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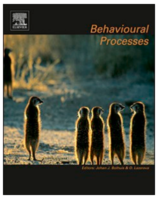

Stable individual differences in vocalisation and motor activity during acute stress in the domestic cat

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ABSTRACT

The behavioural assessment of individual animals in stressful situations should consider measures which are consistent across repeated testing, and therefore truly representative of an individual's behaviour. Here we report a study conducted on 40 neutered adult cats (*Felis silvestris catus*) of both sexes, originating from two animal shelters in Mexico and Hungary. We recorded the responses of the cats to repeated brief confinement trials that mimicked a common situation (confinement in a pet carrier). This test was repeated three times, leaving one week between trials, to assess short-term repeatability. Stable inter-individual differences in two behavioural measures, the number of separation calls and the duration of motor activity, were found, although the inter-individual differences in vocalisation were more pronounced than they were for motor activity. Additionally, the overall number of vocalisations emitted remained stable despite repeated testing, whereas motor activity tended to decrease week to week. There was a negative effect of age on vocalisation rate, and no effect of sex on either behaviour. No correlation between the two behavioural measures was found. We suggest that, in adult cats, vocalisation may be more reliable than motor activity as a behavioural measure of stress.

1. Introduction

The domestic cat (*Felis silvestris catus*) is currently one of the most popular companion animals in the world (American Pet Products Association, 2017; The European Pet Food Industry Federation, 2017). The need to understand its behaviour, cognition and cat-human relations is reflected in special issues and reviews in scientific journals dedicated to these topics in recent years (special issues: Farnworth, 2015; Udell and Vitale Shreve, 2017; reviews: Gartner, 2015; Gartner and Weiss, 2013; Litchfield et al., 2017). Consistent individual differences in animal behaviour (also known as personality) have attracted increasing attention in this regard as a research topic in recent years. The domestic cat is a good model species for such studies due to its rich behavioural repertoire and worldwide availability. In fact, in this field it is by far the most studied feline species, through observation, surveys, and a variety of behavioural tests, such as novel object, handling, and human approach tests (Gartner, 2015; Gartner and Weiss, 2013)

A key interest in the study of individual variation is the stability of individuals' responses to stressful situations (Koolhaas et al., 2007). Separation tests are useful to measure individual differences in behavioural responses to stress in several mammalian species (review: Forkman et al., 2007) e.g. in pigs (Friel et al., 2016; Leliveld et al., 2017), horses (Merkies et al., 2016; Seaman et al., 2002), cattle (Watts et al., 2001), goats (Nawroth et al., 2017) and cats (Hudson et al., 2017, 2015; Iki et al., 2011; Yeon et al., 2011). Separation, or isolation, calls and motor activity are two behaviours which are most commonly used to study stress response since both tend to increase with arousal (Kiley, 1972; Rushen, 2000). Vocalisation reflects the inner, emotional state of the caller (Briefer, 2012; Jürgens, 2009; review: Newman, 2007), and in many mammalian species it is relatively easy to elicit calls through social separation or isolation, e.g. cattle (Boissy and Le Neindre, 1997), cheetahs (Ruiz-Miranda et al., 1998), primates (e.g. Norcross and Newman, 1997), even in species which are not considered to be highly social, such as giraffes (Tarou et al., 2000) and cats (Iki et al., 2011; Yeon et al., 2011). Note, however, that response to extreme stress can elicit freezing behaviour in many species (e.g. Brandão et al., 2008).

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In a previous study on cats, Iki et al (2011) found that adult males showed a strong negative correlation between locomotion and vocalisation behaviour during a stressful situation. However, in that study the cats were tested only once, so there is no information presently available on the stability of these behavioural responses across repeated testing in adults. In kittens, however, the stability of these responses has been evaluated through brief separation trials repeated once a week for the first four postnatal weeks, finding stable individual differences in the frequency of emitting separation calls and in locomotion. Nevertheless, there was no correlation between the two variables, possibly due to differential maturation of the vocal and motor systems in young kittens (Hudson et al., 2015). Like many other altricial mammals, kittens emit separation calls starting immediately after birth and the number of these increases during the first postnatal month, after which time there is a marked decrease (Bánszegi et al., 2017). Locomotor activity, on the other hand, develops more slowly over several months (Levine et al., 1980; Peters, 1983; Villablanca and Olmstead, 1979).

