ini bertujuan untuk menyiasat perubahan tingkah laku dan elektroensefalogram (EEG) akibat tegasan apabila sesekor kucing itu terdedah kepada orang yang tidak dikenali. Sebanyak tiga belas ekor kucing telah digunakan dalam kajian ini, dengan setiap daripada mereka bertindak sebagai kawalan terhadap diri sendiri. Skor tingkah laku subjek dan bacaan EEG diambil apabila kucing bersua dengan individu tidak dikenali yang pertama (T1), orang asing kedua (T2), dan orang asing ketiga (T3). Tegasan dinilai pada setiap titik masa berdasarkan elektroensefalografi kuantitatif dan skor tekanan kucing yang telah ditentusahkan. Keputusan menunjukkan bahawa skor tingkah laku, jumlah kuasa spektrum EEG, keamatan untuk gelombang frekuensi perlahan dalam jalur theta dan delta tidak berbeza antara T1, T2 dan T3. Walau bagaimanapun, kehadiran orang ketiga nampaknya menyebabkan perubahan ketara dalam keamatan gelombang alfa, tetapi bukan antara gelombang beta dan gamma yang berfrekuensi lebih pantas dalam keadaan terjaga. Kajian ini menunjukkan bahawa kucing dapat menyesuaikan diri dengan baik terhadap perubahan tegasan yang disebabkan oleh tiga orang atau kurang, kerana skor tingkah laku median adalah lebih kurang sama di sekitar skor median 3.69 hingga 3.85 ($P>0.05$). Kajian semasa dengan jelas menunjukkan bahawa kehadiran

tiga atau kurang orang yang tidak dikenali di sekeliling mereka akan menjadi tidak penting dalam persekitaran klinikal.

Kata kunci: *tegasan; orang yang tidak dikenali; elektroensefalogram; kucing domestik*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD4999 - Final Year Project.

BEHAVIOURAL AND ELECTROENCEPHALOGRAM RESPONSES OF CATS WHEN EXPOSED TO UNFAMILIAR PERSONS

By

NOR RAIHANAH BINTI RASHID

2022

Supervisor: Prof. Ts. Dr. Goh Yong Meng

Co-supervisor: Dr. Ubedullah Kaka

Unfamiliarity causes significant stress on cats. Encounters with unfamiliar persons, be it veterinary staff or anyone, may cause stress to the cat. In fact, some cats are prone to stress caused by unfamiliar people as a result of reduced human socialisation during developmental phase, or because of genetic factors. Stress can affect the cat's physiological state, which can interfere with veterinary diagnosis. Plus, aggression in stressed cats can pose occupational hazards to the veterinary staff. Hence, it is important to evaluate the degree of stress that these cats are experiencing when approached and handled by strangers, so that the stress response can be mitigated. The current experiment aimed to investigate the behavioural and electroencephalogram

(EEG) changes associated with stress and fear, that occur when cats are exposed to unfamiliar people. A total of thirteen cats were enrolled in this study, with each of them serving as their own control. The subjects' behavioural scores and EEG readings were taken when the cat is exposed to the first stranger (T1), second stranger (T2), and third stranger (T3). Stress was evaluated at each point based on quantitative electroencephalography and a verified Cat Stress Score. Results showed that the behavioural scores, total EEG spectral power, intensities for slow frequency waves in the theta and delta bands were no different among T1, T2 and T3. However, the presence of a third person seemed to cause tangible change in alpha wave intensity, but not among the faster beta and gamma waves in wakeful state. It is postulated that cats adapted well to stress changes caused by three people or less, as the median behavioural score was more or less the same around median score 3.69 to 3.85 ($P>0.05$). The current study demonstrated that the presence of three or less unfamiliar people around cats would be inconsequential in a clinical environment.

Keywords: *stress; unfamiliar person; electroencephalogram; domestic cat*

1.0 INTRODUCTION

In animals, novelty or unfamiliarity can be stressful. For example, changes in the physical environment such as moving into a new house, surrounded by unfamiliar people, and changes in daily routine may all lead to stress (Amat *et al.*, 2016). In cats, the duration and quality of human exposure that they experience during their developmental phases for socialisation strongly affects their behaviour towards people. It has been suggested that the friendliness of a cat may be influenced by the amount of time and quality of human exposure during this period (Lowe and Bradshaw, 2002). However, there could be other factors attributing to the behaviour, including genetic effects, such as paternal and maternal effects (Lowe and Bradshaw, 2002). Moreover, cats with genetic tendency of being brave and curious regarding novelties including unfamiliar people (McCune, 1995) may be more likely to seek out social interactions with people, leading to more socialisation.

In most cases, animals often show fear in the presence of humans, and this could be due to the animal perceiving humans as predators (Stella and Croney, 2016). This can be exacerbated if the human exhibits body language that can cause fear in cats, e.g., leaning over the cat, rapid movements, direct eye contact and excessive petting and handling (Lloyd, 2017). Consistent and positive human-animal interactions can help in improving a cat's welfare (Stella and Croney, 2016).

Cats can experience stress when they are exposed to unfamiliar persons, especially if they were not exposed to socialisation to multiple people during their developmental phases (Collard, 1967). This can be a problem, especially when the cat is brought to the clinic or hospital, as it can put the cat in extreme stress when they are handled by the veterinary staff (Hewson 2008; 2012). Numerous studies have shown that prolonged stress can lead to detriment of the cat's immunity, general health and behaviour (Moberg & Mench, 2000). A stressed cat can also be difficult to handle during medical examination and treatment (Jeyaretnam, Jones, & Phillips, 2000), and stress response may prevent accurate diagnosis by altering the results of samples or data collected (Tynes, 2014). Therefore, investigating the behavioural and EEG changes of cats exposed to unfamiliar people will provide better understanding of alleviating stress levels of cat patients in veterinary clinics or hospitals.

1.2 OBJECTIVE

This also brings the objectives of the study which is to investigate the behavioural and electroencephalography changes associated with stress and fear, that occur when cats are exposed to unfamiliar people.

