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Personality in Guinae pigs *(Cavia aperae* f. *porcellus)* behaviour

Master thesis

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Declaration

I declare that this diploma thesis of Personality in guinea pigs *(Cavia apperea* f. *porcellus)* behaviour was elaborated independently and is based on my own knowledge, consultations with my supervisor and literary resources cited in attached bibliography.

In Prague, dated 17th of April 2014

Mgr. Hana Šimánková

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Abstract

In recent times, the field of personality research focusing on explaining differences in animal behaviour has attracted great interest. But there are still few studies about behavioural differences in domestic guinea pigs (*Cavia apperea* f. *porcellus*). The differences in animals' behaviour are not found only between different species or populations, but also among individuals within a given population. If individual differences in behaviour are constant over time and in various contexts, we find diverse personalities amongst the concerned animals.

The aim of my Master thesis has been to seek personality in domestic guinea pigs. This Master thesis has tried to examine relationships between different behavioural tests, and to describe guinea pigs' behaviour in particular tests from the personality set. The animals were tested in a series of behavioural tests focusing on activity, exploration, and anxiety.

The individual-specific responses to the novel environment corresponded to the animals' performance in the elevated plus-maze. The guinea pigs reacted consistently to two novel objects presented to them in their home boxes. The positive relationship between their approach latencies to novel objects and their house leaving latency in the isolation test was revealed. The center-leaving latency in the presence of the predator was negatively correlated with the parameters obtained from the novel environment test and the elevated plus-maze test. The positive correlation between total numbers of visited squares in two novel environment tests (valid for 15 individuals) was found. The guinea pigs did not differ in their vocal reaction to the predator. We detected almost no influence at all of sex on the animals' performance in the different tests from the personality set.

The obtained results indicate the presence of personality in domestic guinea pigs; the phenomenon, however, needs to be examined in more detail, especially focusing on correlations over time and on connections with physiological parameters.

Keywords: personality, guinea pig, exploration, activity, anxiety, predator, set of behavioural tests, correlation

Abstrakt

Zejména v poslední době se oblast výzkumu personality zaměřená na vysvětlení rozdílů v chování zvířat těší velkému zájmu. Stále však není mnoho studií, které by se zabývaly rozdíly v chování u domácích morčat (*Cavia apperea* f. *porcellus*). Rozdíly v chování zvířat nenacházíme pouze na úrovni druhů nebo populací, ale také mezi jedinci uvnitř populace. Pokud jsou rozdíly v chování konstantní v čase a v různých kontextech, pak u nich nacházíme různou personalitu (osobnost).

Cílem mé diplomové práce bylo hledání personality u domácích morčat. Tato diplomová práce se snažila zkoumat vztahy mezi různými behaviorálními testy a snažila se popsat chování morčat v jednotlivých testech z personalitní sady. Zvířata byla testována v sérii behaviorálních testů zaměřených na aktivitu, exploraci a anxietu.

Jednotlivé specifické reakce jedinců na nové prostředí odpovídaly výkonu ve vyvýšeném křížovém bludišti. Morčata reagovala konzistentně na dva nové předměty, které jim byly předloženy v jejich domovských boxech. Byl nalezen pozitivní vztah mezi latencemi přiblížení se k novým objektům a latencí opuštění domečku v izolačním testu. Latence opuštění středu v přítomnosti predátora byla negativně korelovaná s parametry získanými z testu na nové prostředí a z vyvýšeného křížového bludiště. Byla zjištěna pozitivní korelace mezi množstvím navštívených čtverců v obou testech na nové prostředí, které byly hodnoceny zvlášť pro 15 jedinců. Morčata se nelišila ve své vokální reakci na predátora. Pohlaví jedinců nemělo téměř žádný vliv na jejich výkonnost v jednotlivých testech z personalitní sady.

Získané výsledky z této práce naznačují přítomnost personality u domácích morčat, ale je zapotřebí výzkum ještě dokončit a získaná data vyšetřit podrobněji, zejména jejich korelaci v čase a jejich spojení s fyziologickými parametry.

Klíčová slova: personalita, morče, explorace, aktivita, anxieta, predátor, sada behaviorálních testů, korelace

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1 INTRODUCTION

1.1 Domestic Guinea Pigs (Cavia aperea f. porcellus)

Guinea pigs (*Cavia aperea* f. *porcellus*) are classified in Caviidae family (order Rodentia, suborder Hystricognathi). Guinea pigs are the domesticated form of the wild cavy (Cavia aperea). Guinea pigs have been bread for meat in South America for at least 3000 years. Its precise origin is unknown, but domestication probably occurred just once (Weir, 1974).

The guinea pig pups are precocious. They are born with hair, with eyes and ears open and with fully developed senses. They are able to locomote, thermoregulate and even feed on solid food from birth and they follow their mother (Blatteis, 1975). The gestation period of guinea pigs is about 65 days, especially males approaching sexual maturity around day 75 of age (Romand, 1971; Trillmich et al., 2006). In captivity, guinea pigs breed year-round, with birth peeks in spring (Rood, 1972). Females are polyestrous and a litter size ranges from two to five pups. The young are weaned at 14 - 28 days with weight about 100 g (Rood, 1972; Harkness and Wagner, 1995).

Wild cavies generally associate in small groups (usually consisting of 5-10 individuals), but in a favorable habitat a number of such groups may converge in a large colony (Rood, 1972). Wild cavies occur in a wide variety of habitats – open grasslands, forest edges, swaps, rocky areas and sometimes at mountains up to 4200 m. The diet consists of many kinds of vegetation (Rood, 1972; Grzimek, 2003).

1.2 Personality in Animals

Animals and humans show consistent differences in aggressiveness, shyness, sociability, activity, reactions to stress and environmental factors and other behavioural traits as well as in physiology. These individual differences are unique for each individual. Animal personalities have been documented in a wide variety of animal species (included also invertebrates) (reviewed in Gosling, 2001). Studying of animals' individual differences, animals' personalities have attracted increasing interest from animal behaviour researchers over the past decades. Personality has been studied from a lot of points of view – its relationship with physiological parameters (e.g. Koolhaas et al., 1999; Carere and van Oers, 2004, Cockrem, 2007), reproductive success (e.g. Both et al., 2005;, Dingemanse et al., 2004), cognitive skills (e.g. Dugatkin and Alfieri, 2003; Carere et al. 2003; Sneddon, 2003; Guenther et al., 2013), survival in different environments (e.g. Dingemanse et al., 2004), susceptibility to diseases (e.g. Bolhuis et al., 2003; Murison and Skjerve, 1992) and much more.

Different authors have used variable terms to describe identified individual variability in behaviour of animals such as personality (Gosling and John, 1999; Dall et al., 2004) and other terms that are considered to be interchangeable with term “personality“ such as temperament (French, 1993; Gosling, 2001), individuality (Stevenson-Hinde, 1978 in Gosling and John, 1999), emotionality (Gray, 1965 in Gosling, 2001), behavioral syndrom (Sih et al., 2004), coping style or coping strategy (Koolhaas et al., 1999; Benus et al, 1991), behavioural strategy (Verbeek et al., 1994) or behavioural phenotype (Guenther and Trillmich, 2013). In many cases, scientists preferred to use different names than personality to prevent of being accused from anthropomorphism. For greater clarity, I will throughout the whole master thesis use uniformly the term personality.

But what we can understand under the term animal’s personality? There in no one definition of personality which could be acceptable for researchers of different studying branches (e.g. psychologist, behavioural ecologists,.). Therefore are rather chosen definitions with broader meaning. Personality can be defined as consistent individual differences in behaviour – such as aggressiveness, shyness, sociability and activity which persist over time (within or across generations) and/or across different contexts (Dall et al., 2004). From this definition is important to highlighted the possibility (and/or) of correlation between behaviour across different contexts. It is connected with developmental plasticity. Other broader definition of personality refers to a stable inter-individual variability in behavioural organisation within a particular population (Uher, 2008). Pervin and John (1997) defined the personality as specific characteristics of individual that desribe and explain consistent patterns of feeling, thinking and behaving.

Temperament reflects how individuals react to novel or challenging situations (Wilson et al., 1994). Animals with different temperament differ in their reactions to novel situations, predator avoidance, investment in reproduction and behaviour in a variety of social contexts (Réale, 2000). In a human psychology, temperament is defined as inherited, early appearing tendencies that continue throughout life and serve as the foundation for personality (Wilson et al., 1994).

Behavioural syndrom is a complex of correlated behaviours across situations - in the same context but in different situations (e.g. feeding in environments with different predator pressure), or different contexts in different situations (e.g. aggression towards conspecifics in the absence of predators versus feeding activity in the presence of predators). A population or species can exhibit a behavioural syndrome when each individual showing a behavioural type (e.g.more bold or more shy, or more active vs.more passive type) (Sih et al., 2004).