The ability to reliably assess the behaviour of individual animals in stressful situations using simple, practical and rapid assays would be beneficial in many situations involving farmed, companion and working animals, e.g. during veterinary visits (Pratsch et al., 2018). Individual differences in stress response to an aversive situation (e.g. transport in a pet carrier) are important, as they can make handling difficult and also affect the outcome of examinations and medical diagnoses. For example, in cats, symptoms of stress such as altered heart and breathing rate, elevated blood pressure or drooling can either confound or be confused with symptoms of disease, potentially affecting diagnosis (Pratsch et al., 2018). Good assessment tools should include not only species-adequate test design, but also the selection of behavioural measures which are consistent across repeated testing, and therefore truly representative of an individual's behaviour. In many species, vocalisation rate during isolation or confinement is repeatable and can be easily and reliably measured even in short tests, e.g. in sheep (Wolf et al., 2008) and pigs (Friel et al., 2016), including in kittens of the domestic cat (Hudson et al., 2017, 2015). We are interested in knowing if this pattern is found in adult cats as well, and how quickly in a test individual differences can be found.

It was therefore our aim in the present study to investigate the existence of stable individual differences in the behaviour of adult cats from a heterogeneous population. We recorded individuals' responses to brief social separation on repeated occasions using two behavioural measures: the number of separation calls and the duration of motor activity. We conducted a fine analysis of the data asking the following questions: (i) How stable are individual differences in cats in number of separation calls and duration of motor activity in response to brief social isolation across repeated trials? (ii) How quickly and reliably do any such differences emerge within trials? (iii) Does the expression of these behaviours change with repeated testing? (iv) Is there a relation between the two behavioural measures?

2. Material and methods

2.1. Study sites and animals

We collected data from 40 neutered adult cats between the ages of 8 months (after the age of sexual maturation) and 11 years (mean: 3.92 ± 2.4 *SD* years) from two different animal shelters. Twenty-nine cats (16 female, 13male) were housed at an animal shelter in Mexico City, Mexico and 11 (8 female, 3male) were housed in an animal shelter in Budapest, Hungary. Animals were chosen based on age, good health, ease of handling and remaining in the shelter for the duration of the study.

Both shelters were furnished with cat beds, boxes of assorted sizes with blankets, scratchers, and toys. Water, commercial dried cat food and litter boxes were always available. All the cats had been neutered and received post-operative care by qualified veterinarians within 3 days of entering a shelter, and all subjects participating in the study had been at the shelter for at least 6 weeks prior to the behavioural testing. In both shelters, cats were regularly exposed to and handled by volunteers and staff.

In the Hungarian cat shelter, the total number of cats fluctuated due to cats arriving and leaving, but at any given time approximately 20 cats were housed there. They were in a room 4 x 4 m in size connected to a fenced and roofed outdoor enclosure 3 x 3 m in size. The Mexican shelter was a 4-story house divided into sections; approximately 50 cats were placed in each section, according to how well they tolerated each other. All sections consisted of at least two rooms (approx. 2.5 x 3.5 m each) with access to a fenced outdoor balcony (approx. 2 x 4 m). Each cat was free to roam within its section.