1.3 HYPOTHESIS

The presence of three or less unfamiliar people around cats would be inconsequential in a clinical environment.

2.0 LITERATURE REVIEW

2.1 STRESS

Stress is defined as a biological response induced by the body when a threat is perceived to disrupt homeostasis (Lee, 1965, as cited in Stott, 1981). Stressors are factors that lead to stress, and stress is not detrimental if the animal is able to restore all disrupted functions in time. When animals are stressed, they will resort to survival mechanisms, such as freezing, flight, fidget, fight, or hiss to protect themselves or to cope with the threat.

The model of animal stress defines that the stress response has three stages, the recognition of stressor, biological defence against stressor and the consequence of stress response (Moberg, 2000). First, the stress response starts with the central nervous system detecting a stimulus that could threaten homeostasis. The central nervous system would then develop a combination of biological defence responses: behavioural response, autonomic nervous system response, neuroendocrine response or the immune response.

Distress is when the animal is constantly exposed to stressors, or the animal cannot endure and return to normal baseline state. Thus, this will affect the animal's wellbeing negatively due to emotional and physiological damage. It is worth noting that responses to stress and distress in animals are influenced by genetics, health, positive and negative experiences, particularly during their early developmental period.

2.1.1 BEHAVIOURAL RESPONSE

When animals are faced with a stressor, the first line of response would be behavioural, where they would remove themselves from the stressor. For example, animals would move into the shade to keep themselves cool during hot weather. In terms of social interaction, when encountering peers that are more dominant, the animal may respond by averting their eyes or distance themselves to avoid confrontation. These behavioural responses allow the animal to remove themselves from the stress-inducing stimulus. Signs of behavioural changes when cats are faced with stressors (experimental stressor: handling or relocation to an unfamiliar surrounding) include attempting to escape, exploratory behaviour, immobilisation or defaecation. Often, these signs correlate with physiological changes such as alteration in cortisol secretion and heart rate (Dantzer *et al.*, 1983b; Moberg, 1985a; Mensch and Van Tienhoven, 1986; Broom, 1988; Line, 1987). However, in situations where the animal is unable to escape the threat, they will react passively, by withdrawing or submitting to the threat (Overmeier and Seligman, 1967; Henry and Stephens, 1977; Archer, 1979; Henry, 1982). Examples of the situation would be when they are confined in an unfamiliar cage.

Cats exhibit a variety of behavioural responses to stress that can be evaluated through behavioural scores such as the Cat Stress Score (CSS). Based on the scoring, the stress scores are given from 1, fully relaxed to 7, terrified. The scoring is given by evaluating 11 different categories: body, stomach, legs, tail, head, eyes, pupils, ears, whiskers, vocalisation and activity level (Kessler and Turner, 1997). The results of the CSS is obtained by calculating the mean of each category's scoring. This scoring system also aligns with the ethogram of the UK Cat Behaviour Working Group (as cited in Van Der Leij, 2019). CSS has been used in several studies to estimate stress levels in cats (Gourkow & Fraser, 2006; Tanaka *et al.*, 2012; Stella *et al.*, 2014; Pankratz *et al.*, 2018).

2.1.2 AUTONOMIC NERVOUS RESPONSE

The autonomic nervous system (ANS) is the second line of defence for animals when they encounter stress. The autonomic nervous system affects multiple systems, for example, the cardiovascular system, gastrointestinal system, adrenal medulla and the exocrine glands when the animal is in distress (Sjaastad *et al.*, 2010). The autonomic nervous response is characterized by its swift, specific response (Cannon, 1929; Moberg, 1985). The sympathetic and parasympathetic branches of the ANS makes alterations to the heart rate, vascular resistance, exocrine glands secretion, smooth muscle contraction in the gastrointestinal tract, catecholamines in the adrenal medulla and noradrenergic nerve terminals. When faced with a stressor, the ANS undergoes a pronounced albeit short-lived effect on the heart rate, blood pressure and metabolism. This allows the animal to make rapid physiological adjustments when faced with acute stress (Moberg, 1985).

2.1.3 IMMUNOLOGICAL RESPONSE

Stress can result in different immunological responses, depending on the type of stress. In acute exposure to stress, stressors can actually boost the immune system. On the contrary, in chronic exposure of stress, suppression of the immune system is observed. When an animal is under chronic stress, cortisol levels are elevated, leading to immunosuppression and increases the animal's susceptibility to infections and diseases present in its environment. When the stress response persists for extended time, the period in which the animal is vulnerable to infection also persists (Moberg, 1985). In cases of long-term immunosuppression, the risk of latent viruses manifesting clinically is increased, through reactivation and shedding of virus.

2.1.4 NEUROENDOCRINE RESPONSE

The neuroendocrine response to stress has a longer period of effect to the animal compared to the autonomic nervous system. The main neuroendocrine response to stress is through the activation of the hypothalamic-pituitary-adrenal (HPA) axis. This activation causes the adrenal glands to secrete steroid hormones. In this axis, the hypothalamus secretes corticotropin-releasing hormone (CRH), which regulates the secretion of adrenocorticotropic hormone (ACTH). ACTH triggers the synthesis and release of steroidal hormones from the adrenal cortex. ACTH increases the overall uptake of cholesterol, converting it to cortisol and corticosterone, the glucocorticoid hormones that indicate neuroendocrine response to stress (Matteri *et al.*, 2000).

The somatotrophic axis involves the mechanisms that control growth hormone (GH) secretion and the following physiological responses to the secreted GH. The somatotrophs, specialised cells of the anterior pituitary gland produce and release GH. The effects of GH include the stimulation of liver to produce and release insulin-like growth factor-I (IGF-I). In rats, there has been reports of reduction in GH and IGF-I due to stressors (Armario *et al.*, 1987; Straus, 1994; Peisen *et al.*, 1995). These responses divert energy from growth to survival, as a reduction in IGF-I minimises growth when the animal is under stress, preserving the energy for survival purposes (Matteri *et al.*, 2000).