Coping style is characterized as coherent set of behavioural and physiological stress responses that is consistent over time. The coping style is characteristic to a certain group of individuals (Koolhaas et al., 1999).

There are two possible points of view on the concept of personality. Animals with different personality characteristics could be divided into groups - personality types. Usually they are divided into 2 types – with extreme characteristics in evaluated behavioural traits. Sometimes also intermediate type is involved. We can find different names for this two behavioural types which usually reflecting the focus of tested parameters.

Proactive vs. reactive types are mainly divided according animals' reaction to a stressful stimuli (aggressive stimuli included) which reflecting their emotionality. Proactive individuals seem to be more aggressive and bold and also form routines easily. Their exploration of new environment is active. Compared with reactive individuals, they behave cautiously to external stimuli and respond better to changing environments. Their behaviour is more flexible. Proactive and reactive personality types have been documented in rats (*Rattus norvegius*) (Koolhaas et al., 1999; Benus et al. 1991) pigs (*Sus scrofa domestica*) (Hessing et al., 1994) or in farm mink (*Mustela vison*) (Malmkvist and Hansen, 2002) etc.

Personality types bold and shy reflect animals' willingness to risk. Bold individuals risk more, are more active and also in general disperse more. Shy types are typically more cautious and hesitate for a longer time (Wilson et al., 1994; Réale et al., 2000). Bold and shy types have been reported for example in pumpkinseed sunfish (*Lepomis gibbosus*) (Coleman and Wilson, 1998), poeciliid (*Brachyraphis episcopi*) (Brown a Braithwaite, 2005), and bighorn sheep (*Ovis canadensis*) (Réale et al., 2000).

The criterion for division of animals in slow explorers and fast explorers is their explorative behaviour. Usually, it is studied how animals react towards novel objects and unknown environments. Fast explorers quickly explore novel environments and also hesitate for shorter period to explore novel objects placed in their home environment. They are more aggressive and readily form routines. Exploration of slow explorers is slower, but more thorough. They show a lower level of aggression, quickly adjust their behaviour to environmental changes and better cope with defeat (Verbeek et al. 1994; 1996; Marchetti and Drent 2000, Drent et al., 2003, van Oers et al, 2005). Fast and slow explorers have been found mainly in birds – in family paridae: great tits (*Parus major*) (Verbeek et al., 1994, Drent et al., 2003, van Oers et al, 2005) and blue tits (*Cyanistes caeruleus*) (Ježová, 2008; Herborn et al., 2010) but also in other animals group – e.g. convict cichlids (*Amatitlania nigrofasciata)* (Jones and Godin, 2010).

Sitters and rovers are classified according to the distance that the fruit flies (*Drosophila melanogaster*) move away from a food resource after feeding on it. Sitters spend more time circling around the food after feeding. By contrast, rovers fly away from it. Each of this type is favoured in environments with different distribution of food sources (Pereira et al., 1993; Sokolowski et al.,1997).

When looking carefully at behavioural traits connected with described personality types (see above) it is possible to find some similar characteristics between some of them. I summarized these common behavioural traits for each of personality type in a Table 1. I used collective term "Fast" as an equivalent for proactive, bold, fast explorers and rovers and "Slow" for reactive, shy, slow explorers and sitters.

Parameter	1.type: **Fast** (proactive, bold, fast explorers, rovers)	2.type: **Slow** (reactive, shy, slow explorer, sitters)
Latency to approach novelty	↓	↑
Exploration	fast, superficial	slow, thorough
Risk-taking behaviour	↑	↓
Aggressiveness	↑	↓
Activity	↑	↓
Routine formation	↑	↓
Stress response	↓	↑
Coping behaviour	↑	↓
Dealing with defeat	↓	↑
Flexibility	↓	↑

Table 1: The comparison of different behavioural parameters in 2 personality types (Fast and. Slow).

Other possible expression of the personality is a conception of continuum. This continuum displays behavioural variation in humans and animals on axis. Characteristics of individual are “distributed” along this axis with extremes in measured parameters on both ends. Traditionally, it is used the term “shy-bold continuum” which was established according individuals propensity to take risks. Individuals differ in their average degree of shyness and boldness (Wilson et al., 1994).

Personalities are genetically determinated (i.e., personality traits are heritable) (Drent et al., 2003), but there is a growing evidence for the influence of the early physical and social environment in shaping individual differences in the personality. Such influences may begin prenatally (e.g. nutritional or endocrine conditions) or postnatally (e.g. influenced by the presence of siblings). Huge effects of ecological circumstances and interspecific interaction were also found (e.g. unstable environment, different distribution of resources or predation pressure). Early developmental environment affected personalities due to the strong selection pressures. Animals with different personalities differed in a surviving or in a reproduction rate. Youngs need to be adapted to different niches than adults, but such adaptations often disappeared later in life. These findings refer to a developmental plasticity of personality traits (Trillmich and Hudson, 2011).

1.3 Personality in Guinea Pigs

This chapter is mainly based on recent articles which are directly related to personality in domestic guinea pigs (Zipser et al., 2013) and wild cavies (Guenther and Trillmich, 2013; Guenther et al., 2013; Guenther et al., 2014).

1.3.1 Domestic Guinea Pigs (Cavia aperea f.porcellus)

There is a lack of studies focused on individual differences in domestic guinea pigs. One of the first complex studies concentrated on domestic guinea pigs is the research of Zipser and co-workers (2013). They studied dimensions of personalities in domestic guinea pigs (in adult males). They concentrated mainly on emotionality, social behaviour and cortisol-stress reactivity which represented dimensions of animal personalities. These domains could be also use to investigate consistencies in different contexts. Dominance status was also examined. Animals were tested in the set of tests consisted of an open field test, a dark-light test, a step-down test (3 tests for emotionality evaluating), a male/female interaction test (which reflected a social behaviour) and cortisol reactivity test (measured from blood samples). Animals were held in colonies and they were tested when reached minimum of 6 months (=adulthood). Experiments were repeated after 8 weeks which allowed them to examine stability of personality traits over time.

The open field was represented by a square enclosure (1 x 1 m) with a floor divided into 16 virtual squares (a=25 cm). Evaluated parameters were virtual squares crossed per time (exploration) and time percentage spent in the central part of enclosure (anxiety-like behaviour). In the wooden dark-light box, animals could explore the illuminated part and also the dark part. Measured behavioural patterns were a first latency to leave the dark section of box and a total time spent in the light area (anxiety-like behaviour) and a number of times the individual entered the light area (exploration). In the step-down test, the enclosure was fit up with the tower (23.5 cm high) and the animal was put on a platform of this tower. Recorded parameter was the latency to step down the platform and reach the floor (risk-taking behaviour). Social behaviour was tested when a single tested male interacted with oestrus females. Unknown females from different colony were placed in the males' enclosure. Evaluated parameters were frequencies of courtship and sexual behaviour. The latency to show sexual and courtship behaviour and also latency to mount were measured. The experiment was repeated with different females. In the cortisol-reactivity test, the cortisol concentrations in plasma titres from blood samples were measured. Three blood samples were collected – first sample when the animal was caught from its home box and other two samples after placing the animal in an unknown enclosure. There exists a relationship between a facing to novelty and a cortisol level. Animal which is put into an unknown environment (acting as a stressor) shows increasing cortisol level in blood (De Boer et al., 1990; Hennessy et al., 2006).

Zipser et al. (2013) were the first to prove that the concept of animal personality is applicable to domestic guinea pigs. They pointed to the fact that it is not important to study only behaviour itself, but also to take into account physiological parameters in a personality research. In their study, the cortisol reactivity and social behaviour were stable over time (Figure 1). The male dominance status also did not change over 8 weeks. But authors did not find any correlations among emotionality during two testing phases. None of tested parameters (3 domains of emotionality, social behaviour and cortisol reactivity) were correlated. They argued that although no identified stability of behaviour and stress reactivity across different context was found in this study, it did not necessarily mean that correlations across different contexts do not exist. It could be explained by fact that correlations of behavioural traits across context can be presented only during some phases in ontogeny, or in certain environmental situations or in certain animal populations (Sih et al., 2004). No individual consistency over time in domestic guinea pigs could be also explained by changing selection pressures during domestication (Price, 1984).