2.2. Experimental procedure

At each site, the confinement trials were performed in a single closed room which was unfamiliar to the animals, located in the same building where they were housed and no more than 10 m away from the rooms that they typically occupied. In the Hungarian shelter, the testing room was 3 x 4 m in size, with a white tiled floor and walls and white ceiling, furnished with steel counters, and with a curtained window; in the Mexican shelter, the testing room was 1.5 x 2 m in size, with a flat finished, unpainted concrete floor, walls and ceiling, and without furnishings. Both rooms were illuminated by electric light. During trials, no other animals or humans were allowed to enter the test room nor the room adjacent to it to reduce potential auditory and olfactory disturbance. For each trial, all cats from the same shelter were tested on the same day in a randomized order between 13:00 h and 18:00 h once a week for three consecutive weeks.

One experimenter chose a cat from the housing area, briefly stroked it and picked it up. The experimenter then carried the cat, in their arms, into the test room. This procedure typically took less than one minute, and cats rarely vocalised during it. Once inside the test room, the first experimenter, with the help of a second experimenter, placed the cat inside a standard commercial pet carrier (42 × 61 x 38 cm), which was a closed plastic box with a steel-wire front door, and small ventilation holes along the walls. The carrier, with the cat inside, was then placed facing away from the door, after which the experimenters left the room and the trial began. The confinement test lasted two minutes; once this time had elapsed the cat was released from the pet carrier and returned to its home room carried in the arms of the first experimenter. All trials were recorded with a video camera (GoPro© Hero3 +, GoPro, Inc., USA) which was set up 60 cm from the carrier allowing a clear view of its interior. To facilitate observation, a red light was mounted on the ceiling of the carrier. The carrier was cleaned between trials with isopropyl alcohol.

2.3. Behavioural recording and analysis

Using Solomon Coder software (Péter, 2015), the following behaviours were scored for each cat from the video recordings: vocalisation (the timing and total number of calls emitted by each individual during each separation trial) and duration of motor activity (displacement of any of the limbs on the floor or on the sides of the carrier; quantified in seconds at intervals of 0.5 s by analysis of video footage for each individual in each trial).

2.4. Statistical analysis

Statistical analyses of the data were carried out using the program R, version 3.3.1 (R Development Core Team, 2016). To assess inter-observer reliability, two observers independently scored the cats' behaviour for a subset of 30 trials (25% of the total), from 5 and 17 cats from the Hungarian and Mexican shelters, respectively. Inter-observer reliability was assessed using weighted Cohen's Kappa for vocalisation data and a one-way intraclass coefficient (ICC) for motor activity data using the R package *irr* (Gamer et al., 2013). Agreement between the two independent observers was high both for number of vocalisations (weighted $\kappa = 0.962$, $p < 0.001$) and duration of motor activity ($ICC = 0.891$, $p < 0.001$).

Prior to analysis, motor activity was normalized using a Box-Cox transformation (Shapiro-Wilk test of normality: $W = 0.98$, $p = 0.084$) using the R package *MASS* (Venables and Ripley, 2002). We tested for significant effects of the experimental setup (shelter of origin), and of individual characteristics of the cats (age, sex and their interaction) on each of the two measured behaviours (vocalisation and motor activity) by fitting generalized linear mixed models (GLMMs) using the *lme4* package (Bates et al., 2015) in R. For each behavioural measure, we included the following variables into the full model. As fixed effects, we included sex, shelter (in Mexico or Hungary), trial number (1–3), age (as a covariate), the interaction of sex × age, and the interaction of trial number × age. As a random factor, we included individual identity. We applied backward stepwise reduction of the full models beginning with non-significant interactions followed by non-significant fixed effects when $p > 0.05$. Individual identity as a random factor was included in all models to account for repeated measures of individuals.

Across-trial and within-trial repeatability, that is, the amount of variance explained by the individual divided by the total phenotypic variance (Sokal and Rohlf, 1995), of each of the two behaviours (number of vocalisations and transformed duration of motor activity) was calculated using intra-class correlations obtained through GLMM-based calculations for count (Poisson-distributed) and LLM-based calculations for normalized data, respectively, using the R package *rptR* (Nakagawa and Schielzeth, 2010). Individual identity was always used as a random factor, and the fixed effects found to have a significant effect in the previous analysis were included where applicable. *P*-values were calculated by 1000 permutations and 95% confidence intervals (95% *CI*) for parameter estimates were assessed by 1000 bootstrapping runs. A variance decomposition analysis, where the proportions of the total variance in the expression of the two behavioural variables due to within-individual variance and between-individual variance, was also carried out using the rptR package.

