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Intraspecific variation of breathing rate during stress response in *Microtus arvalis* (Mammalia: Rodentia)

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Abstract. Recently, personality traits have been determined in the common vole on the basis of behavioural tests. Both bold as well as shy individuals have been recorded. The aim of the present study was to verify whether variations in the behavioural manifestations in the common vole are also accompanied by variations in the values of usually monitored stress parameters. This study investigated variations in the breathing rate of the common vole, *Microtus arvalis* (Pallas, 1778), and whether these variations are consistent with regard to repetition and stress state. We also investigated possible relationships between breathing rate variation and behavioural personality traits of the tested individuals. Breathing rate values were measured using a device consisting of a respirometric chamber connected to a sensitive pressure sensor and an oscilloscope. Resting breathing rate (RBR) and stress breathing rate (SBR) were measured, and the percentage of stress breathing increase was calculated. The locomotor activity of the observed voles in the Open Field test was used as a behavioural personality trait. It was found that the common vole shows consistent inter-individual differences in breathing rate in all measured parameters. A very close relationship between the individual SBR value and the relative increase of the stress breathing with the personality locomotor trait of the tested animals was demonstrated. Higher values of SBR and stress increase percentage were measured in individuals that appeared to be "shy" in the Open Field test (i.e. anxious animals easily subject to stress). The relationship points to a connection between behavioural and physiological traits, which corresponds with the concept of "coping style" and the distinction between proactive and reactive individuals.

Key words. Common vole, *Microtus arvalis*, breathing rate, stress value, repeatability, coping style.

INTRODUCTION

Behavioural intraspecific differences are not only a manifestation of random variation around the adaptive value of a trait (Carere & Locurto 2011), but a reflection of the close relationship between the behavioural profile of an individual and its life strategy (life-history) (see e.g., Wilson 1994, Réale et al. 2000). Intraspecific behavioural differences that are consistent over time, across situations and contexts are usually referred to as personality. From the point of view of behavioural ecology, two concepts of personality are currently accepted (Réale et al. 2007, 2010). Animal personality in the broad sense is defined as consistent inter-individual differences in a selected type of behaviour e.g., reaction to a predator (Réale 2007). This concept is most often used in studies dealing with the effect of selection on evolutionarily crucial behaviours, e.g., in response to a predator (bold-shy trait/dimension) or in obtaining food (fast-slow trait/dimension).

On the other hand, the narrow sense personality concept, Réale (2007) works with sets of mutually correlated behaviours (personality traits/dimensions), which together create a multidimensional characteristic of the psychological properties of each individual. This concept approaches the concept of personality in human psychology (Pervin & John 1997) and in physiological studies (referred to here as “coping style”, Koolhas et al. 1999). It is defined as a set of behavioural and physiological responses to stress that are unique to a given individual and whose nature is consistent over time and across different situations (de Ruiter et al. 1992, Sgoifo et al. 1996, Koolhaas et al. 1999). This latter concept has so far been applied to various animal species, such as the rat, *Rattus norvegicus* (Berkenhout, 1769), the house mouse, *Mus musculus* Linnaeus, 1758, the wild pig, *Sus scrofa* Linnaeus, 1758, and the rhesus macaque, *Macaca mulatta* (Zimmermann, 1780) – see the overview by Koolhaas et al. (1999).

In general, proactive rodent individuals are characterised by lower reactivity of the hypothalamus-pituitary-adrenal axis (hereafter referred to as the HPA axis), manifested by a low level of glucocorticoids in the plasma during stress and higher sympathetic reactivity expressed by a high level of catecholamines during stress. In contrast, reactive individuals show higher HPA axis reactivity and higher parasympathetic reactivity.

In a selection study on domestic mice, *Mus musculus domesticus* Schwarz et Schwarz, 1943, individuals were selected on the basis of attack latency into the lines “short attack latency” (SAL, fast attacking) and “long attack latency” (LAL, slow attacking, Benus et al. 1989). SAL individuals were more aggressive, explored new environments faster, but were also more prone to routine behaviour. LAL individuals, on the other hand, were less aggressive, slower to explore new environments, but more attentive to details and more plastic in behaviour (Benus et al. 1990). SAL and LAL mice have also been found to differ in testosterone levels, with SAL individuals showing higher levels than LAL individuals (de Ruiter et al. 1992). The link between personality and physiological characteristics is also demonstrated by a study conducted on a wild population of alpine marmots, *Marmota marmota* (Linnaeus, 1758), which found a positive relationship between interest in new stimuli and respiratory and heart rates (Ferrari et al. 2013).

During stress, due to the influence of external and internal factors (stressors), the internal balance – homeostasis – is disturbed (Barreto & Volpato 2004, Reeder & Kramer 2005). Stressors can be physical, psychological or combined in nature. Internal body stressors include e.g., hypoglycemia, anoxia; external ones include e.g., cold, heat, movement (work) activity or injury. Psychological stressors include stimuli that affect emotions (cause fear, anger, anxiety). In response to the stressor, a stress response occurs, which is a set of physiological and behavioural reactions and serves to neutralize the impact of the stressor and restore homeostasis (Reeder & Kramer 2005). Since it is necessary to mobilize as much energy as possible in these situations, there is an increase in the activity of the neural-autonomic system and humoral secretion (Barreto & Volpato 2004, Reeder & Kramer 2005, Renwrantz & Spielvogel 2011). The most important physiological responses to stress in mammals stimulate the sympathetic nervous system and the HPA axis.