The gonadotrophic axis involves the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Matteri *et al.*, 2000). They are produced by gonadotrophs, specialised cells in the anterior pituitary gland. In female animals, LH induces ovulation and exerts trophic effects on the corpus luteum; while FSH induces maturation of the ovarian follicles, maintains the ovarian size and stimulates oestrogen production. In male animals, LH induces androgen productions from Leydig cells in the testes, and FSH stimulates the Sertoli cells which are vital for sperm production. There have been studies where they show that acute physiological stress can cause LH to increase temporarily (Siegel *et al.*, 1981; Sakamoto *et al.*, 1991). Meanwhile, chronic stress can cause decrease in gonadotropin secretion and failure of the animal to reproduce (Moberg, 1991; Rivier and Rivest, 1991).

2.2 CAT-HUMAN RELATIONSHIP

Cats were domesticated around 10,000 years ago, as they lived near human settlements and became predators to rodents (Vigne *et al.*, 2004) that commonly roam around the area. As a result, they have successfully integrated into the human population. Cats were excellent candidates for domestication as they exhibit behavioural characteristics favourable for domestication as proposed by Zeder (2012): (1) Hiearchical group structure; (2) Promiscuous mating system, males dominant over females and sexual signals are exhibited by movement or posture; (3) Formation of social bonds through imprinting, the acceptance of females towards their young after parturition; (4) Wide environmental tolerance in terms of habitat choice; and (5) Low reactivity to humans or sudden changes in environment and may seek human's attention for food and shelter.

Cats communicate with humans through non-verbal communication, where they use their senses, such as smell, hearing, touch and eyesight to communicate (Koyasu *et al.*, 2020). Cats do use gaze to communicate with humans, for example when cats encounter a novel object, they can read the human's facial expression or behaviour or lead the human's gaze to the object (Merola *et al.*, 2015). Cats have also adapted their voices to communicate with humans, as they meow at humans (Mertens and Turner, 1988), even though meowing is typically used only for kitten-queen communication (Bradshaw and Cameron-Beaumont, 2000). On the other hand, cats are also sensitive to human voices. In fact, cats can distinguish between their owner's voice and a stranger's voice (Saito and Shinozuka, 2013). In a habituation-dishabituation test, cats show a decreased orienting response to unfamiliar voices, and increased orienting response (head and ear movements) to their owner's voice (Adachi *et al.*, 2007).

Furthermore, cats also communicate with humans through smell, as they often show rubbing behaviour or allorubbing. Cats like to be petted by human along the temporal gland, around the forehead region where pheromones are secreted (Soennichsen and Chamove, 2002; Behnke, 2019). Cats also often use their forehead to allorub against conspecifics and their human counterparts (Hart, 1977; Soennichsen and Chamove, 2002; Behnke, 2019). In cats, allorubbing between conspecifics signifies attachment behaviour (Bradshaw, 1992; Soennichsen and Chamove, 2002; Behnke, 2019). As cats do engage in the same behaviour with their human counterparts, this suggests that the owner's presence and possibly scent is comforting to them (Soennichsen and Chamove, 2002). It has also suggested that when cats rub against human, it is essentially greeting the human (Crowell-Davis *et al.*, 2004).

2.2.1 FACTORS AFFECTING CAT BEHAVIOUR TOWARDS STRANGERS

Young kittens have a sensitive socialisation phase, which is thought to appear between their second to seventh weeks of life (Karsh and Turner 1988). If the kitten's exposure to humans is delayed after the kitten is 7 weeks old, socialisation is less effective. Hence, if the kitten was not adequately exposed to humans and handled by them during its sensitive socialisation period, it may develop behavioural inhibition (Lowe & Bradshaw 2002), where it is a temperament characterised by strong reactions to novelty, such as distress and increased escape attempts. However, the study also suggested that the third and fourth months of a kitten's life may also be vital in determining its behaviour towards humans (Lowe & Bradshaw 2002).

The amount of people exposed to the kittens during the sensitive period can also affect the friendliness of the cat. According to a study, kittens that were handled by multiple people tend to be less cautious of strangers than those exclusively handled by the same one person (Collard 1967). The time duration in which the kittens were exposed to the stranger also affects the friendliness of the cat. A study showed that kittens were quicker to approach a person and allowed the person to hold it for longer duration when the kittens were handled for 40 minutes per day, compared to kittens that were handled for 15 minutes per day (Karsh 1984).

Genetics also play a role in determining the friendliness of a cat, where both the paternal and maternal side can influence it. A study (McCune 1995) has shown that socialised kittens that had friendly tomcats as fathers were more friendly to unfamiliar people. Plus, they were also less distressed when the stranger approached and handled them. On the other hand, the direct influences of the queen's behaviour on her offspring could affect the kitten's behaviour towards humans (Lowe and Bradshaw, 2002). If a queen is human-socialised and calm, it may lessen the kitten's distress and allow them to become bolder to explore their environment, including approaching a stranger. On the other hand, if the queen is shy and not human-socialised, she may cause her kittens to become distressed when exposed to humans without her presence. The queen may also hide her kittens after parturition, delaying the kitten's contact with humans (Turner 1995).

2.3 ELECTROENCEPHALOGRAPHY

Electroencephalography (EEG) is a recording of continuous electrical activity in the brain. It is a non-invasive and reliable method for stress evaluation by examining the cerebrocortical function. The cerebral cortex's role is to process the afferent nociceptive input which leads to perception of pain or stress response of the body. Studies have shown that behavioural responses correlate with the EEG spectral frequencies relating to noxious stimuli in animals (Chen *et al.*, 1989, Gibson *et al.*, 2009, Imlan et at., 2020).