To test for an association between the number of vocalisations and the duration of motor activity, mixed models separating between-individual and within-individual slopes were used. Since one behavioural variable must be handled as a predictor and the other as a response, two models were built: one where number of vocalisations was the response variable, and another where (normalized) duration of motor activity was the response variable. In each model, the other behavioural variable used as a predictor was included in two ways: averaged by individual (to eliminate within-subject variation and examine between-individual variation) and centred within subjects (to eliminate between-individual variation and examine only within-individual variation; van de Pol and Wright, 2009). Also included as fixed effects were sex, shelter of origin, trial number and age, as well as the interactions between each of these fixed effect and the behavioural predictor variables (both averaged by individual and within-subject centred); individual identity was included as a random effect. We applied backward stepwise reduction of the full models beginning with non-significant interactions followed by non-significant fixed effects when $p > 0.05$. Individual identity as a random factor was included in all models to account for repeated measures of individuals.

2.5. Ethics note

Throughout the study, animals were kept and treated according to the guidelines for the use of animals in research as published in Animal Behaviour (ABS, 2016), as well as the relevant legislation for Mexico (National Guide for the Production, Care and Use of Laboratory Animals, Norma Oficial Mexicana NOM-062-200-1999) and Hungary (European Communities Council Directive of 22 September 2010, 2010/63/EU).

3. Results

3.1. Repeatability of individual differences in behaviour

Age was found to have an effect on the number of vocalisations (Appendix Table A1), where older cats vocalised less. No effects of sex, trial number, the interaction of age * sex, the interaction of trial*sex, nor the shelter to which each cat belonged were found on the number of vocalisations during the study (Appendix Table A1). Therefore, age was included in the repeatability analysis as a fixed effect in addition to individual identity as a random factor. Individual differences in the number of vocalisations emitted by the cats ($n = 40$) across the three trials of the confinement test were highly repeatable (intra-class repeatability: $R = 0.913$, $CI = [0.803, 0.964]$, $p = 0.001$). Average between-individual variance (3.826 ± 1.106 *SE*) was higher than the average within-individual variance (0.386 ± 0.099 *SE*).

Trial number (one to three) had a negative effect on the duration of motor activity; no effects of age, sex, the interaction of age * sex, the interaction of trial*sex, nor the shelter to which each cat belonged were found on the duration of motor activity during the study (Appendix Table A2). Therefore, in the repeatability analysis, only trial number was included as a fixed factor and individual identity was included as a random factor. Although individual differences in motor activity ($n = 40$) were repeatable across trials, the repeatability value was low ($R = 0.423$, $CI = [0.209, 0.601]$, $p = 0.001$), because the average between-individual variance (0.254 ± 0.085 *SE*) was lower than the average within-individual variance (0.347 ± 0.056 *SE*).

3.2. Emergence of individual differences in behaviour during the trials

In regard to our second aim, which was to see how quickly individual differences emerged during trials, inter-individual differences in the number of vocalisations and the duration of motor activity were already significantly repeatable between trials after the first 10 s of testing (vocalisation: $R = 0.78$, $CI = [0.400, 0.949]$, $p = 0.001$; motor activity: $R = 0.226$, $CI = [0.019, 0.423]$, $p = 0.013$; Fig. 1). The repeatability of the number of vocalisations increased slightly after the first 20 s and remained stable until the end of the test, whereas the repeatability of motor activity increased slowly until ˜90 s into the test, whereafter it remained stable (Fig. 1).