The first system ensures a very quick response – after activation of the sympathetic nervous system catecholamines adrenaline and noradrenaline are released. An increase in their levels immediately causes an increase in breathing rate, heart rate and blood pressure (Rietmann et al. 2004, Reeder & Kramer 2005, Renwrantz & Spielvogel 2011). The second system is slower – after activation of the paraventricular nucleus of the hypothalamus, neurons begin to synthesize corticotropin (“releasing” hormone) and vasopressin. Corticotropin travels to the adenohypophysis and adrenocorticotropin (hereinafter referred to as ACTH) is released into the bloodstream. ACTH then acts on the adrenal cortex. Its influence results in the secretion of glucocorticoids, which increase available energy through increased glyconeogenesis, reduced glucose utilization, and reduced sensitivity to insulin (Reeder & Kramer 2005).

As indicated above, there is an increase in respiratory rate in response to stress. Respiratory frequency is thus often used in research as an indicator of stress (e.g., in fish, Barreto et al. 2003, Barreto & Volpato 2004, Bell et al. 2010, in birds, Carere & Oers 2004, Oers & Carere 2007, Davit et al. 2011, and in mammals Jürgens et al. 1996). However, a reaction to a stressful situation can be quite individual (Bell et al. 2010). Intraspecies differences exist primarily in the rate of increase in the level of catecholamines (Koolhaas et al. 1999), and it can be assumed that individuals more easily subject to stress will breathe faster (Carere & Oers 2004, Bell et al. 2010, David et al. 2012), e.g., three-spined sticklebacks (*Gasterosteus aculeatus* Linnaeus, 1758) from populations with high predation pressure showed greater changes in respiratory rate than individuals from low-predation populations (Bell et al. 2010). In the case of "fast" and "slow" selected lines of great tits (*Parus major* Linnaeus, 1758), a striking relationship between the change in breathing rate and the personality type of individuals was found. Slow individuals had a higher breathing rate than fast individuals in both measurements at the beginning and at the end of the test (Carere & Oers 2004).

In our laboratory, we demonstrated the presence of consistent intraspecific behavioural variations (personality traits) in the common vole (Lantová et al. 2011, Urbánková et al. 2020), but have not yet tested the link to physiological parameters. From the above overview, this test would be beneficial and offer complementary information on how animals experience various behavioral tests and how it relates to personality traits. The following questions were answered:

(1) Does the common vole show intraspecific variation in resting and stress breathing rate?
(2) Are individual differences in breathing rate consistent over time?
(3) Is intraspecific variation of breathing rate related to intraspecific variation of a behavioural trait?

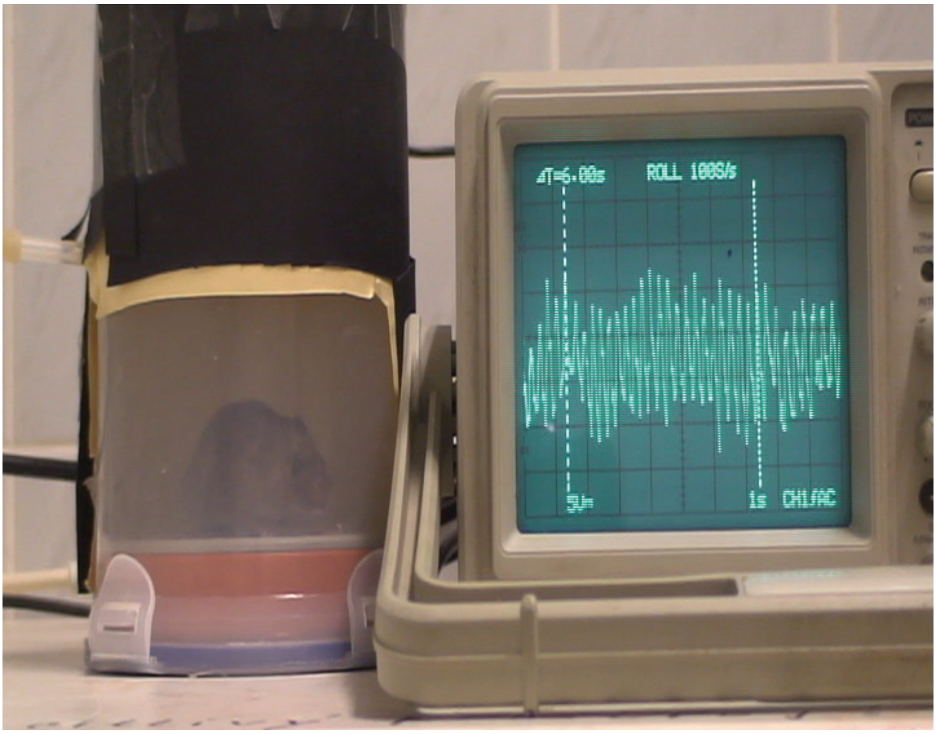

Fig. 1. Recording of the first measurement for vole ID 134; 21 breaths were detected in the video recording during a six-second period; multiplying the respiratory frequency by 10 gives SBR=210 breaths×min^{-1}.