EEG is able to quantify changes in the signals from the basic brain waves (Alpha, Beta, Theta and Delta). Each frequency band represents a different physiological state. For Alpha and Beta waves, it represents consciousness and wakefulness. We can observe the retinal activity by looking into the Alpha waves, while stress and pain can be observed by looking into the Beta waves. On the other hand, Delta and Theta waves are dominant in the unconscious state, including during anaesthesia and sleep. The changes in the EEG power spectrum have been widely used to evaluate the response of animals to noxious stimuli. Examples of the power spectrum include median frequency (F50), spectral edge frequency (F95) and total power of EEG (Ptot) (Gibson *et al.*, 2007, Murrell *et al.*, 2007, Kongara *et al.*, 2014).

Filtering out unwanted noise is critical to obtain relevant EEG information. Unprocessed electroencephalogram contains many disturbances due to its sensitivity to electrical and mechanical interferences, muscle tremors, movement of the body, etc. Therefore, processing techniques such as quantitative EEG can overcome this. Quantitative EEG can demonstrate that the brain wave spectrum changes according to the brain activity and its intensity (Gibson *et al.*, 2007, Ploner *et al.*, 2014). When an animal is awake, the higher frequency waveforms are elicited (Alpha and Beta waves), as cognition is functioning and there is response to stimulus. Meanwhile, when the animal is anaesthetised or asleep, the slower frequency waveforms are elicited (Theta and Delta waves), as the cognitive centres of the brain are suppressed and there is increase in reticular activation system (RAS) activities.

One of the key challenges in EEG is the fact that EEG has poor spatial resolution (Ploner *et al.*, 2017). Spatial resolution refers to how accurately the measured activity is localized in the brain (Kimberly & Lewis, 2007). However, EEG has good temporal resolution, which refers to how closely the measured activity corresponds with the timing of the actual neural activity (Kimberly & Lewis, 2007). EEG can record activity in neurons within milliseconds (ranging from 3-500 Hz) (Le Blanc *et al.*, 2016; Morton *et al.*, 2016).

Some EEG requires invasive procedures, such as surgery to put the EEG screws and electrodes in the animal's head, while some EEG setup do not need to undergo invasive procedures, such as recording with on-skin electrodes. Overall, EEG is the most direct way to measure neuronal activity but has limitation in spatial resolution (Da Silva & Seminowicz, 2019). Despite the shortcomings, EEG remains a method of choice for quick determination of stress.

Overall, EEG is still a new diagnostic modality in the research field involving animals of various species. Therefore, it is advised that EEG is done in conjunction with other stress evaluation methods, such as blood sampling, urine sample, behavioural scoring methods, as it can better support the outcome of the research study.

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

The study was approved by the University Putra Malaysia Institutional Animal Use and Care Committee (UPM/IACUC/AUP-U029/2022). Thirteen healthy adult domestic cats weighing 3-7kg were used as test subjects for this experiment. Cats are deemed clinically healthy on the basis of a general physical examination. Three students from Universiti Putra Malaysia were present as part of the simulation.

3.2 EXPERIMENTAL PROCEDURE

The experiment was carried out on 13 cats. Out of the 13 cats, 6 cats are male and while 7 cats are female and of the age group 1 year old to 6 years old. Each cat was exposed to 3 scenarios: T_1, T_2, T_3, where data is collected at each point. Electroencephalography leads were attached using surface gel electrodes, placed on the left ramus, and prefrontal areas between the ears of the cat. Behaviour of the cat was evaluated in real time based on the Cat Stress Score (Kessler and Turner, 1997).

T_1 (baseline): Data was recorded with the animal exposed to the first unfamiliar person

T_2: Data was recorded with the animal exposed to the second unfamiliar person

T_3: Data was recorded with the animal exposed to the third unfamiliar person

3.3 EEG ANALYSIS AND CAT STRESS SCORE

The electroencephalogram was recorded using a computer installed with Chart 5.0 recording software and connected to Powerlab 4/20 data recording system (ADInstruments Pty Ltd, Australia).

The electroencephalogram (EEG) was recorded at a sampling rate of 1 kHz and raw EEG was resampled with low pass filter of 200 Hz into delta frequency (0.1 - 4 Hz), theta frequency (4.1 - 8 Hz), alpha frequency (8.1 - 12 Hz) and beta frequency (12.1 - 20 Hz). The raw EEGs are then analysed in serial epochs of 1 second duration, with quantitative changes noted everytime a new unfamiliar person was introduced at T1, T2 and T3. Waveforms were subjected to Fast Fourier Transformation (FFT), and then the relevant intensities, voltages at every frequency band were extracted using the Chart 5.0 software. Root mean square (RMS) for each of alpha, beta, delta and theta waves, median frequency (F50) and total power (Ptot) to the exposure of to 1 person (T1), two persons (T2), as well as three persons (T3), were calculated for consecutive non-overlapping 1-s epochs. There are no pre-exposure data as the cat must be handled by a minimum of one person to measure the EEG, or even during stress score evaluations. The datasets were compared across the number of unfamiliar persons (1, 2 or 3 people), and changes resulted from the exposure. The behaviour of cats was determined from a scale of 1 to 7, using Cat Stress Score in Table 1.

Cat Stress Score (Kessler and Turner, 1997) was evaluated by grading the animals' behaviour based on real time observations during the procedure (See Appendix A). Cat stress scores were noted for all animal subjects at T1, T2 and T3, as well as immediately prior to the experiment.