3.3. Overall change in performance of behaviours with repeated testing

In regard to our third aim, which was to determine whether the expression of the behaviours changed with repeated testing, we found that the number of vocalisations emitted did not vary between trials (Appendix Table A1), but the duration of motor activity did (estimate ± SE = -0.237 ± 0.066, $p = 0.0005$; Appendix Table A2). Paired Bonferroni post-hoc tests showed that motor activity decreased after the second trial (Fig. 2).

3.4. Correlation between vocalisation and motor activity

In regard to our fourth aim, the two models we constructed gave similar results (Appendix Table A3). There was no significant correlation in either of the models between the number of vocalisations and the duration of motor activity at the between-individual level. Although in one of the models (with duration of motor activity as the response variable) the *p*-values indicated that there was a significant relationship between the two behaviours at the within-individual level, the model estimates were extremely small (0.024 ± 0.01), so we do not interpret this result as indicative of a meaningful relationship between the

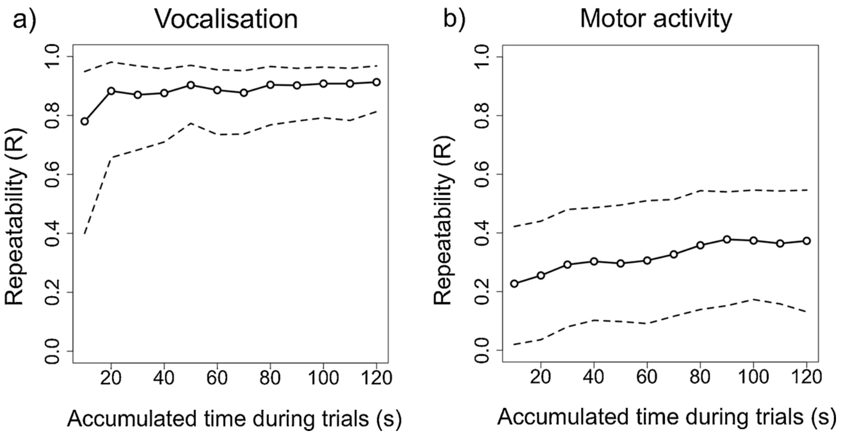

Fig. 1. Progression of repeatabilities of (a) the number of vocalisations and (b) the duration of motor activity of individual cats across the three, 2-min trials carried out over three weeks. Analyses are based on cumulatively increasing 10-second-long segments of the three trials of the study. Circles represent the intra-class correlation coefficient (*R*), expressing the degree of repeatability across the three trials. Dotted lines give the 95% confidence intervals of *R*, based on 1000 bootstraps. All *R* values in (a) and (b) are statistically significant.

variables. Likewise, although in the model where the number of vocalisations was the response variable the interaction of age * duration of locomotion (within-subject centred) was significant with a model estimate of −0.004 ± 0.002, and the interaction of trial * duration of motor activity (within-subject centred) was significant with a model estimate of 0.012 ± 0.005, the model estimates were again so small that we do not interpret these results as meaningful. The same is true in the model in which duration of motor activity was the response variable, where the interaction between trial * number of vocalisations (averaged by individual) was significant with a model estimate of 0.012 ± 0.005, which again is extremely small, and we do not interpret this as a meaningful result.

4. Discussion

4.1. Stable inter-individual differences

The cats in this study showed high stable inter-individual differences in the number of vocalisations emitted during repeated periods of brief confinement, and to a higher degree than was previously found in kittens (Hudson et al., 2017, 2015). Stable differences in vocalisation have also been reported for adult cats in different test situations, e.g. in novel environments (Adamec et al., 1983). The duration of motor activity, on the other hand, showed significant but lower repeatability between trials; the average within-individual variance was greater than the average between-individual variance. The repeatability of motor activity in adult cats that we report here is lower than that of locomotor activity reported in kittens in a similar test situation (Hudson et al., 2017), suggesting that there could be an age-related change in inter-individual differences in activity, although whether these differences were due to developmental, experiential or environmental factors cannot be determined without further study.