MATERIAL AND METHODS

Vole individuals

The common voles, *Microtus arvalis* (Pallas, 1778), originating from the first generation of young individuals caught in the wild (České Budějovice, 48.9778° N, 14.4414° E) were used for the tests. In total, there were 30 individuals (11 males and 19 females) aged about 12 weeks. Voles were kept individually in polycarbonate breeding boxes 58×36×20 cm (VELAZ Praha) with wood shavings, hay, and a plastic tube as a shelter (l=15 cm, d=4 cm). Commercial pellets for rats and mice, as well as pellets for guinea pigs and rabbits (VELAZ Praha), fresh carrots, and water were available *ad libitum*. All individuals were individually marked on the breeding boxes. The laboratory conditions were stable, with a room temperature of 18–20 °C under a L:D 12:12 photoperiod, the same conditions as for their parents.

Breathing rate measurement

In small terrestrial mammals, measuring the breathing rate is quite difficult. In a study by Jürgens et al. (1996), the breathing rate of the pygmy white-toothed shrew, *Suncus etruscus* (Savi, 1822) was measured using electrocardiography (ECG) and verified using an auto-focusing laser system that measured chest movements. Another possibility is the use of a piezoelectric transducer, which can be combined with an ECG and thus a record of breathing and heart rate can be obtained simultaneously (Zehendner et al. 2013). In our study, the breathing rate was measured using a respirometric chamber with a volume of 3 dm^3 connected to an electronic pressure transducer recording pressure changes in the chamber. Changes in electrical voltage from the transducer were displayed on the oscilloscope screen (Fig. 1). The chamber was equipped with a double bottom, into which was inserted a filter paper soaked in a solution of potassium hydroxide (concentration of 1 $mol \times l^{-1}$), which served as a carbon dioxide absorber. The concentration of oxygen in the air is also important, a drop in concentration should not be more than 4% (see e.g., Nilsson 2010). We relied on data that a small mammal the size of a vole consumes 3 ml of oxygen per 1g of body weight per hour. Our experiment did not exceed the stated critical limit by far.

The tested vole was moved from its breeding box to the respirometric chamber using a plastic tube (home shelter). Breathing rate was measured for a total of 20 minutes and was recorded by a video camera. The tested individual was startled by a sound signal immediately after being placed in the chamber to obtain an individual stress breathing rate (SBR) value. After calming down (falling asleep, lying down), the resting breathing rate (RBR) was read. The latency of this behaviour as well as urination was also recorded. Each individual was weighed before and after all tests. After each animal, the chamber was washed with diluted alcohol and water then dried. Each vole was tested a total of three times (BM – breathing measurement 1–3). The interval between the first and second measurement was 24 hours, the third measurement was performed one month after the second. Output parameters (SBR, RBR, sedation latency) were evaluated from the video recording of each measurement, see Fig. 1. On the video recording, 21 breaths were recognized in a period of six seconds, by multiplying by 10, a respiratory rate of 210 $breaths \times min^{-1}$ was obtained.

Personality trait

Personality trait was determined using open field tests (OFT; Hall 1934, Archer 1973). Restricted amount of data were used from the study Urbánková et al. (2021) where 5 tests were carried out with two-month intervals. The tested voles were placed in an opaque apparatus with dimensions of 75×75 cm, which was uniformly illuminated by an 18 W fluorescent lamp. The animals were transferred from the breeding box to the experimental apparatus in a plastic tube, which served as a shelter in the breeding box. The OFT took place for three minutes and was recorded by a digital camera (Panasonic Color CCTV Camera WV-CP500/G) placed 150 cm above the testing arena. Analysis of the video recordings was carried out using the "Modular tracking system 1.07" program, which was able to track the movement of the animal in the test apparatus. The voles were tested between 9 a.m. and 4 p.m. in a random order, because voles are animals with a polyphasic circadian activity with alternating 2–3 hours of moving and rest (Gerkema et al. 1993). After the end of each test, the apparatus was wiped with diluted alcohol and water, then dried with a cellulose cloth. The output information was the length of the trajectory given in meters. For organizational reasons, it was possible to take data from 13 tested animals (4 males and 9 females) – see Table 3.

Statistical analyses

To gain insight into the parameters we were working with, principal component analysis (PCA) implemented in CANOCO software was used to show their relationships (ter Braak & Šmilauer 2012). For determination of the personality trait, we used the original values of the distance moved in three OFT using the lmer function in R 4.0.2 (R Core Team 2020) using lme4 package. These analyses were done to generate the random intercepts (personality trait) for each individual (Urbánková et al. 2021). We used each OF test round (1–5) as an independent variable, ID for random effect and assessed the consistency repeatability (R_C) after Biro & Stamps (2015). We used an updated version of rptR package (Stoffel et al. 2017) enabling us to compute repeatability according to the distribution of each dependent variable, to assess intra-individual variation.