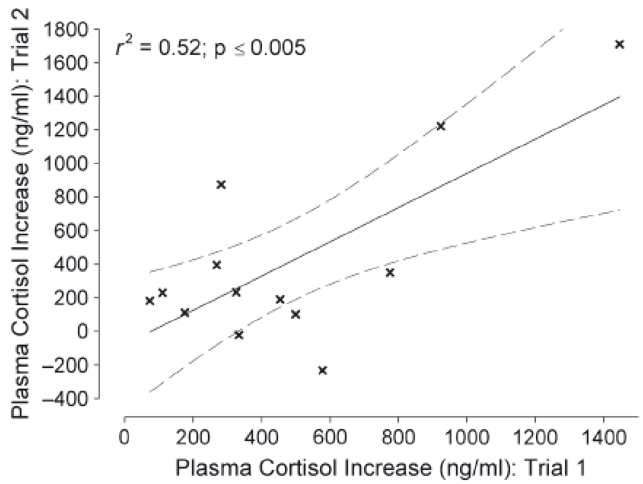

Figure 1: The significant correlation over time (8 weeks) in domestic guinea pigs. Plasma cortisol response after exposure to an unfamiliar environment in the cortisol-reactivity test (showing initial value to a maximal reaction value). X´s –single individuals (n=13). (Zipser et al., 2013).

1.3.2 Wild Guinea Pigs (cavies) *(Cavia aperea)*

Personality was also studied and found in wild guinea pigs (*Cavia aperea*). Guenther and Trillmich (2013) studied relationships between behavioural and physiological parameters and ontogenetic stages in wild cavies. Their study investigated the influence of ecological conditions (changing prenatal photoperiod - simulating spring or autumn) on developing personality (phenotype). They found strong influence of prenatal photoperiod on personality and stress response.

Wild cavies were involved in research focused on testing behavioural and physiological parameters. Three different behavioural tests were used in this study. The open field test (OF) was divided into two parts. In the first part the animal was placed in the center of arena under the shelter. It could voluntarily leave and explore the arena. In the second part, the shelter was removed and the animal was forced to explore the arena for other 10 minutes. Measured parameters were the exploring distance, the number of touched marked cells on the floor and the shelter leaving latency (in the case that the animal left the shelter during the first part of the experiment). The long field test (LF) was used to observe voluntary exploration in an apparatus consisting of a standard housing enclosure with added corridor (5-m long) and two light sensors. The latency to start exploration and the number of trips in the corridor were recorded. The last experiment of the behavioural set was the novel object test (NO). Three different objects were presented to a single animal in its home box during ontogeny. First object was a 4-cm big green egg cup introduced in enclosure of 20-day-old juveniles, second object was a yellow plastic duck (8 cm) used at maturation and a last presented object represented by a red plastic pig (10 cm) in adults. The object was placed in the home box for 60 minutes. Recorded patterns were the latency to leave the shelter, the latency to contact with novel object and the number of contacts with the object within 15 minutes after the animal first left the shelter. The physiological test involved collecting blood samples and measuring of cortisol concentration in the novel environment.

For personality (behavioural phenotype) evaluating were chosen 3 variables – the latency to start exploration in the long field test, the distance explored in the open field test and the latency to contact with the novel object - which were the most distinguishing variables between animals. The consistency of results was compared between 3 different ontogenetic stages (juvenile, mature and adult). Different results were obtained in various ages. A significant repeatability was found in two out of three behavioural tests in juveniles. Juveniles that started early exploring in the long field test, also reached more distance in the open field test. And also the number of trips made in the long field test correlated with the distance explored in the open field test. These two behavioural tests did not correlate with the novel object test. Both the latency to start exploration in the long field test and the distance explored in the open field test were consistent over six months. The latency to interact with novel object was not repeatable. So the emotionality was repeatable over time. This is opposite to results obtained in study on domestic guinea pigs where no consistency over time was found regarding emotionality (Zipser et al., 2013.) Results in charts of behavioural traits obtained from 3 tests in 3 different ontogenetic stages are shown in Figure 2. In mature as well as in adult animals no correlations between any of the variables were found.

Strong effect of ecological circumstances (in this case prenatal photoperiod) on personality development of wild cavies was proved. Heavy females which were born in autumn (poorer conditions) were less explorative and more shy whereas spring (in good conditions) born heavy females developed more explorative and bolder personality types –they explored the corridor in long distance test more and also were more explorative in the arena in the open field test. Interestingly, all males and also females with lower weight did not differ in their personality types.

Results revealed a high developmental plasticity in the personality. Individual behavioural differences were linked to differences in the predicted life history of animals. Repeatable early personality traits changed over different ontogenetic stages. Animals changed their personality type in response to the photoperiod they experienced during ontogeny. These personality traits completely disappeared in adult animals. It could be explained by the fact that animals live under different selection pressure during their life. The selection pressure acts most strongly in juveniles (Guenher and Trillmich, 2013). Sachser et al. (2011) found that brain structure and physiological processes change during development and parts of brain related to anxiety behaviour fully develop only in adolescence. Developing of this structure could also affect personalities of animals.

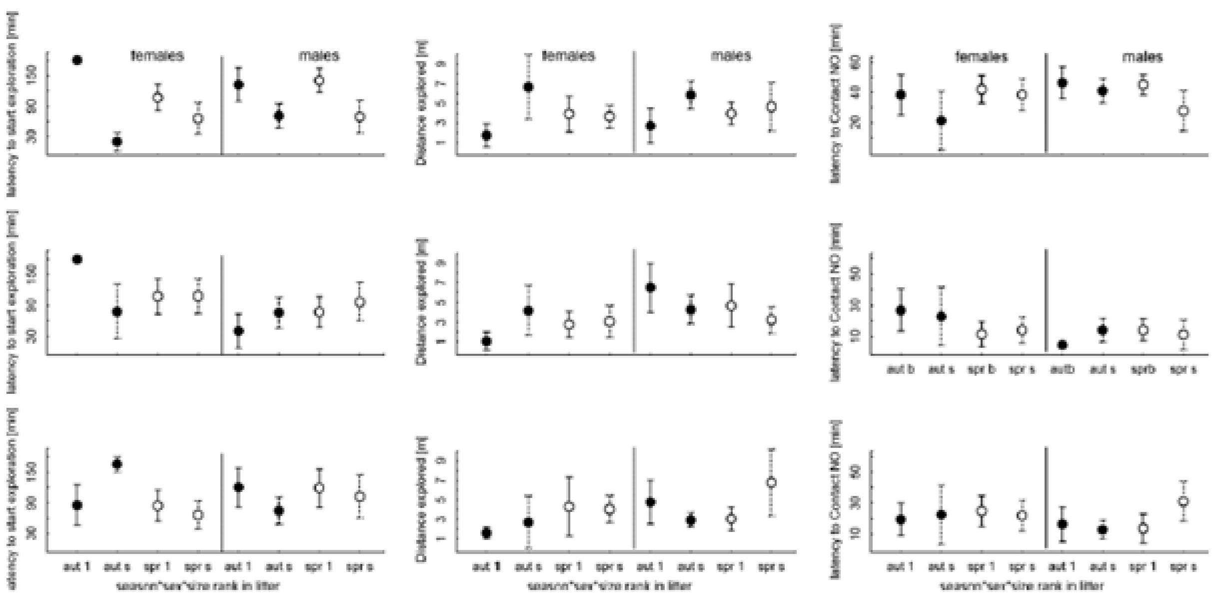

Figure 2: Different behavioural traits among 3 life stages. The upper row shows behavioural responses of juveniles toward the LF test (left), the OF test (middle) and NO test (right). The middle row displays the same behavioural traits in sexually mature animals and bottom row shows adults. Aut=autumn, spr=spring, 1=heaviest juvenile in litter, 2-5=smaller juveniles in litter. (Guenher and Trillmich, 2013).

A following work in the field of the ontogeny of personality is study that highlighted the importance of studying environmental factors which influence animals during early ontogeny – they could adaptively shape their behaviour and physiology. Guenther et al. (2014) studied developmental plasticity of personality traits - how the personality of wild cavies *(Cavia aperea)* developed when exposed to increasing and decreasing photoperiod before and after birth. Notable plasticity in the development of personality traits was found and also a relatively high degree of temporal consistency in most traits. The basal cortisol level, the resting metabolic rate and the fearlessness were stably correlated across two different ontogenetic stages and these correlations became tighter over time. On the other hand some correlations disappeared over time or were presented only after maturation. Strong correlation was found between exploration and basal cortisol level and between boldness and resting metabolic rate, but these correlations changed with time, they disappeared in mature animals. Conversely, two behavioural traits – exploration and boldness were correlated only in mature animals (Guenher et al.2014).

Learning performances were another topic studied in relation with personality in wild guinea pigs. Guenther et al. (2013) found a relationship between individual differences in personality and individual differences in cognitive performances. They examined 3 personality traits – boldness, activity, and aggressiveness and their correlations with individual learning, associative learning speed, and behavioural flexibility (assessed by reversal learning). In the association learning task, animals learned to discriminate between three cylinders with different symbols according to which they find a reward. The animal reached the learning criterion when chose rewarded cylinder correctly in eight out of ten consecutive trials. For the reversal learning, one of the former unrewarded symbols was chosen as the new rewarded symbol. The same learning criterion was used to measure success in this task.