Cat Stress Score - Kessler MR, Turner DC. (1997)

a: Active cat i: Inactive cat

Score	Body	Stomach	Legs	Tail	Head	Eyes	Pupils	Ears	Whiskers	Vocal	Activity
1. Fully Relaxed	Laid out on side or on back	Exposed, slow ventilation	Fully Extended	Extended or loosely wrapped	Laid on surface with chin up or on surface	Closed or half, may be blinking slowly	Normal	Half-back (normal)	Lateral (normal)	None	Sleeping or resting
2. Weakly Relaxed	i: laid ventrally or half on side or sitting a: standing or moving, back horizontal	Exposed or not, slow or normal ventilation	i: bent, hind legs may be laid out a: when standing, extended	i: extended or loosely wrapped a: when standing extended	Laid on surface with chin up or on surface	Closed, half opened or normal opened	Normal	Half-back or erected to front or back and forward on head	Lateral or forward	None	Sleeping, resting, alert or active. May be playing
3. Weakly tense	i: laid ventrally or sitting a: standing or moving, body behind lower than in front	Not exposed, normal ventilation	i: bent a: when standing extended	May be twitching I: on the body or curved backwords	Over the body, some movement	Normal opened	Normal	Half-back or erected to front or back and forward on head	Lateral or forward	Meow or quiet	Resting awake or act active exploring
4. Very Tense	i: laid ventrally, rolled or sitting a: standing or moving, body behind lower than in front	Not exposed, normal ventilation	i: bent a: when standing hind legs bent in front extended	I: close to the body a: curled forward dose to the body	Over the body or pressed to body, little or no movement	Widely open or pressed together	Normal to partially dilated	Erected to front or back, or back and forward on head	Lateral or forward	Plaintive meow, yowling, growling or quiet	Cramped sleeping, resting or alert may be actively exploring, trying to escape

5. Fearful, Stiff	i: laid ventrally, or sitting a: standing or moving, body behind lower than in front	Not exposed, normal or fast ventilation	i: bent a: bent near to surface	i: close to the body a: curled forward dose to the body	On the plane of the body, less or no movement	Widely opened	Dilated	Partially flattened	Lateral or forward or back	Plaintive meow, yowling, growling or quiet	Alert, may be actively trying to escape
6. Very Fearful	i: laid ventrally or crouched directly on top of all paws, may be shaking a: whole body near to ground, crawling, may be shaking	Not exposed, fast ventilation	i: bent a: bent near to surface	i: close to the body a: curled forward dose to the body	Near to surface, motionless	Fully opened	Fully dilated	Fully flattened	Back	Plaintive meow, yowling, growling or quiet	Motionless, alert or actively prowling
7. Terrified	Crouched directly on top of all fours, shaking.	Not Exposed, fast ventilation	Bent	Close to the body	Lower than the body, motionless	Fully opened	Fully dilated	Fully flattened back on head	Back	Plaintive meow yowling, growling or quiet	Motionless

Table 1: Cat Stress Score (adapted from Kessler and Turner 1987)

Source from Kessler MR, Turner DC. (1997) Stress and adaptation of cats (*Felis silvestriscatus)* housed singly, in pairs, and in groups in boarding catteries.

3.4 STATISTICAL ANALYSIS

The results for cat stress score and median frequency of EEG result were expressed as means ± standard error, while the EEG wave frequency band results and total power were expressed as percentage difference between T1, T2, and T3. All datasets are checked for their conformance to normality prior to parametric analysis. The data were analysed using repeated measure ANOVA at 95% confidence level, utilising the univariate function of the IBM SPSS version 25.0 software (IBM-SPSS Inc, USA). Significantly different means were then elucidated using Least Significant Difference test for post hoc comparisons.

4.0 RESULTS

Thirteen cats weighed 3 to 6 kg were included in this study during the sampling period. Out of 13 subjects, 6 are males, 7 are females, ranging from 1 to 6 years old. All of them were from multi-cat households.

CAT ID	AGE (YEAR)
ANGEL	6
PEMAISURI	6
DIABLO	5
EDDY	5
FIONA	5
HERSHEY	4
KOKO	2
LOLO	2
MAMA	2
NONO	2
OLLIE	1
BROWNIE	1
BLACKIE	1

Table 2: List of participant cats with their respective ages

4.1 CAT STRESS SCORES

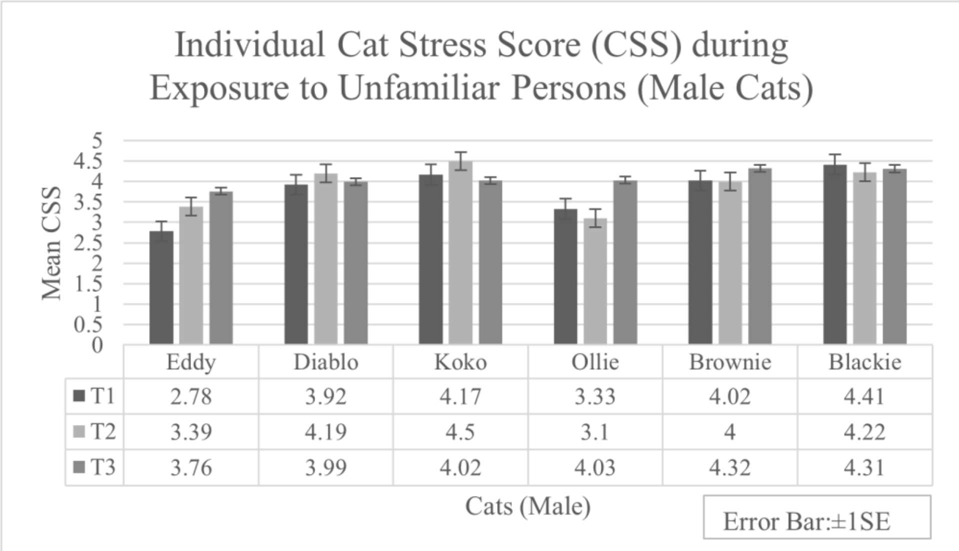

Figure 1: Individual Male Cat Stress Score during Exposure to Unfamiliar Persons

Figure 1 shows the individual male cat stress scores when exposed to unfamiliar persons. The stress scores obtained at each point in the study did not differ significantly ($P>0.05$) in each male cat. This shows that the male cats adapted well to the unfamiliar persons.