The same statistical procedure (LMM) was used for evaluation of the personality trait in the breathing rate categories and in the evaluation of the significance of the independent variables for the response variables of the three breathing

Table 1. Overview of the measured parameters

parameters	mean	median	minimum	maximum	SD
RBR (min^{-1})	141.5	140.0	100.0	210.0	22.1
SBR (min^{-1})	218.4	210.0	170.0	290.0	28.3
increase (%)	34.8	37.0	0.0	59.3	11.7
weight 1 (g)	26.5	23.3	15.1	50.0	9.0
weight 2 (g)	26.0	22.9	15.4	48.7	8.7

RBR (min^{-1}) – resting breathing rate, SBR (min^{-1}) – stress breathing rate, increase (%) – calculated from resting (100%) and stress values, weight 1 (g) and weight 2 (g) – weight before and after breathing measurement

rate categories. Figures were created in Statistica 13 (TIBCO Software Inc. 2017). Except for the relative change in breathing rate, no parameter had a normal distribution, so the parameters were logarithmically transformed. For all tests, we considered the cut-off for statistical significance as $P<0.05$.

RESULTS

The measured values are presented in Table 1. The values for RBR ranged from 100 to 210 min^{-1} with mean value 141.5 min^{-1} and median 140 min^{-}1. The values for SBR ranged from 170 to 290 min^{-1}. The percentage values of the stress increase ranged from 0.0 to 59.3%, the mean value

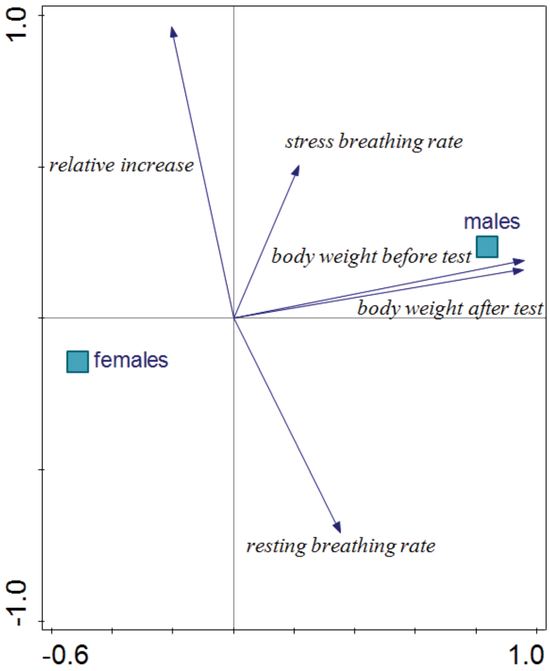

Fig. 2. Ordination graph of PCA for the measured parameters. Explained cumulative variations according to the axes 1, 2, and 3 were 41.4, 76.7, and 99.2%, respectively.

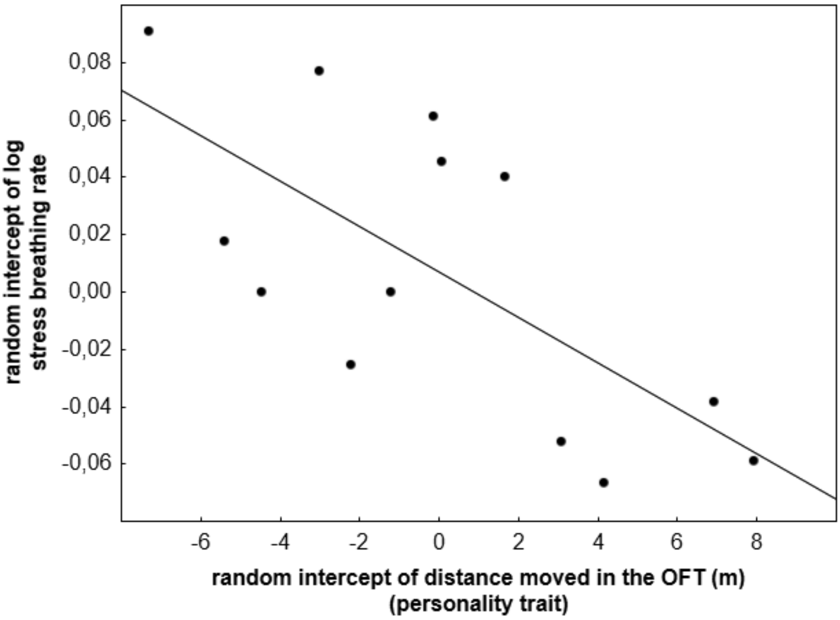

Fig. 3. Correlation between the random intercept of distance covered during the OFT and the stress breathing rate during the stress test (r=–0.7069, p=0.0069). The longer distance voles cover, the lower stress relative breathing rate increase is caused under stressful situations.

was 34.8 and the median 37.0. Body weights before the test ranged from 15.1 to 50.0 g with a mean value of 26.5 g and median 23.3 g. Body weights after the tests ranged from 15.4 to 48.7 g with mean value 26.0 g and median 22.9 g.

Using the PCA we got an overview of the relationships between the measured parameters. The relative increase in breathing rate during a stressful situation showed the opposite trend to resting breathing rate (Fig. 2). The lower the resting rate, the higher the stress increase. Sex factor along the first axis had a negligible effect on the mentioned physiological parameters. The body weights of the animals before and after the experiment showed an almost identical course, i. e. the test had small effect on the animals. Both weight vectors showed weak correlation with breathing parameters. The influence of sex and body weight was verified also by LMMs.