Activity was measured as all locomotive behaviour (a change of body position in space) for period of 5 minutes during the first week of the initial association learning. The boldness was scored as a reaction to novel objects (a yellow plastic duck and a smaller green egg cup) presented in the testing apparatus. Measured parameter was the latency until the cavy first touched the novel object. Aggressiveness of tested individuals was assessed during two confrontations with two unfamiliar cavies – a very shy male and the bold one. The number of aggressive interactions was scored.

The aggressiveness was negatively correlated with the activity and positively correlated with the boldness. There was a positive relationship between activity and boldness. Boldness, activity, and aggressiveness were significantly repeatable over time. The strong positive relationships between three personality traits and the learning speed were found. More active and aggressive cavies which also touched a novel object faster, performed better in the associative learning task. The behavioural flexibility was negatively correlated with the aggressiveness. In the reversal learning, the less aggressive cavies were faster to reach the learning criterion. There was no correlation between activity, boldness, and reversal learning speed.

The social environment of pregnant and lactating wild cavies can shape the behavioural profiles as well as the endocrine development of their youngs. Females that had lived in a stable social environment during pregnancy and lactation had sons that displayed higher amounts of aggressive and attentive behaviour and they were more socially interactive. Sons whose mother had lived in an unstable social environment during pregnancy and lactation showed infantilized behavioural profiles with a higher amounts of play behaviour, lower amounts of aggressive behaviour and delayed gonadal development. Delayed gonadal and behavioural development in sons of mother from unstable environment could represent an adaptive mechanism for wild male cavies in social instable environment. Their behaviour ("behaviour camouflage strategy") might decrease the risk of fights and injuries until these males have gained full adult body weight. The pregnant and lactating females might shape the behavioural and endocrine profiles of their offspring via hormonal and/or behavioural mechanisms according the social situation they experienced during pregnancy and lactation. And thanks to that, their youngs can be more successful in current social environment (Siegeler et al., 2011). The infantilization of sons of mother from unstable social environment was also found in domestic guinea pigs (Kaiser and Sachser, 2001).

Data based on personality research in guinea pigs acquired over last nearly years confirmed that we are able to find individual differences in wild cavies as well as in domestic guinea pigs. Both, the criterions of stability of personality traits over time and also between different contexts not be necessarily reach to prove existence of personality, because the developmental plasticity plays important role on creating personality during ontogeny. Some acquired differences in personality traits could be found only in some developmental stages. It was proved that it is essential to measure physiological parameters together with behavioural traits when studying personality (Zipser et al., 2013). Results obtained from research of the relationship between the personality and learning abilities supported the hypothesis that cognitive types reflect personality types (Guenther et al., 2013).

1.4 Personality Tests Used in Rodents Studies

1.4.1 Novel Environment Test (Open Field Test) (NE)

The open field is one of the most commonly used apparatus in many behavioural studies. It was introduced by Hall (1934 in Gosling, 2001). This test was originally designed to provide a standardized index of emotionality in rats and mice. This index was based mainly on measuring of an animal's level of activity and its rate of defecation. At first, the apparatus consisted of a flat elevated platform.

In this test, the animal is placed into a new unfamiliar environment from which escape is barred and its reactions are observed, usually for 5-15 minutes. Typically, the open field consists of enclosed arena (of different sizes) with floor divided into network of squares (inner squares in the center of the arena and outer squares) (Figure 3). Between measured parameters belong presence or absence of defecation and urination, number of entered inner and outer squares, proportion of time spent in the center of arena and by walls (thigmotaxis), proportion of activity and passivity, monitoring the trajectory of movements, number of jumps, amount of rearings, latency to leave the first square, latency to enter into an unknown environment (in the case of free exploration) (Hall, 1934 in Gosling 2001; Simon et al. 1994; Archer, 1973; Eilam, 2003; Eilam, 2004; Gerano and Schmidek, 2000; Takahashi et al., 2005).

Different researchers argued what this test is really focused on. Typically, the open field is considered to be an exploratory test. An overall level of locomotion and time spent in the center of the arena are considered to be measures of exploratory behaviour. Sometimes it is difficult to distinguish which behaviour elements belong to exploration and which to activity (Brown and Nemes, 2008). But in conditions with using a light in a testing room, the increased light itself and also the open space are very stressful for animals (results reflecting emotional responds of individuals). And in this case, the locomotor activity in the open field reflects more attempts to escape from an open space than exploratory activity itself (Gerano and Schmidek, 2000). When changing the light condition – testing in the dark – increased exploration and decreased defecation and urination are observed (Archer, 1973). Other authors find it problematic to evaluate the open field test as exploratory in the fact, that placing animals into unfamiliar environment does not allow them to exhibit their motivation to explore an unknown open space and that this test evokes a strong anxiety response (Birke and Sadler, 1986; Renner, 1990 ex. Brown and Nemes, 2008).

There are two possible realization of this test – to put the animal into an empty unknown arena (= forced exploration) or to allow the animal to access from a known into an unknown environment (= free exploration). During the free exploration animals have a free choice between two or more environments and at least one of these environments is familiar for them. The open field with added shelter represents the free exploration (Welker, 1957; Brown and Nemes, 2008; Hughes, 1997).

When animals are placed into the arena in the open field test, they show a series of behaviours such as sniffing, running, rearing and grooming. Stretching (stretch-attend) is usually exhibited at the beginning of the test and it is connected with risk-taking behaviour and approach-avoid conflict. During the test this behaviour is decreasing and rearing and grooming are increasing (Takahashi et al., 2006). Rearing has been viewed as a behaviour expressed when animals habituate to the environment (Vadasz et al., 1992). Thigmotaxis also gradually decreases during the open field test. If the decline does not occur, this indicates a fear of open spaces (Simon et al., 1994).

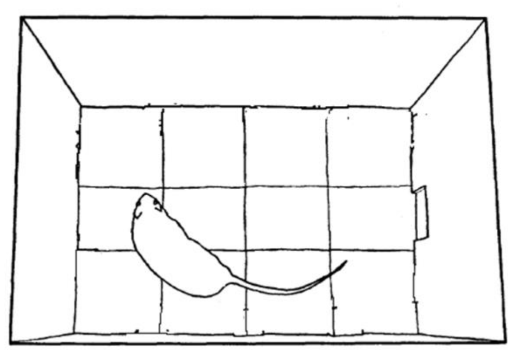

Figure 3: The novel environment test (Welker, 1957).

1.4.2 Hole Board Test

The hole board test is in a broad meaning a variation on open field test. It measures responses of an animal to an unfamiliar environment. But we can find big differences between them. The first difference is that in the hole board test, the flour has holes in it (in open field test, lines divided the floor into a network of squares). The second difference is that each of these tests focused on different behavioural traits. The hole board test is originally designed to investigate explorative motivation in rodents. This apparatus was first introduced by Boissier and Simon (1962) to distinguish between exploration and overall animal's activity. The first design of the hole-board test consisted of an elevated squared desk with 16 equally spaced holes of 3 cm in diameter. Different new object were placed under the desk (Boissier and Simon, 1962; Huges, 1997).

The hole board apparatus is generally consisted of an enclosed arena with wooden or plastic plate at the bottom with a variable number of holes in the ground, where animal can insert its head (Figure 4). This behaviour is called "head-dipping" and it could reflect exploratory behaviour independent on activity (Boissier and Simon, 1962; Huges, 1997; Abel, 1995; Fernandes et al., 1999; Brown and Nemes, 2008). Direct exploration (or neophilia) is reflected by the frequency and duration of a head-dipping. This measurement is supposed to be independent from the general locomotor activity (File and Wardill, 1975; Ljungberg and Ungerstedt, 1976). Results obtained from the hole-board test can be interpreted in the case of high levels of a head-dipping as neophilia or low anxiety level and in an opposite situation, when low levels of a head-dipping are evaluated as lack of neophilia or reflecting a high anxiety conditions (Takeda et al., 1998).

File and Wardill (1975) modified the number of holes in the hole-board test. They used only 4 holes, instead of 16. The main reason was to reduce the high density of holes which did not allow animals to move without coming in contact with any hole. This was problematic, because it was unable to discriminate between exploration and activity. They concluded that the apparatus with 16 holes seemed to provide only a measurement of general activity and that the 4-hole version gave information about directed exploration.

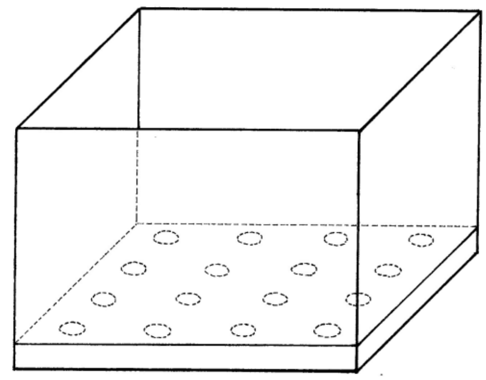

Figure 4: The hole board test.(Müllerová, 2010).