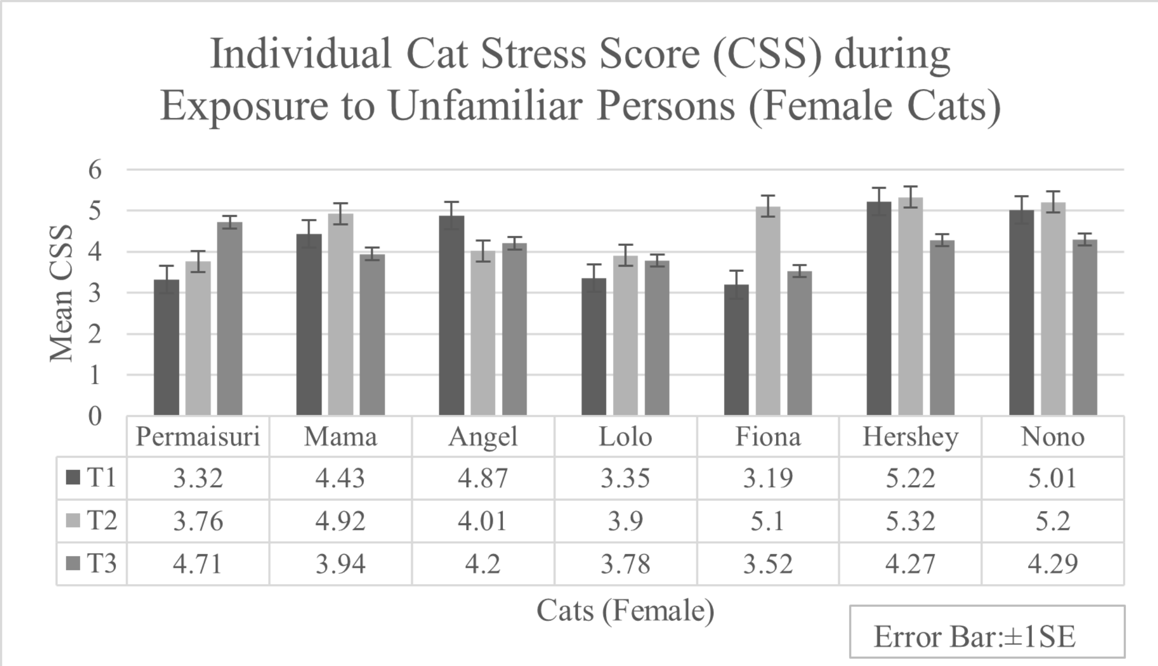

Figure 2: Individual Female Cat Stress Score during Exposure to Unfamiliar Persons

Figure 2 shows the individual female cat stress scores when exposed to unfamiliar persons. The stress scores obtained at each point in the study also did not differ significantly (P>0.05) in each female cat. This shows that the female cats adapted well to the unfamiliar persons, albeit they were generally more stressed than the males.

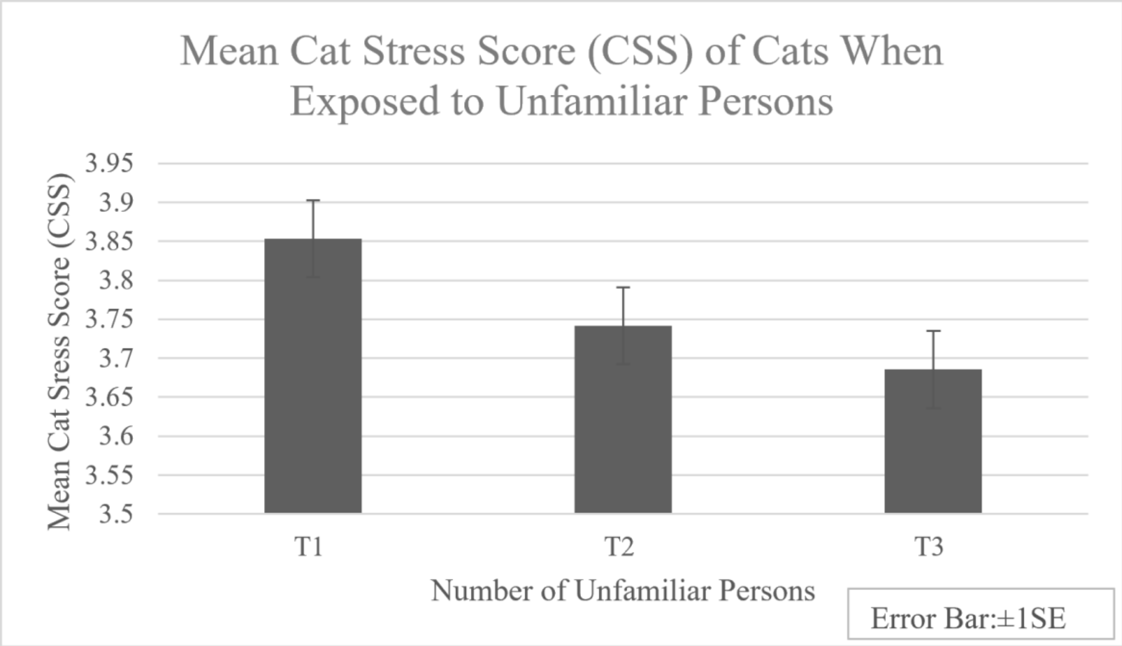

Figure 3: Mean Cat Stress Score of Cats during Exposure to Unfamiliar Persons

From the results, the cat stress scores remained consistent after the cats had become accustomed to the unfamiliar person, and the EEG machines and procedures ($P>0.05$). The median stress score for all cats was 3.82 (with mean of 3.76) for T1, T2, and T3.