Based on LMM of the physiological parameters (RBR, SBR, and relative breathing rate increase), we tested for the significance of the fixed and random effects, shown in Table 2, the random intercepts are sorted in Figs. S1–3. In Table 3, this high variation of individuals is characterised by consistency and repeatability R_C=0.58, 0.77, and 0.60, respectively. Besides the measured parameters, we also included distance moved in OFT in the evaluations, taken with the author's kind permission (Urbánková et al. 2021) from the data pool of the personality trait stability study (Tables 2 and 3). Unfortunately, we could use data for 13 voles only. Their IDs are listed in Table 3. Random intercepts of distance moved in OFT were considered as certain personality traits and correlations with the physiological parameters were determined (Table 3). A statistically insignificant value r=0.1414 was found for the resting breathing rate. Statistically significant correlation was determined in the correlation of personality trait and the random intercept of stress breathing

Table 2. Significance of the fixed and random effects in LMM for four response variables. Presented tests compare each model with the reduced model on the preceding row

response variable	model	AIC	BIC	log lik	df	p
distance	~ trial	456.30	462.82	–225.15		
	~ trial + (1 | ID)	443.22	451.92	–217.61	1	0.0001
	~ trial + sex + (1 | ID)	445.11	455.98	–217.55	1	0.734
	~ trial + weight + (1 | ID)	444.37	455.25	–217.19	1	0.357
log RBR	~ trial	–216.25	–208.86	111.13		
	~ trial + (1 | ID)	–241.30	–231.44	124.65	1	<0.000
	~ trial + sex + (1 | ID)	–239.42	–227.09	124.71	1	0.736
	~ trial + weight + (1 | ID)	–240.12	–227.79	125.06	1	0.367
log SBR	~ trial	–254.52	–247.12	130.26		
	~ trial + (1 | ID)	–310.36	–300.49	159.18	1	<0.000
	~ trial + sex + (1 | ID)	–308.98	–296.65	159.49	1	0.428
	~ trial + weight + (1 | ID)	–311.46	–299.13	160.73	1	0.078
relative increase	~ trial	–122.42	–115.02	64.21		
	~ trial + (1 | ID)	–149.47	–139.61	78.74	1	<0.000
	~ trial + sex + (1 | ID)	–147.51	–135.18	78.75	1	0.853
	~ trial + weight + (1 | ID)	–147.48	–135.15	78.74	1	0.920

AIC – Akaike information criterion; BIC – Bayesian information criterion; log lik – log-likelihood *estimates*; df – degrees of freedom; p – significance level.

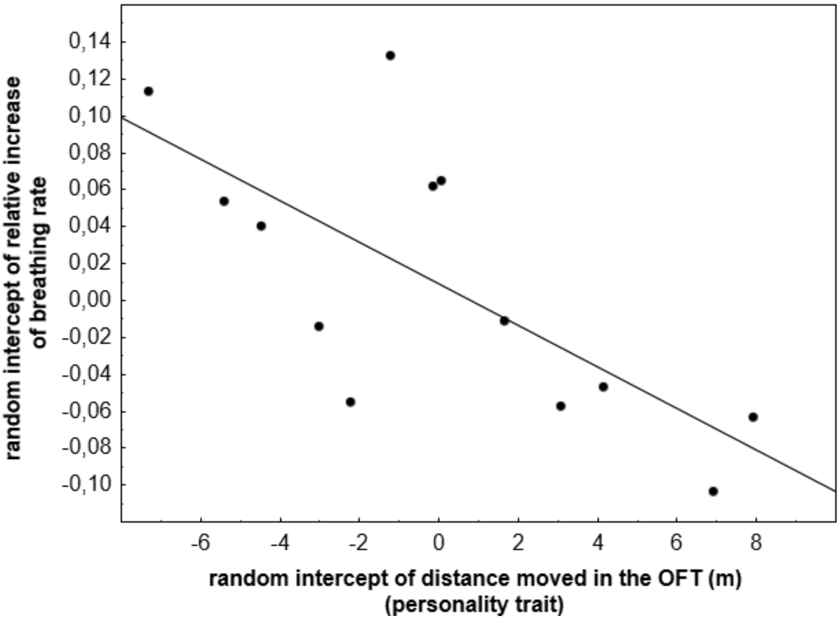

Fig. 4. Correlation between the random intercept of distance covered during the OFT and the relative increase of stress breathing rate during the stress test (r=–0.7069, p=0.0069). The longer distance a vole covers, the lower relative breathing rate increase is caused under stressful situations.

Table 3. Intercepts of random effects of LMM in one behavioural parameter considered personality trait and three physiological parameters

ID	sex	distance personality	log RBR	log SBR	breathing increase
5	♀	–1.2188	–0.0957	–0.0001	0.1324
47	♂	6.9250	0.0315	–0.0384	–0.1039
60	♂	–2.2169	0.0160	–0.0252	–0.0550
64	♀	–3.0250	0.0816	0.0771	–0.0142
71	♀	1.6490	0.0466	0.0404	–0.0115
72	♂	–7.3028	–0.0014	0.0911	0.1134
75	♂	0.0804	–0.0120	0.0455	0.0645
76	♀	–4.4826	–0.0261	–0.0001	0.0401
77	♀	3.0749	–0.0082	–0.0523	–0.0577
81	♀	4.1523	–0.0273	–0.0665	–0.0468
88	♀	–0.1572	0.0160	0.0614	0.0618
90	♀	–5.4174	–0.0180	0.0175	0.0537
92	♀	7.9390	0.0154	–0.0590	–0.0631
R±SE		**0.43±0.14**	**0.58±0.10**	**0.77±0.07**	**0.60±0.10**
p		<0.0001	<0.0001	<0.0001	<0.0001
r			0.1414	**–0.6889**	**–0.7069**
p			0.6450	0.0092	0.0069