1.4.3 Light/Dark Box Test

The light/dark box test is based on the innate aversion of rodents to brightly illuminated areas. It also reflects their spontaneous exploratory behaviour in response to a novel environment and a light (mild stressors) (Crawley and Goodwin, 1980; Bourin and Hascoët, 2003). It is typically consisted of two parts – a small part: one third of this box is the dark compartment (regarded as a safe area) and a large part: two thirds of the box represented by the light compartment (illuminated aversive area). The opening between the two compartments is small, usually not more than 7 cm. Sometimes, a tunnel between compartments is used (Belzung et al., 1994). In this experiment is evaluated the number of transitions between the light and dark compartments reported as an index of activity-exploration. The aversion could be measured as a proportion of time spent in each part of the box (Belzung et al., 1987). The latency of the first passage from the light compartment to the dark one and sometimes the rearing activity are also reported (Hascoët and Bourin, 1998). Hascoët and Bourin (1998) find as the best way how to express results the percentages of time spent in each compartment and also the movements (exploratory behaviour) measured in both parts. Results could be affected by inner factors such as strain of animals, age, weight, or by external factors like conditions in laboratory (Bourin and Hascoët, 2003). The light/dark box can be modified in many ways. One modification can be changing the size of the compartments (Young and Johnson, 1991; Belzung et al., 1994, Malmberg-Aiello et al., 2002), duration of the test (for example recording for 10 minutes (Young and Johnson, 1991), for 5 minutes (Hascoët et al., 1999) or for 3 minutes (Shimada et al., 1995)), also the appearance of the apparatus could differ (a corridor-type runway with outer and inner walls used in work of Shimada et al. (1995)).

1.4.4 Elevated Plus-maze (EPM)

The elevated plus-maze is an apparatus which is widely used to evaluate rodents' anxiety. It is based on the natural aversion of rodents of height and open spaces. In this test, the animal is placed at the center of the elevated plus-maze with its head facing away from the observer. The apparatus consists of four elevated arms –two of them are open and they are orthogonal to other two which are enclosed by walls. The arms are separated by a central square. The maze is raised off the ground (Figure 5). The open arms combine elements of unfamiliarity, openness and elevation. The animal is allowed to explore freely this apparatus. The standard length of this experiment is 5 minutes. The number of entries to any of the arms and also the duration of the stay in each arm are recorded during the experiment.

The main evaluated parameters are entries to open arms (expressed in percentages) and percentages of time spent on the open arms. The percentages are counted from all entries to open and closed arms and from the total time spent on both types of arms. These measured values express the anxiety. The total number of entries to closed arms reflects motor activity (Lister, 1987; File, 2001). The time spent in the central square with sitting or cautious exploration (a head poking) reflects a decision making and/or risk assessment behaviour (Rodgers and Johnson, 1995; Trullas et al., 1991). Other information obtained from analysis of behaviour in elevated plus-maze test can be scanning (investigating/exploring the edges of arms) (Cruz et al., 1994), immobility (Pellow et al., 1985) and stretch-attend posture (Wall and Messier, 2000).

Design of the elevated plus-maze is based on the research in rodents (Montgomery, 1955). His research focused on a relationship between a fear and an exploration activity. He did not use the model with two open and two enclosed arms in his studies, but he used Y shaped maze with different number of arms. He found that rodents preferred closed arms to open ones and that they were more active in closed, protected areas. This preference was independent on the ratio of the number of closed and open arms (Montgomery, 1955). Why do animals prefer closed arms to open ones? Reasons for that are not clear. File (2001) summarized that main factor for avoiding open arms could be the fear from new, open and elevated spaces. Other reason is the impossibility to stay close to walls (thigmotaxis) (Treit et al., 1993). But in the contrary to this finding, rats (*Rattus norvegicus*) still avoid open arms even though they are enclosed by transparent plexiglass which can allow them to stay close to walls (thigmotaxis). Certain is that the open space represents for animals an aversive stimulus. Other design of elevated maze with four arms in X shape was used in work of Handley and Mithani (1984). The current appearance of the maze in plus shape was modified by Pellow et al. (1985) who studied rats.

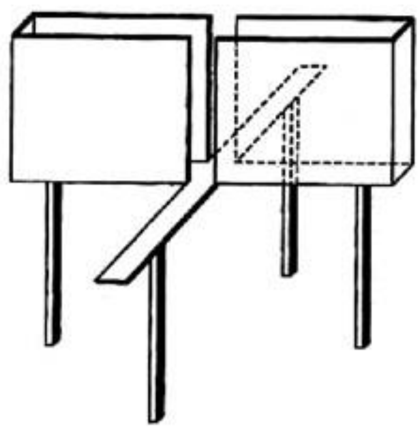

Figure 5: The elevated plus-maze (Müllerová, 2010).

1.4.5 Elevated T-maze (ETM)

The elevated T-maze is a modification of the elevated plus-maze with only one closed arm by lateral walls introduced by Graeff and co-workers (Graeff et al, 1993).This test focused on separation of conditioned from unconditioned fear. The device has one closed and 2 open arms of equal dimensions elevated from the floor. Open arms are joined by a central square to the closed arm (Figure 6). The animal is placed at the distal end of the enclosed arm and open arms are invisible from this part. The anima has to poke its head beyond the wall of closed arm to see whole apparatus. Leaving the enclosed area represents for an animal a negative experience because of open space and heights (Pellow et al., 1985). When the experiment is repeated, animals stop leaving the close arm (protected area). This behaviour could be interpreted as unconditioned learned fear based on negative experience. The latency to leave the enclosed area is evaluated as an inhibitory avoidance. Placing the animal at the end of open arms results in the escaping reaction (Graef et al, 1993; Graef et al, 1998; Zangrossi and Graeff, 1997; 1994 Viana et al, 1994). This test is mainly used in studies of anxiolytic compounds and anxiety neurobiology (Sena et al., 2003).

Not only the elevated plus-maze or the elevated T-maze but also other types of maze, for example the radial maze with different number of arms (Olton and Samuelson, 1976) are used in a personality research (Lantová et al., 2011).

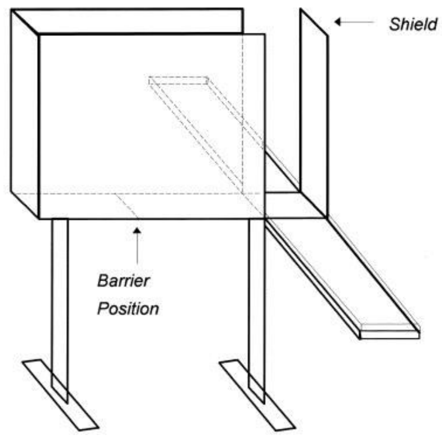

Figure 6: The elevated T-maze (Zangrossi and Graeff, 1997).

1.4.6 Novel object test

The novel object test is focused on reaction to a novel, non-food object. Paying attention to new stimuli is really essential for responding to a constantly changing environment (Berlyne, 1960). Reactions to novelty cause an approach–avoidance conflict. This is a conflict between exploration which is essential for surviving (in case of food finding etc.) and fear of novelty manifesting as an avoidance of potentially threatening situations (Montgomery, 1955; Berlyne, 1960; Powell et al., 2004). New objects are usually placed into a home cage (Figure 7), known environment for animals or it could be placed in the open field.

Roy and Chapillon (2004) compared different behavioural tests and concluded that the novel object test is a good tool for distinction of emotional processes from general activity of animal. The exploration of novel objects together with the risk assessment could give information about the emotional reactivity. Typical first rodent´s reaction after presenting a novel object is approaching, sniffing and quick contact with the object. This behaviour also refers to a mild exploratory activity. For some animals is also typical observing a second reaction – play-like behaviour following the first exploratory reaction. Between playing behaviour occurred traits such as moving around the object, holding the objects in mouth or biting the object. Reactions to similar objects are weaker after repeated presentations (Kim et al., 2005). There are many external factors that influence approach and exploration of new objects. Some of these include a manipulation with animal by an experimenter and a degree of familiarity with the experimenter (previous handling),a prior exposure to a novel environment, a degree of familiarity with the object (Powell et al.,

2004) or environment complexity (an environmental enrichment affects habituation to novelty) (Zimmermann et al., 2001). Animals consider a known object when is placed at a novel position as a new object (Besheer and Bevins, 2000).