4.2 ELECTROENCEPHALOGRAMS

	Percentage difference to the Total EEG Power (uV) at baseline (T1) by individual cats		
Cat ID	T1	T2	T3
Permaisuri	0	+56	+22
Angel	0	-25	+11
Blackie	0	+7	0
Brownie	0	+10	+1
Diablo	0	-8	-11
Eddy	0	+1	-10
Fiona	0	+7	+12
Hershey	0	+5	+21
Koko	0	+9	+9
Lolo	0	+15	+3
Mama	0	-11	0
Nono	0	+19	+6
Ollie	0	-1	+9
Overall percentage change(+/-)	-^{ns}	+3.8	+7.1

^{ns} Means within row DID NOT differ significantly at P<0.05
Positive values indicate increase and negative values indicate decrease over baseline value (100%)
T1 = 1 person, T2 = 2 persons, T3 = 3 persons

Table 3(a) : Total EEG Power (uV) differences in percentage from baseline value (T1)

	Percentage difference from baseline (T1) power (uV) among dominant waveforms of the wakeful state								
Wave form	Alpha wave			Beta wave			Gamma wave		
Cat ID	T1	T2	T3	T1	T2	T3	T1	T2	T3
Permaisuri	0	+62	-42	0	+3	+19	0	+16	+15
Angel	0	+140	+129	0	+4	-3	0	+11	+4
Blackie	0	+22	+18	0	+1	+2	0	+8	0
Brownie	0	-32	-20	0	0	+1	0	+5	+1
Diablo	0	-10	+3	0	-5	-8	0	+9	+1
Eddy	0	-10	+25	0	-7	+10	0	+11	-2
Fiona	0	-43	-32	0	+12	+17	0	+25	-3
Hershey	0	-55	+10	0	+77	+32	0	-9	-5
Koko	0	+6	+13	0	+23	+16	0	-3	-28
Lolo	0	+78	+79	0	-18	+10	0	-16	-46
Mama	0	+12	+10	0	-5	+11	0	-11	+1
Nono	0	+55	+50	0	-9	-9	0	+13	+1
Ollie	0	+10	+16	0	0	-3	0	+5	0
Overall percentage change(+/-)	-[a]	+14.4[a]	+25.8[b]	-[ns]	+6.1	+6.3	-[ns]	+5.2	+5.9

[a,b] Means within column of wave form with different superscripts differed significantly at $P<0.05$
[ns] Means within column of wave form DID NOT differ significantly at $P<0.05$
Positive values indicate increase and negative values indicate decrease over baseline value (100%)
T1 = 1 person, T2 = 2 persons, T3 = 3 persons

Table 3(b) : Electroencephalography results by Alpha, Beta & Gamma waveforms

	Percentage difference from baseline (T1) power among dominant waveforms of the non-wakeful state					
Wave form	Delta wave			Theta wave		
Cat ID	T1	T2	T3	T1	T2	T3
Permaisuri	100	+1	0	100	+3	0
Angel	100	0	+2	100	0	0
Blackie	100	-2	+8	100	0	0
Brownie	100	+10	-1	100	+1	+10
Diablo	100	-1	-1	100	-5	0
Eddy	100	-2	-3	100	-8	0
Fiona	100	-6	-2	100	0	-6
Hershey	100	-16	+10	100	0	-5
Koko	100	+10	+10	100	+2	0
Lolo	100	+27	-2	100	+13	0
Mama	100	-1	0	100	+25	0
Nono	100	0	0	100	+1	0
Ollie	100	0	+1	100	-1	0
Overall percentage change(+/-)	-^{ns}	+1.6	+1.8	-^{ns}	-0.6	+2.7

^{ns} Means within column of wave form DID NOT differ significantly at P<0.05
Positive values indicate increase and negative values indicate decrease over baseline value (100%)
T1 = 1 person, T2 = 2 persons, T3 = 3 persons

Table 3(c) : Electroencephalography results by Delta & Theta waveforms

	Median Frequency values (Hz)		
Cat ID	T1	T2	T3
Permaisuri	3.32	3.76	4.71
Angel	4.87	4.01	4.20
Blackie	4.41	4.22	4.31
Brownie	4.02	4.00	4.32
Diablo	3.92	4.19	3.99
Eddy	2.78	3.39	3.76
Fiona	3.19	5.10	3.52
Hershey	5.22	5.32	4.27
Koko	4.17	4.50	4.02
Lolo	3.35	3.90	3.78
Mama	4.43	4.92	3.94
Nono	5.01	5.20	4.29
Ollie	3.33	3.10	4.03
Mean ± SD ^{ns}	4.00 ± 0.77	4.38 ± 0.67	3.96 ± 0.35

^{ns} Means within row DID NOT differ significantly at $P<0.05$
T1 = 1 person, T2 = 2 persons, T3 = 3 persons

Table 3(d) : Electroencephalography results by Median Frequency Values

The quantitative electroencephalogram (EEG) recorded five types of waves. In conscious animals, alpha, beta and gamma waves are more dominant; while in unconscious animals (for example, anaesthetized or sleeping animals); delta and theta waves are more dominant. Total power (Ptot) is the total intensity of energy contributed by the various waveforms. Median frequency (MF) is the frequency below which 50% of the total power of the EEG is located. An increase in MF is frequently associated with increasing stress and pain levels. Percentage difference for the total EEG power (uV), total power of each wave forms within their frequency bands (alpha, beta, gamma, delta and theta), were derived using the root mean square method (RMS). The percentage difference was then calculated using the RMS values.

EEG spectral analysis indicated that except for alpha wave intensities (Tables 3(a), (b), (c)), all the power intensities were largely the same across T1, T2 and T3 ($P>0.05$). Likewise for the median frequency, there were no changes across T1 to T3. It should be pointed out that despite being dominant waves during non-wakeful or unconscious states, the delta and theta waves do appear in wakeful animals. However, in wakeful animals, the total EEG power and spectral frequency contribution would be rather limited as compared to the alpha, beta and gamma waves. However, Table 3(b) showed that power intensities for the alpha wave actually increased significantly from T1 to T2 (14.4%) and then T3 (25.8%) ($P<0.05$). This could have been associated with eye activity of the cats, as some of the cats closed their eyes when being pet by the handlers during experimentation.

5.0 DISCUSSION

This study provides us vital information on the stress experienced by cats when they encounter unfamiliar persons. The current study also showed us that generally cats can still adapt well to the stress of being approached by three or less unfamiliar people in a clinical environment.