RBR – resting breathing rate, SBR – stress breathing rate, log – logarithm of base 10, R – repeatability, SE – standard error, r – correlation coefficient, p – significance level, bold – significant value.

rate (r=–0.689, p=0.009, Fig. 3). The voles with a higher breathing rate under stressful conditions show a shorter movement trajectory in the OFT. Higher correlation was determined with the relative stress increase (r=– 0.707, p=0.007, Fig. 4). This shows that the longer the distance a vole covers in the OFT, the lower relative breathing rate increase is caused under stressful situations.

DISCUSSION

Determined values and stress increase of breathing rates

The values for RBR ranged from 100 to 210 min^{-1} with mean value 141.5 min^{-1} and median 140 min^{-1}. The determined values correspond with other published data. Breathing rate corresponds with metabolic rate and in mammals also to the weight of the animals (Crosfill & Widdicombe 1961). In mammals such as the cat, the respiratory rate ranges from 20 to 50 min^{-1} and in the dog from 10 to 40 min^{-1} (Kleinman & Radford 1964). The breathing rate of smaller mammals, such as mice, ranges from 80 to 200 min^{-1}, in hamsters and rats from 60 to 110 min^{-1}, in guinea pigs from 70 to 100 min^{-1} (Kleinman & Radford 1964) and laboratory mice about 200 min^{-1} (Sahai et al. 2021). In the pygmy white-toothed shrew, the average respiratory rate is around 661 min^{-1} (Jürgens et al. 1996). For the cinereus shrew (*Sorex cinereus* Kerr, 1792) weighing 3.4 g, it is 800 min^{-1} (Morrison et al. 1953 ex Calder 1968).

The values for SBR ranged from 170 to 290 min^{-1}. The percentage values of the stress increase ranged from 100% to 171%, whereas the mean value was 134.8% and the median 137.0%. The determined values correspond to the published data and approximately mirror common stress intensity. In mice, the mean breathing rate increased from 180 to 246 (137%) after a drug (acetazolamide) was injected (Sato et al. 2018). In injured rats, the mean values increased from about 110 min^{-1} to 230 min^{-1} (209%; Baran et al. 2020).

In mice, a changed air composition increased the breathing rate by about 150 % to over 200 % from basic breathing rate of about 150 min^{-1} (Receno et al. 2019). Tube restraint in mice changed the breathing rate from 220 to 300 min^{-1} (136%) and after bicuculline (plant alkaloid) injection from 250 to 380 min^{-1} (152%; Li et al. 2020).

Intraspecific variation and consistent individual differences

In the common vole, intraspecific variation of the breathing rate was found. Variation was found in all parameters, in resting breathing rate, stress breathing rate, and stress breathing relative increase. Study of intra- and inter-individual variations and their consistency of behavioral and physiological parameters, especially in mammals, has been given attention for a long time (Hayes & Jenkins 1997). As can be seen from the previous chapter, the observed intraspecific variation corresponds to that reported in the literature. The main sources of this variation could be caused by differences in sex and weight of the individuals. However, none of the mentioned factors had a significant influence on the observed breathing rate parameters (see Fig. 2 and Table 2). Although the breathing rate is a physiological parameters that contribute to maintaining the narrow range of homeostasis of the internal environment, under natural conditions, heterozygotes tend to be favored, especially for polymorphisms in natural populations (Parsons 1998). This finding is consistent with studies that have also found intraspecific variation in breathing rate in birds e.g., in great tit (Carere & van Oers 2004, Fučíková et al. 2009) or in zebra finch (David et al. 2012). The variation was the same or similar to that observed in the case of other physiological parameters (overview in Koolhaas et al. 1999).

We found that the resting breathing rate (R_C=0.58), and stress breathing rate (R_C=0.77), as well as, the percentage stress increase (R_C=0.60) have a high degree of repeatability at the level of the individual. This indicates that the observed intraspecific differences/variation are not the result of imprecise measurement or changing laboratory conditions, but rather a manifestation of the individual characteristics of the tested voles. This result is consistent with other studies demonstrating the consistent nature of individual differences in breathing rate values. A study on zebra finches reported high repeatability (R=0.66) in individual respiratory rate values (David et al. 2012). The repeatability of respiratory rate values in individuals may be related to the length of interval between individual measurements and their timing in the ontogeny of the monitored individuals, as follows from the study by Fučíková et al. (2009). There, the repeatability of the stress breathing rate in the great tit was measured, first at the age of 14 days and again at six months of age. The detected value (R=0.34) is about half compared to the values discussed above. However, as already mentioned, this difference is most likely caused by different experimental design. In our study and the study by David et al. (2012) the breathing rate of adult animals was measured. Fučíková et al. (2009) measured baseline values relatively shortly after hatching, and these can change significantly during maturation and reduce the repeatability value. For other physiological indicators of stress, similarly high values can be found in other studies. For example, the repeatability values of stress heart rate and corticosterone level at the individual level in the goose were R=0.84 and 0.77, respectively (Kralj-Fišer et al. 2007, 2010). For glucocorticoid metabolites, high repeatability was found also in rats (R=0.51) (Vobrubová et al. 2021). This is understandable because these parameters are related to the internal environment where high stability is required to maintain homeostasis. However, the repeatability of physiological parameters strongly depends on intervals between individual measurements and their duration to ensure stability (Ferrari et al. 2013, Karlíková et al. 2018). With behavioural expressions of stress responses, the situation is somewhat different, here the general repeatability is approximately 37% (Bell et al. 2009). In voles, depending on the specific behavioural parameter, the repeatability ranged between 20% to 63% (Lantová et al 2011, Herde & Eccard 2013, Urbánková et al. 2020).