Different types of novel objects were presented to rodents – e.g. a small cube wrapped with paper in mice (Kim et al., 2005), wood blocks, a white PVC pipe, rolls of cotton, balls of tin foil and hard plastic PEZ dispensers in rats (Lewis et al., 2003), differently coloured Mega Bloks® pieces in mice (Powell et al., 2004), a clear smooth glass water, a blue smooth metal rectangular can, a set of yellow and red smooth plastic squares, a blue grooved plastic toy barrel in mice, a white cardboard box and or a grey smooth plastic rectangle with a hole in the center (Heyser and Chemero, 2012), a white cylinder in mice (Kazlauckas et al., 2005), an egg green cup, a yellow plastic duck, a red plastic pig in wild guinea pigs (Guenther and Trillmich, 2013).

Figure 7: The exploration of a novel object (a plastic duck).

1.4.7 Concentric Square Field (CSF)

The concentric square field represents a multivariate test in which a tested animal can freely choose which of different environments wants to explore. The complex testing arena consists of differently elevated spaces, ground floor zones, sheltered and open areas, differently lightening regions, enriched places to explore and also a hole-board device (Figure 8). The main goal of this apparatus is to bring the experimental setting closer to the natural conditions and try to model situations which might animals meet in their natural habitat. This complex test enables to measure activity, explorative behaviour, avoidance of open areas and also risk-taking behaviour which can give together a behavioural profile of the animal. It was designed for studying of an exploring activity and a risk-taking behaviour. In some cases, it is better to use this one complex test rather than battery of behavioural tests, where the experience obtained from previous experiments can influence results in following tests. This test enables to analyze behavioural traits depending on each other and also their interaction (Meyerson et al., 2006, Ohl et al., 2001b, Roman et al., 2007)

. Other test focused on measuring of more complex behaviour traits is a modified hole board test. The modified hole board test combines classical hole board test and the open field test. Observed behavioural parameters are exploration, anxiety, excitement, intake and inhibition of food and also locomotor activity which together give information about behavioural dimensions in animals (Ohl et al., 2001a, 2001b)

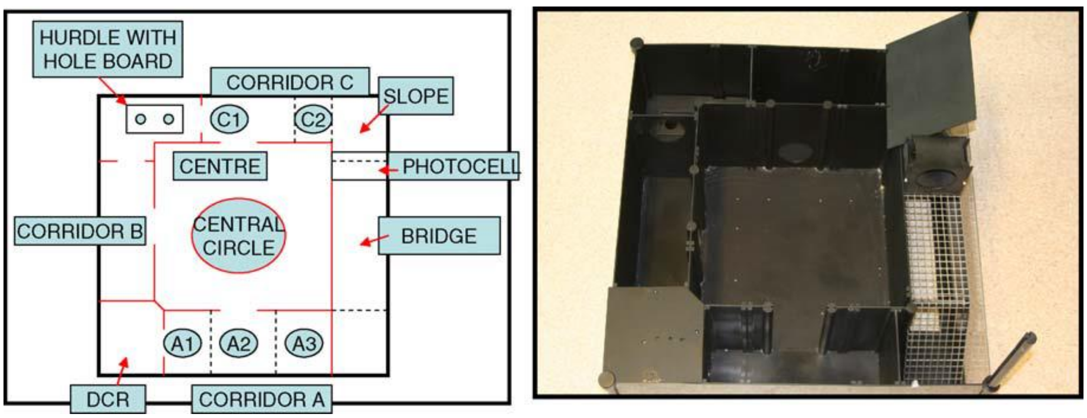

Figure 8: The concentric square field (Meyerson et al., 2006).

1.4.8 Tail Suspension Test (TST)

The review of Cryan et al. (2005) started with a quote of Mark Twain: “A man who carries a cat by the tail learns something he can learn in no other way.” This quote describes very well the main purposes of the tail suspension test. Scientists started to use this test mainly to detect and describe antidepressant activity in mice. We can get a lot of information about way how can tested animals cope with an inescapable stressful situation such as suspending an animal by its tail. The principle of TST is to hold an animal by its tail (manually or mechanically) (Figure 9) in an uncontrollable fashion for a short time and observing its reaction. Typical reaction is after initial escape-oriented movements to develop an immobile posture. The recorded parameter is the total time spent immobile in comparison to time of active movements. It could be measured manually or through an automated device (Steru et al., 1985). The advantage of an automated apparatus is assessing other values such as a power of movement and an energy of movement (Steru et al., 1987; Porsolt et al., 1987). Because we observe animal's behaviour under stress situations and we are able to find different responses such as immobile freezing or actively trying of escaping, this test could also be possibly used for evaluating different animals personality (Cryan et al., 2005; Kazlauckas et al., 2005).

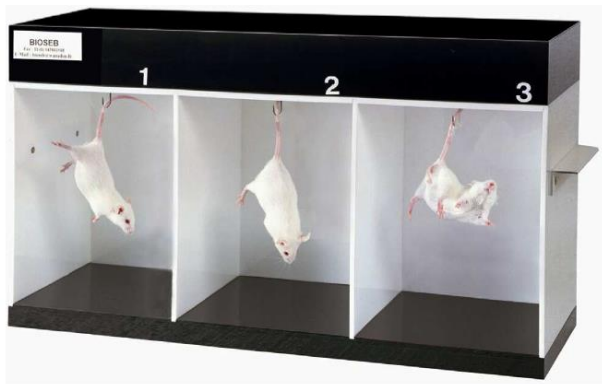

Figure 9: The automated tail suspension test apparatus (Cryan et al., 2005).

1.4.9 Suok test (ST)

The suok test (also called "ropewalking") and its modification a light/dark suok test (LDST) (Figure 10) were introduced by Kalueff et al. (2005). This test measures the fear of heights and instability and the fear from novelty. The light/dark ST modification represents an aversion to brightly lit environment. It combines principles of several rodent behavioural tests based on anxiety such as the light/dark box, the open field and the elevated maze. It is based on exposure to a long elevated horizontal metal stripe (usually 2-3 m long) – a rod (tested in mice) (Kalueff and Tuohimaa, 2005) or to an alley (in case of rats) (Kalueff et al., 2005). ST provides information about behavioural characterization in rodents, including assessment of their anxiety, activity, and neurological phenotypes subsequently rated as high and low anxiety types. Typical for high anxiety types is higher motor incoordination measured by the number of falls and missteps. This test could also distinguish between different types of anxiety - light-induced, socially-induced and a drugs-induced. The suok test was named after a brave little ropewalker girl in Yury Karlovich Olesha's novel "The three fat men" (Kalueff et al., 2005).

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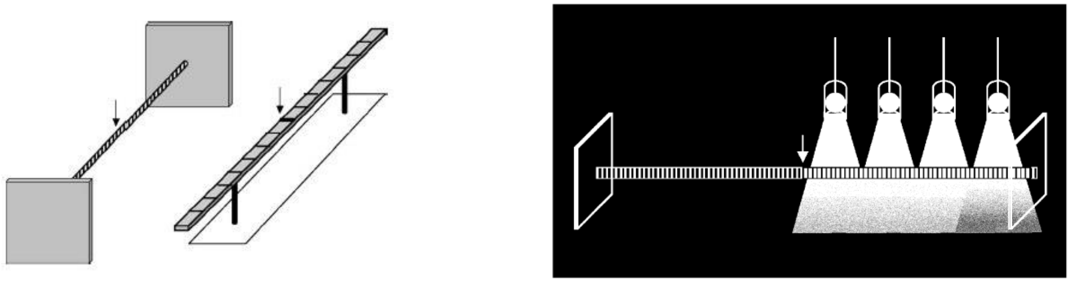

Figure 10: The suok test (ST) and its light-dark modification (LDST) (Kalueff et al., 2005).

1.4.10 Step Down Test

The step down system is based on the step-down scheme in which an animal is dropped on an elevated platform. The latency to step down the platform is measured as a reflection of risk-taking behaviour. The latency is measured as the time when the animal placed all four paws on the floor of the enclosure (Zipser et al., 2013).

1.4.11 Other Tests

There are other more tests which have been used in different studies focused on individual variability in rodents. I just shortly mention some of them. The personality research in rodents is in many studies concentrated on measuring aggressive behaviour. The individual level of aggressive behaviour is strongly related to the way that animals react to various other environmental challenges (Koolhaas et al., 1999). According to the attack latency, the animals were divided into SAL type (Short Attack Latency) and LAL type (Long Attack Latency) (De Ruiter et al.,1992; Van Oortmerssen and Bakker, 1981; Benus et al., 1992). Proactive and reactive types in rodents were determined according their strategy in a defensive burying test. The defensive burying test allows the animal a choice between proactive and reactive coping, when proactive individuals try to actively bury the harmful and noxious objects, when reactive individuals become immobile and freeze (Sgoifo et al., 1996; Koolhaas et al., 1999; Sluyter et al., 1996; Wada and Makino, 1997; De Boer and Koolhaas, 2003). Aggressive and social behaviours were tested also by using the reflection in the mirror (mirror-image stimulation). Observed parameters were interactions between animals and their reflections in the mirror. Measured factors were the approach latency, the avoidance behaviour and the sociability. Animals with low scores in second and third factor were considered as aggressive (Armitage, 1986a, b, Armitage and van Vuren, 2003; Blumstein et al., 2006).