The present results have shown that there were no significant changes in the mean cat stress score after T1, T2 and T3. In all of the scenarios, the cats were weakly tense (CSS: 3.69 to 3.85). It is believed that while the cats are tense, their stress level is still within acceptable range and does not cause extreme distress in cats. When looking into the mean cat stress score of male cats when exposed to unfamiliar persons, it can be seen that the male cats were more relaxed and able to adapt well to the exposure of unfamiliar persons. On the other hand, the female cats were initially more stressed throughout the experiment but were still able to adapt to the exposure of unfamiliar persons.

Similar responses were reflected in the quantitative electroencephalography. Except for alpha wave intensities, all the power intensities were largely the same across T1, T2 and T3. This shows that the cats were not significantly stressed when exposed to three or less unfamiliar persons. The power intensities for alpha wave increased significantly, from T1 to T2 (14.4%), and then T3 (25.8%). This could be associated with the eye activity of the cats. During the experiment, the handlers may have petted the cat and this caused the cats to close their eyes. Alpha wave measures the electrical activity at the visual cortex. When the eyes are closed, it allows all electrical activity to concentrate to the visual cortex. Hence, this leads to an increase in alpha waves when the eyes are shut. There were also no significant changes across T1 to T3 for median frequency (MF) and total power of EEG (Ptot). This means that there were no significant changes to the stress level and overall intensity of brain activity of the cats.

6.0 CONCLUSIONS

In conclusion, it can be said that the cats adapted well to stress changes caused by three or less unfamiliar persons. The presence of three or less unfamiliar persons around them would be inconsequential in a clinical environment.

7.0 RECOMMENDATIONS

All the findings suggest that presence of unfamiliar persons exerts a significant level of stress to the cats. However, the cats are able to cope with the stress as shown by the behavioural scoring and EEG analysis results. To further mitigate stress in cats when exposed to unfamiliar persons in a clinical environment, it is suggested that cat owners acclimatise their cats by conditioning them to the new environment and veterinary staff frequently with routine scheduled checkups. Furthermore, veterinary personnel can also practice low stress handling techniques so that cat patients are more comfortable and less stressed. The current study also showed that exposing cats up to an unfamiliar crowd of three persons would probably not affect outcomes of clinical examination in a clinical setting. This is comforting as the crowd size is easy and practical to manage in a practice setting.

The limitation of the current study is that it was conducted only with three unfamiliar persons, and it is still unknown whether a number of more than three unfamiliar persons would significantly affect the stress level of cats in a clinical environment. However, it should be noted that in a clinical setting, cats would typically be examined in the presence of not more than three persons, not including familiar family members of the feline patient, in a private practice. Nevertheless, the current work showed the potential value of increasing the sample size of cats and by recruiting more unfamiliar persons. This should be done so that we can determine the number of unfamiliar persons that will affect the cat's stress level significantly, and thereby determining the threshold of unfamiliar crowd size of unfamiliar persons that could be extremely stressful to cats.

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9.0 APPENDICES

1. Appendix A – Cat Stress Score Data

T1

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B1(	B11
EDDY	3	3	3	2	3	3	4	3	3	2	4
DIABLO	4	3	4	2	3	4	4	4	3	5	5
KOKO	6	5	5	4	3	4	4	4	4	5	5
NONO	4	4	3	3	3	4	4	4	4	3	3
OLLIE	5	5	5	5	4	2	4	3	3	3	3
HERSHEY	4	3	4	5	5	5	5	4	5	4	5
FIONA	4	5	4	4	3	4	5	4	4	3	4
LOLO	5	5	4	4	3	3	4	3	4	3	3
ANGEL	4	5	4	3	3	3	4	4	5	3	4
MAMA	4	4	4	4	3	3	3	4	4	6	5
PEMAISUR	3	4	4	3	3	3	5	3	5	3	5
BROWNIE	4	5	4	5	5	3	5	4	5	5	4
BLACKIE	3	3	3	3	3	4	4	3	4	3	4

T2

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B1(	B11
EDDY	3	3	3	2	3	2	4	4	3	2	3
DIABLO	3	3	4	2	3	3	4	4	3	4	4
KOKO	6	5	4	4	3	3	4	4	4	5	4
NONO	4	5	4	4	3	3	4	3	5	3	4
OLLIE	5	5	4	4	2	3	3	3	3	3	3
HERSHEY	4	5	4	4	3	4	4	4	3	3	4
FIONA	5	5	4	4	4	3	4	4	4	3	3
LOLO	4	5	3	4	3	4	5	3	5	3	4
ANGEL	4	5	4	3	3	3	4	4	5	3	5
MAMA	4	4	4	4	3	3	3	4	3	5	5
PEMAISUR	4	4	4	3	3	4	5	4	4	3	5
BROWNIE	4	5	4	4	4	4	4	5	4	5	4
BLACKIE	3	3	3	3	3	3	4	4	3	3	5

T3

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B1(	B11
EDDY	3	3	3	2	3	3	4	4	3	2	3
DIABLO	3	3	4	2	3	3	4	4	3	3	4
KOKO	6	5	5	4	3	4	4	4	4	5	4
NONO	4	5	4	4	4	3	4	4	5	3	3
OLLIE	5	5	4	4	2	2	2	3	3	2	2
HERSHEY	4	4	4	4	4	4	4	3	3	3	3
FIONA	5	5	4	4	4	2	4	4	4	3	2
LOLO	4	5	3	4	3	4	4	3	5	3	4
ANGEL	4	5	4	3	3	3	4	4	5	4	5
MAMA	4	4	4	3	3	3	3	4	4	5	5
PEMAISUR	4	4	4	3	3	4	5	4	3	3	5
BROWNIE	4	5	3	3	4	3	4	5	4	4	4
BLACKIE	3	3	3	3	3	4	4	4	3	4	5

Categories of body parts for Cat Stress Score evaluation	
B1 – BODY	B7 – PUPILS
B2 – STOMACH	B8 – EARS
B3 – LEG	B9 – WHISKERS
B4 – TAIL	B10 – VOCAL
B5 – HEAD	B11 – ACTIVITY
B6 – EYES	