4.2. Change in inter-individual differences during trials

The repeatability of the number of vocalisations was high even after the first 10 s of the trials, and remained high thereafter which is again consistent with findings in kittens (Hudson et al., 2017), although the repeatability values in adults were higher than those in kittens. This is perhaps not surprising, since kittens' vocalisation in response to social separation changes rapidly in early age, which can drive down repeatability (Bánszegi et al., 2017). Repeatability of the duration of motor activity in the present study, on the other hand, was low in the first 10 s of the trial, and after a slow increase it appeared to plateau (at a low value) after 90 s, suggesting that even in a longer trial it would not become highly repeatable. This was different to the pattern observed in kittens, where repeatability of locomotor activity increased markedly until ˜100 s into trials and then dropped (Hudson et al., 2017).

4.3. Change in behaviour with repeated testing

Previous works by Adamec et al. (1983) and Candland and Nagy (1969) showed that upon repeated testing, cats vocalised somewhat less frequently in later trials. However, those tests were 15 and 10 min long, respectively, and in the case of Adamec et al. (1983), this decrease was observed only after the first 8 min of the test. In the present study the cats were confined for only 2 min, which perhaps was not long enough

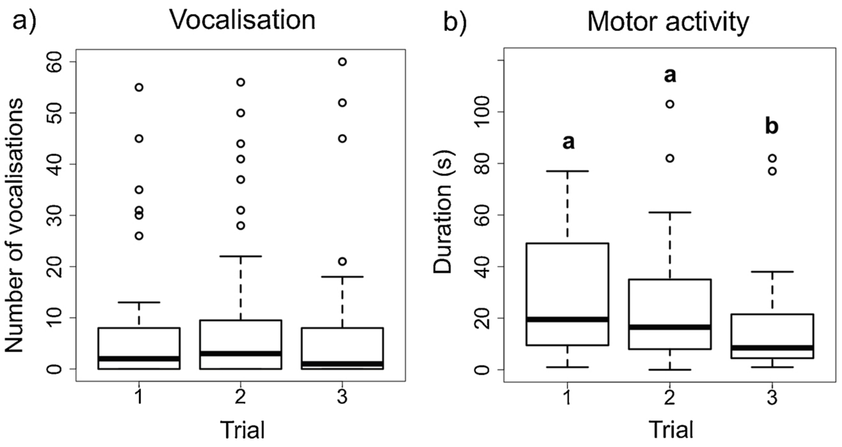

Fig. 2. a) The number of vocalisations emitted during the trials remained stable across repeated testing. b) The duration of motor activity decreased with repeated testing. Each boxplot depicts medians with inter-quartile ranges; whiskers extend to maximum 1.5 times the inter-quartile range. Different letters above boxes indicate significant differences following paired Bonferroni post-hoc tests.

to see a general change in the number of vocalisations week to week, since a decline in vocalisations has only been observed in long (> 10 min) tests. However, we did find a general decrease in the duration of motor activity over the three weeks of testing, supporting the findings of Candland and Nagy (1969) regarding activity. This general decrease in activity could be interpreted in different ways. One possible interpretation is that the animals habituated to the test, although this is an unlikely explanation since habituation should then also have been seen in the rate of vocalisations, which, however, remained stable from week to week. Another possibility is that the cats' motor activity was expressed in response to a combination of different motivations which changed with repeated testing, e.g. exploration or general activity.

4.4. Lack of correlation between number of separation calls and duration of motor activity

In kittens tested repeatedly from 1 to 4 weeks of age, no correlation was found between the number of vocalisations and amount of locomotion in an isolation test by Hudson et al. (2015), who suggested that this could be due to different rates of maturation of the locomotor and vocal systems. However, even in the fully mature cats used in the present study, no such correlation was found. This is in contrast to the results of Iki et al. (2011), who found a strong negative correlation between locomotion and vocalisation in adult male cats during a spray shower. The different results could be due to the characteristics of the study population (degree of socialization to humans and/or conspecifics, population density, housing conditions, or sex) or to characteristics of the negative stimulus: the motivation to move during confinement may differ from that during a spray shower.