2 AIMS OF THE THESIS

The main objective of this thesis was to determine, if it is possible to find personality, individual variability in domestic guinea pigs (*Cavia aperea* f. *porcellus*) based on the tested personality set. Aims of this study were to use different behavioural tests that were processed to identify personality in different animal species and also added tests that are not usually used in this context and to answer questions such as:

1. Do the domestic guinea pigs differ in their explorative activity, anxiety-like behaviour and stress response?
2. Which of these tests are suitable for determining personality in domestic guinea pigs and which not?
3. What is the relationship between different tests used in this study?
4. Is there any influence of sex on measured parameters?

Hypotheses

H1: I hypothesized that domestic guinea pigs will differ in their exploratory behaviour, activity, anxiety and stress response. So we are able to distinguish more explorative, more active and more stress resistant animals and also animals which will be less explorative and active and also will strongly react to stress stimuli as revealed by Dellu et al. (1992) in rats, Kazlauckas et al. (2005) in mice, Guenther and Trillmich (2013) in wild cavies.

H2: I hypothesized that at least some of tested behavioural traits will be correlated - between different contexts (and/or over a short time period) (Sih et al. 2004; Réale et al. 2007, Guenther and Trillmich, 2013).

H3: I do not expect that males and females will considerably differ in tested behavioural parameters because of their low age.

3 MATERIALS AND METHODS

3.1 Animals

Twenty-four juveniles of guinea pigs *(Cavia aperea* f. *porcellus)* - 13 males and 11 females – were involved in this Master thesis. Guinea pigs of the breeding stock ID number 2176003 from breeder Lukáš Sobota from Městec Králové were used for the experiments. The age of the tested animals in the time of their transfer to the Demonstrational and Experimental Stable varied between 7-10weeks. The minimum age of the tested animals was 10-11 weeks.

The animals were housed in plastic boxes- enclosures (57 cm x 37 cm x 20 cm) with a wire mesh top (Figure 11). They were housed in standard conditions, woodchips being used for flooring. They also had a water bottle and hay that served as food and also as shelter. They were kept in groups of 3-4 members (Figure 12). The animals got used to the composition of the new group and also to the conditions in the Demonstrational and Experimental Stable, where all the experiments were held, and this with a minimum of 3 weeks before the start of the first experiment.

Their diet consisted of hay, vegetables (carrot, cucumber) and fruit (apples), dried bread and pellets for rodents with an addition of vitamin C (BIOSTAN). The guinea pigs were provided with vegetables, fruit and pellets once a day during the morning hours. Drinking water and hay were provided *ad libitum*. Housing light conditions were set according to the outdoor photo-period. The temperature was maintained at 19 °C, humidity was about 60%.

All the tested animals were transferred to new breeders after the last experiment from behavioural set was carried out.

Figure 11: The home box.

Figure 12: The group of guinea pigs (4 females).

3.2 Demonstrational and Experimental Stable

All experiments were carried out in the Demonstrational and Experimental Stable– in the Experimental section (Figure 13) and all the tested guinea pigs were kept there during the whole research period. The Demonstrational and Experimental Stable are part of the Demonstrational and Experimental Center. This workplace center was founded January 1st, 2011.

The Demonstrational and Experimental Stable (N 50°7', E 14°22') are located in the area of The Czech University of Life Sciences in Prague (Figure 13). These accredited Stable (No. 58176/2013-MZE-17214) are divided into the demonstrational section where livestock, the laboratory, and the exotic animals are presented to students and the public. The important role of the demonstrational part is education, and many subjects are taught in a lecture room. The second part of this complex – the experimental part – serve mainly the purposes of different physiological, nutritional and ethological research projects. The Demonstrational and Experimental Stable were established in 1998 and reopened after reconstruction in 2013**.**

Figure 13: The Demonstrational and Experimental Stable.

3.3 Equipment

Vocal reactions to predator were recorded on Marantz Intros PMD620 Portable Media Recorder (Figure 14) in wav files (sample rate: 44.1 kHz, 16 or 24-bit resolution, frequency response: 20 Hz to 22.000 Hz ±1.0 dB; range > 87 dB). All experiments were recorded on camera Sony Everio.

Figure 14: The Marantz Intros PMD620 Portable Media Recorder used in the predator test.

3.4 The Sequence of Tests:

The personality set of experiments consisted of the isolation test, the novel environment test (the open field test) – the forced exploration (repeated in some animals – 15 individuals), the novel object test (which was repeated with three new and different plastic objects), the elevated plus-maze test and the predator test. The basic set of experiments was divided into 3 days. The first day, the animal was separated from its group and its reactions were recorded in the isolation test. The second day the novel object was put into the home enclosure of the animal during the morning hours (the first novel object test with the pink ball) and the novel environment test was performed during the afternoon. The third day, the novel object test was repeated with the yellow plastic duck and the elevated plus-maze was also performed. After testing all individuals, the predator test and the third novel object test with the orange plastic pig were carried out. Approximately 1 month after performing the basic set, the novel environment test was repeated with 15 individuals. All experiments of this thesis were realized under authority of the Project of experimentation.

3.5 The Personality Set of Tests:

3.5.1 Isolation Test

Before the start of the personality set of experiments, the animals were housed in groups of 3-4 individuals of the same sex for a minimum of 3 weeks. For an individual implementation of experiments it was necessary to separate the tested animals from their group. To reduce the stress from isolation, we housed the other separated animals in the same room. In this test we observed the animals' first reactions after isolation. The guinea pig was placed into a new plastic enclosure of the same size as its home box, but enriched by a plastic house (23 x 15 x 26 cm) and a small pile of hay close to the plastic house (i.e., a protected area). On the opposite site of the box we placed food (pellets) in the corner (i.e., the open area) (Figure 15). The guinea pig was placed in the plastic house at the beginning of the experiment (Figure 15). We measured the first house-leaving latency and the proportion of time spent in the open and in the protected area. We also evaluated time spent feeding in the open area and the number of rearings in a time span of 30 minutes. The house leaving latency was scored from the whole duration of the video recording (60 minutes). If the animal did not leave the house, it was given a score of 3700s. The isolation test was the first test from the personality set.

Figure 15: The plastic box used in the isolation test and the detail of the plastic house.

3.5.2 Novel Environment Test (Open Field Test) - Forced Exploration

The exploration tendencies of animals placed into a new, unknown environment,was observed in the novel environment test. The open field test was used to evaluate particular behavioural traits in guinea pigs (Tobach and Gold, 1966; Porter et al., 1973).

This experiment was recorded in a plastic transparent arena about the size of 60 x 45 x 43 cm (Figure 16). The floor was divided into 12 squares with a side of 15 cm. These squares allowed me to evaluate the animals' activity in open space. I differentiated the inner squares (two squares in the central part of the arena) and the outer squares (ten squares along the walls). The experiment started with the launch of the animal into the center of the arena. The novel environment was carried out in daylight.

I recorded the selected behavioural traits in the Observer XT 8.0 program (see Table 2) for a time phase of 60 minutes. I also counted the number of inner and outer squares visited by the animals and the latency of the first square visited, apart from the central part of the arena, and the number of boluses (excrements) and urinations. This experiment was repeated with fifteen guinea pigs approximately 1 month after the first personality set experiment. The duration of the second experiment was 30 minutes. When comparing both novel environment tests, the first ten minutes and also the 30 minutes of both tests were used for analysis.

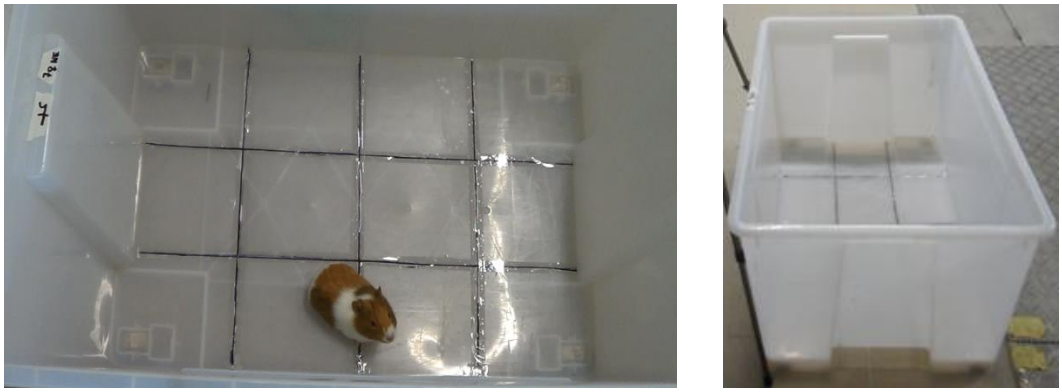

Figure 16: Pictures of plastic arena used in the novel environment test (open field test).