Correlation of physiological and behavioural variation

Resting breathing rate (RBR) represented by random intercept (personality trait) was not related to the random intercept (personality trait) of the trajectory length traveled by the animal in the Open Field tests. The rationale may be that intraspecific differences in behaviour are most pronounced in stressful contexts (Koolhaas et al. 1994). In our case, this is in the OFT, where the animals are not in a resting state. At the inter-population level, this finding can also be supported by the results of a study conducted on the three-spined stickleback, where no differences were found in the basal breathing rate in populations living in environments with different predation pressure (Bell et al. 2010). Stress breathing rate (SBR) represented again by random intercept was negatively related to the random intercept of length of the trajectory traveled by the vole in the OFT. A similar trend was found in the percentage increase from RBR to SBR. Voles that reacted to the induced stress (manipulation and acoustic startle) with a greater increase in breathing rate displayed behaviour as anxious ("shy" personality type) in OFT. Their locomotor activity was reduced by the stress induced during exposure in the OFT. The obtained results point to the fact that stress seems to induce a more intense physiological response in individuals of the shy behavioural type, which in our study was manifested by a rapid increase in breathing frequency. We can find similar reactions in great tits, where breathing rate was significantly higher in "slow" individuals (analogy of the shy personality type) than in "fast" ("bold") birds (Carere et al. 2001, Carere & van Oers 2004). However, as already indicated in the introduction, the results of studies carried out so far focusing on the relationship between breathing rate and personality traits do not completely match. One such study is on zebra finches (David et al. 2012). The authors experimentally investigated to what extent handling stress was correlated with personality in female zebra finches, as assessed from a suite of repeatable behavioural traits. Although breathing rate was repeatable across individuals, it was not related to any behavioural trait, suggesting that it cannot be used to quickly predict personality, at least in zebra finches. The following study fits well into this seemingly contradictory picture too.

Fučíková et al. (2009) showed a completely opposite relationship, where fast great tits showed a higher stress breathing rate compared to slow individuals. This corresponds to Koolhaas et al. (1999) that formulated a more general view that proactive coping rodents show, in response to stressful stimulation, a low HPA-axis reactivity (low plasma corticosterone response), but high sympathetic reactivity (high levels of catecholamines). In contrast, reactive coping rodents show higher HPA axis reactivity and higher parasympathetic reactivity. However, it should be also noted that the activity of the sympathetic nervous system in general and the level of plasma noradrenaline, in particular, are connected with the metabolic and cardiovascular demands associated with physical activity, which could explain the positive correlation between proactive coping and sympathetic reactivity (Koolhaas et al. 2010, Kanitz et al. 2019). A similar correlation was also observed in the bank vole. Bolder and less flexible individuals had lower faecal glucocorticoid metabolite levels than shyer, more flexible individuals (see Mazza et al. 2019). This inconsistency may be caused, as already mentioned above, by a different experimental design. In our study common voles could mirror an emotional stress and not a physical one, because they were held in a small respiration box and soon after insertion displayed quite limited locomotor activity. It could therefore be thought that the original coping style concept effectively needs replacement for a new coping style model with two axes (Mazza et al 2019).

Despite the correlation of breathing rate with a personality trait was relatively high, a generalisation is highly restricted due to the low number of evaluated individuals. Besides correlation with higher number of behaviourally tested animals, also data on faeces pellets and urination produced during all tests should be involved in the analysis. However not only the quantity, but also the precise timing of voiding. The precise knowledge of timing is important also for the breathing rate increase because it is important to distinguish the fast response mediated above all by neural pathways and the reaction mediated by above all hormonal substances. These differences could be associated with personality traits as was mentioned above. The finding that breathing rate could indicate a personality trait would be very valuable, not only from the point of view of behavioural ecology, but also for other aspects. Undisputed advantages include the time-saving way in which individual values of this parameter could be obtained, but above all its non-invasiveness. This is beneficial not only from an ethical point of view, but also from a methodological point of view. So far, the only method that approximates the measurement of breathing rate with its advantages is determining the values of stress hormones from excrement (Vobrubová et al. 2021). In this case, however, it is always difficult to compare the results between individuals with regard to the difference in behaviour over a longer period of time, for which the faeces are analysed.