The lack of correlation between motor activity and vocalisation is consistent with other reports in cattle (Van Reenen et al., 2004, 2005; Van Reenen et al., 2013), where it was suggested that the two behaviours could reflect two different underlying traits. Higher levels of vocalisation might be due to some individuals' stronger reactions to isolation or confinement, possibly due to the social aspect of the isolation (Müller and Schrader, 2005) or as a general fearful reaction (De Passillé et al., 1995), whereas high activity could be due to the animals having an active, rather than passive coping style in stressful situations (Koolhaas et al., 1999; Van Reenen et al., 2013). A similar explanation has been proposed by Iki et al. (2011) for individual differences in locomotion and vocalisation in cats exposed to a stressful situation. Alternatively, Van Reenen et al. (2013) also propose that locomotion may not be linked to an emotional reaction, but rather that it could reflect a general measure of activity or exploration, or (what seems more likely) that it cannot be exclusively attributed to a single underlying trait (Rushen, 2000). Due to the lack of correlation between the two variables in our results, we propose that in adult cats, and as considered previously in kittens (Hudson et al., 2015), they are due to (at least) two different neurophysiological mechanisms.

4.5. General discussion

Activity is one of the most common behavioural responses measured in stressful test situations and has been found to be repeatable in a variety of species, although its repeatability tends to be low (Bell et al., 2009). Additionally, whether high levels of activity actually indicate stress has been questioned (Rushen, 2000). It has been suggested that whether activity accurately reflects stress is most likely species-specific (Candland and Nagy, 1969), and most likely depends on the set-up of a particular test as well. The number or rate of vocalisation is less commonly used, although in several species it has also been found to be a good marker of stress and is repeatable, e.g. in cattle (Van Reenen et al., 2004; Van Reenen et al., 2005, 2013), pigs (Fraser, 1974), horses (Seaman et al., 2002), sheep (Torres-Hernandez and Hohenboken, 1979), kittens (Hudson et al., 2017, 2015), and adult cats in the present study. In addition, some vocalisations have been linked to negative emotions (Brudzynski, 2007; Jürgens, 2002; Newman, 2007), as both vocal and physiological responses to stress are partially controlled by the same central neuroendocrine systems (Jürgens, 2009).

Vocalisation is increasingly recognized as a useful way to quickly and reliably measure the emotional state of individuals, both in experimental settings and in applied situations. For example, distress calls have seen applied use in welfare (Grandin, 1998; Manteuffel et al., 2004) and in pharmacological studies (Brudzynski, 2015). Although current methods of behavioural stress assessment in cats include vocalisation, such as the Cat-Stress-Score (CSS; Kessler and Turner, 1997) which combines the assessment of posture, facial expression, and behaviour, the interpretation of vocalisation remains somewhat ambiguous. The CSS rates both the presence *and* absence of vocalisation as indicative of high stress in cats, which suggests that there may be individual differences in how cats react behaviourally to stress (though we would like to note that the CSS was developed to assess stress over a period of several days rather than in response to short-term, acute stressors). In other areas of research, however, vocalisation in cats is a promising candidate for the behavioural assessment of stress, although the relationship between the two in cats is still unclear: one recent study found that vocalisation during confinement in a pet carrier was reduced by the administration of trazodone, an antidepressant and anxiolytic drug (Stevens et al., 2016); whereas another study found the opposite effect, where vocalisation during isolation in a room increased with the administration of the anxiolytic drug diazepam (de Rivera et al., 2017). The validation of vocalisation behaviour as a behavioural indicator of stress with additional measures, such as physiological variables of stress response or qualitative vocal analysis, could make for faster, easier and non-invasive assessment of stress reactivity in cats and potentially other animals.

In the present paper, we cannot attribute the vocal response we observed in the cats solely to confinement, isolation or, for example, the exposure to a novel environment. To disentangle the effect of each of these conditions as stressors further research is needed. Additionally, the underlying motivation and expression of behavioural responses to stress may change over development: for example, very young kittens placed in a novel environment will still vocalise even in the presence of their mother or a littermate, whereas older kittens (approaching weaning age) placed in novel environments tend to vocalise much less (Bánszegi et al., 2017), and particularly if they are in the presence of a familiar conspecific (Rheingold and Eckerman, 1971). The interpretation of behavioural responses to stress must always be carried out with caution as they will usually reflect a mix of motivations, although vocalisation in many species has been experimentally linked to hypothalamic–pituitary–adrenal (HPA) axis activity and a stress response (Rushen, 2000).

4.6. Conclusions

We found stable inter-individual differences in adult cats' behavioural responses to repeated brief confinement tests. Vocalisation rate was highly repeatable and it remained stable from week to week, whereas the duration of motor activity had very low repeatability values and tended to decrease with repeated testing. Our findings suggest that, in cats, vocalisations may be more reliable than motor activity as indicators of stable differences between individuals, and potentially a better measure of stress response. An important next step is to carry out repeated measurements across development, as it is still unknown whether individual differences in these behaviours are repeatable across different life stages. Behaviours may change as animals grow and mature, especially in altricial species, as developing offspring acquire skills and experience, exhibit more adult-like behaviours, and become self-sufficient (Stamps and Groothuis, 2010; Trillmich and Hudson, 2011). Nevertheless, since individual differences in the rate of separation calls was highly repeatable in adult cats and was previously found to be repeatable in kittens, and more so than levels of motor activity (Hudson et al., 2015), it appears to be a good behaviour to study personality across different life stages.

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Appendix A

Models to check for significant effects of covariates

Table A1
Models investigating the effect of study location and characteristics of the cats on the number of vocalisations. Bolded effects were significant and were thus later included in the repeatability analysis.

Vocalisation		
Effect	Estimate ± SE	*p*
age	**−0.455 ± 0.163**	**0.005**
age*sex	−0.073 ± 0.318	0.819
trial*sex	−0.132 ± 0.078	0.089
sex	−0.025 ± 0.684	0.970
trial	0.007 ± 0.038	0.849
shelter	−0.913 ± 0.772	0.237

Table A2
Models investigating the effect of study location and characteristics of the cats on the duration of motor activity (normalized). Bolded effects were significant and were thus later included in the repeatability analysis.

Motor activity		
Effect	Estimate ± SE	*p*
trial	**−0.237 ± 0.066**	**0.0005**
age*sex	−0.054 ± 0.086	0.531
trial*sex	0.107 ± 0.136	0.436
shelter	−0.084 ± 0.241	0.730
sex	−0.024 ± 0.202	0.906
age	−0.046 ± 0.040	0.257

Table A3
Mixed models resulting after backwards stepwise selection investigating the relationship between number of vocalisations and duration of motor activity at the between-individual level (with data averaged by individual) and within-individual level (data within-subject centred) with a) number of vocalisations, and b) duration of motor activity as the response variables, respectively. Individual identity was included as a random effect.

a) Number of vocalisations		
Effect	Estimate ± *SE*	*p*
duration of motor activity, within-subject centred	−0.001 ± 0.011	0.94
age	**−0.457 ± 0.167**	**0.006**
trial	0.025 ± 0.041	0.539
age * duration of motor activity, within-subject centred	**−0.004 ± 0.002**	**0.037**
trial * duration of motor activity, within-subject centred	**0.012 ± 0.005**	**0.009**
b) Duration of motor activity		
Effect	Estimate ± *SE*	*p*
number of vocalisations, averaged by individual	−0.019 ± 0.012	0.1
number of vocalisations, within-subject centred	**0.024 ± 0.01**	**0.02**
trial	**−0.339 ± 0.073**	**0.00001**
trial * number of vocalisations, averaged by individual	**0.012 ± 0.005**	**0.013**

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.beproc.2019.05.022.

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