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SUPPLEMENT

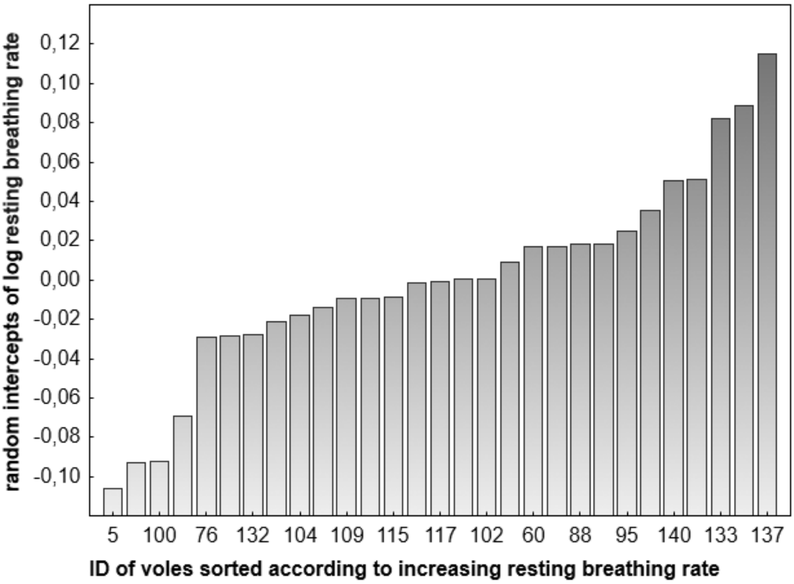

Fig. S1. Resting breathing rate (RBR, min^{-1}) was log-transformed and evaluated by linear mixed model (LMM). The bars show random intercepts that represent specific individuality traits of the measured physiological parameter. The figures are the IDs of the tested voles, n=29.

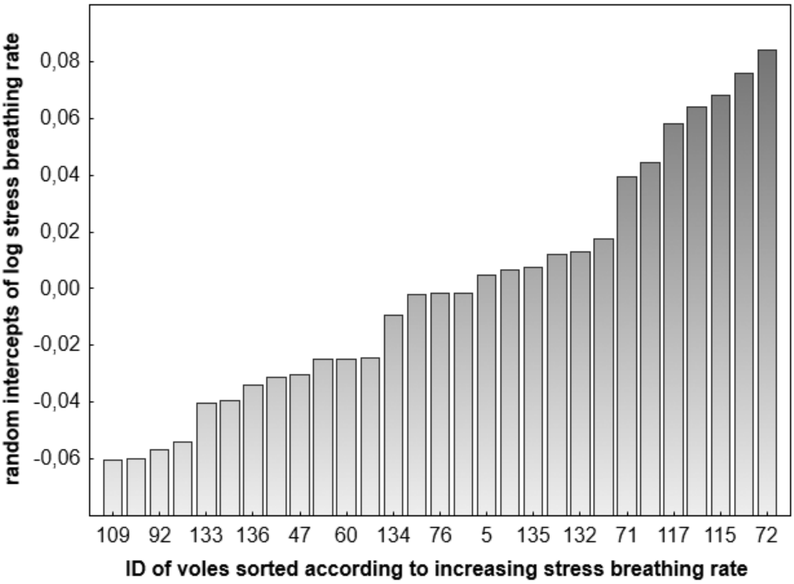

Fig. S2. Stress breathing rate (SBR, min^{-1}) was log-transformed and evaluated by linear mixed model (LMM). The bars show random intercepts that represent specific individuality traits of the measured physiological parameter. The figures are the IDs of the tested voles, n=29.

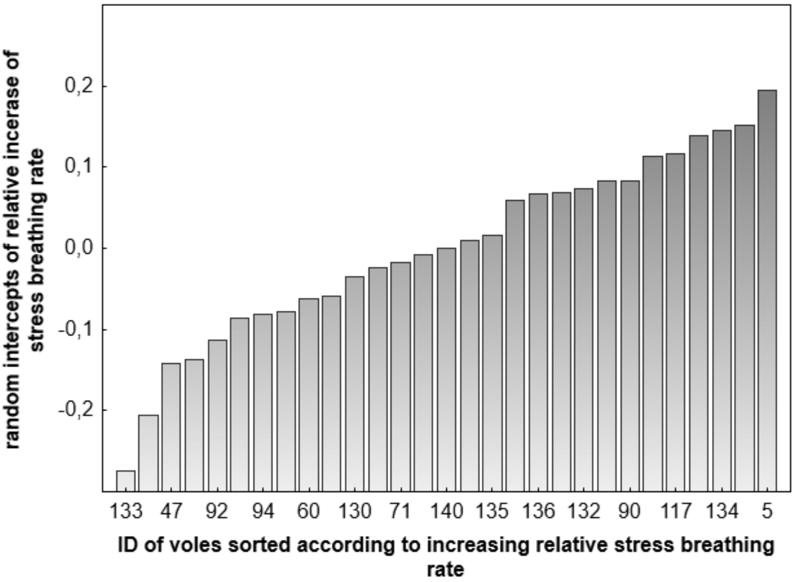

Fig. S3. Relative increase of stress breathing rate was evaluated by linear mixed model (LMM). The bars show random intercepts that represent specific individuality traits of the measured physiological parameter. The figures are the IDs of the tested voles, n=29.