Recording of selected behavioural traits
Exploration and locomotion: (walking and running around the arena)
Sitting, resting
Defecation (presence or absence of boluses and urinations)
Rearings (standing on the hindlimbs with or without touching the wall)
Jumping
Grooming

Table 2: Behavioural parameters evaluated in the novel environment test.

3.5.3 Novel Object Test

The novel object test is used to measure animals' reactions to novel (non-food) objects in a known environment (home enclosure). We observed animals' behavioural reactions to three different objects. The first object that we used was a plastic pink ball (5 cm in diameter) (Figure 17). We placed it into the guinea pig's home cage. This experiment was recorded for a period of one hour to give animals enough time to decide whether they wanted to explore the new object or not. The first ten minutes of the video recording were evaluated in detail for particular behavioural traits (Table 2). If the animals did not explore and get close to the pink ball, their approach latency was 3700 s.

The second tested object was a yellow plastic duck (6 cm in diameter) (Figure 17). I placed it in the same position as the pink ball. This test followed the day after the first novel object test and its duration was 30 minutes. The behavioural parameters were measured in the whole time span of 30 minutes. The maximal approach latency was 1900 s.

The last object – the orange plastic pig (11 cm in diameter) (Figure 17) was used in a different context – in a group context. I offered it to the group of guinea pigs in which its members were used to being in and I observed their reactions to this object, and also the interactions (biting and chasing) among the group members, for a time period of 10 minutes.

Selected behavioural traits (see Table 3) were recorded in the Observer XT 8.0 programme. For further analysis I selected some of them (the first approach latency, the duration of interest in the novel object, the total time spent biting and manipulating the novel object and the number of rearings).

To be able to compare these three tests, the first ten minutes were separately evaluated from each test and also the maximum latency was the same for all the three tests (700 s). This comparison allowed me to examine the short-time correlation between all the novel object tests.

Figure 17: Three different objects used in novel object tests.

Recording of selected behavioural traits
Lack of interest
Observing and sitting close to the object (10 cm and closer)
Manipulating and biting the object (active exploration)
Feeding
Sitting and resting
Grooming
Rearings (standing on the hindlimbs with or without touching the wall)

Table 3: Behavioural parameters evaluated in the novel object tests.

3.5.4 Elevated Plus-Maze

The last experiment from the basic personality set was the elevated plus-maze test which measured the relationship between fear (of heights and open space) and exploration activity. A guinea pig was placed in the central square of the elevated plus-maze with 2 open and 2 closed arms. The arms of the apparatus (50 cm x 10 cm) were raised 50 cm above the ground; the dimensions of the central part were 10 x 10 cm. This apparatus was made from plywood (Figure 18). The animal had 30 minutes to explore the tested device. I measured the behavioural traits recorded in Table 4. After the end of any experiment the apparatus was cleaned with a spirit (ethanol) to prevent any behaviour influence caused by the odour of another individual.

Figure 18: The apparatus used in the elevated plus-maze test.

Recording of selected behavioural traits
Entries to closed arms
Entries to open arms
Center-leaving latency
Central square crossing (yes/no)
Time spent in closed arms
Time spent in open arms
Time spent in central part of maze
Rearings (standing on the hindlimbs with or without touching the wall)
Grooming
Sitting and resting
Head poking (sitting in central square, head directed to the open arm)

Table 4: Behavioural parameters evaluated in the elevated plus-maze.

3.5.5 Predator Test

This test was focused on risk-taking behaviour in the presence of a predator. A guinea pig was placed in the center of a glass aquarium (39 x 52 x 50 cm) (Figure 19). The bottom of the aquarium was covered with paper divided into 12 squares (13 x 13 cm). I observed the animal's behaviour in the presence of a predator –- a female dog (*Canis lupus f. familiaris*) – a Border Collie for a period of 2 minutes. The predator was running around the aquarium and was also barking on command of its breeder - Bc. Veronika Strnadová. I recorded the behavioural reactions, such as the number of squares visited, the center-leaving latency (the latency of the first square visited outside the center) and the acoustic reaction to the dog on a Marantz Intros PMD620 Portable Media Recorder (Figure 14). The predator test was realized with the help of Ing. Aneta Baklová and Bc. Veronika Strnadová. Ing. Aneta Baklová was responsible for the vocal analysis of the sounds recordings and Bc. Veronika Strnadová for the manipulation with her dog.

Figure 19: The testing apparatus (a glass aquaruim) used in the predator test.

3.6 Data analysis

3.6.1 Acoustical Analysis

The recorded call was quantified by bioacoustical software Avisoft-SASLab Pro, version 5.1.0 (Avisoft Bioacoustics, 2010). The sound was visualized as spectrogram of the following parameters: FFT length: 512; frame size: 100%; window: Hann; bandwidth: 129 Hz; frequency resolution: 86 Hz; overlap: 87.5%.

3.6.2 Statistical Data Analysis

Statistical data analyses were done in software STATISTICA 10.1 (StatSoft, Tulsa) and CANOCO (Version 4.5). Data was considered as significant when $p < 0.05$ and highly significant when $p < 0,01$. For all tests, a significance level (alfa) of 0.05 was selected. Videotapes were digitised and subsequently evaluated using the behavioural observation and analysis programme The Observer XT 8.0.

All data were tested for normal distribution by descriptive analysis of histograms and application of the one sample Kolmogorov-Smirnov test. In cases in which criteria for normal distribution were not met the corresponding data set was transformed (log10) to achieve normal distribution. If this still did not result in normally distributed data, nonparametric statistics were used.

For correlations analyses between tested parameters, Spearman Rank Correlations were used for non-normally distributed data. Correlation matrices were used to correlate more variables with each other in the novel environment test - numbers of total squares visited in the first 10-, 30- and 60-minute periods).

To demonstrate the differences between males and females in all personality tests I used non-parametric (Mann-Whitney U test) or parametric statistics (One-way ANOVA). To compare three approach latencies to novel objects was used non-parametric Friedman ANOVA.

The relationships (correlations) between parameters obtained from individual tests from personality set were tested by PCA analyses. The tested variables are shown in a Table 5.

TotSqNE	Total number of visited squares (Novel environment test)
OutSqNE	First square visited outside the central area (Novel environment test)
HouLeaIZ	First house-leaving latency (Isolation test)
OutTimeIZ	Time spent in open parts of enclosure (Isolation test)
OutSqPRE	First square visited out of the center in the presence of the predator (Predator test)
SqNuPRE	Number of squares visited in the presence of the predator (Predator test)
ApNO1	First approach latency to the novel object no. 1 –the pink ball (Novel object test 1)
IntNO1	Total time spent interesting in the novel object no.1 - the pink ball (Novel object test 1)
ApNO2	First approach latency to the novel object no. 2 – the yellow duck (Novel object test 2)
IntNO2	Total time spent interesting in the novel object no. 2 - the yellow duck (Novel object test 2)
ApNO3	First approach latency to the novel object no. 3 – the orange pig (Novel object test 3)
IntNO3	Total time spent interesting in the novel object no. 3 - the orange pig (Novel object test 3)
ArmsEPM	Total number of visited arms (Elevated plus-maze)
CenCroEPM	Crossing the central part of the maze (1) or not (0) (Elevated plus-maze)
DungsNE	Number of boluses (Novel environment test)
UrineNE	Number of urines (Novel environment test)
TotSqNE2	Total number of visited squares (Novel environment test 2)
Weight	Body mass

Table 5: Variables (and their shortages) used in the PCA analysis.

4 RESULTS

4.1 Isolation Test

The isolation test, when guinea pigs were separated from their group and placed under the plastic house, was recorded for a time period of one hour to give the animals enough time to leave the shelter. Only 5 out of 24 animals did not leave the house during that period. The guinea pigs which left the house earlier spent more time in the open parts of the plastic box (Spearman Correlations: R_S= -0.680, p=0.0003). The animals which spent more time in the open space also spent more time feeding (Spearman Correlations: R_S =0.915, p<0.00001) (Figure 20). The guinea pigs which left the protected area (house) earlier also had a higher rearing activity (Spearman Correlations: R_S= - 0.589, p=0.0025).

In comparison with the time spent in the open space, the females spent more time out of the protected area, but those results were just barely significant (One-way ANOVA: F=4.232, Df= 1, p=0.0517) (Figure 21). The house-leaving latency (Mann-Whitney U Test: U=49.5, N1=11, N2=13, p=0.213) and the rearing activity (U=68.0, N1=11, N2=13, p=0.862) did not differ between males and females.

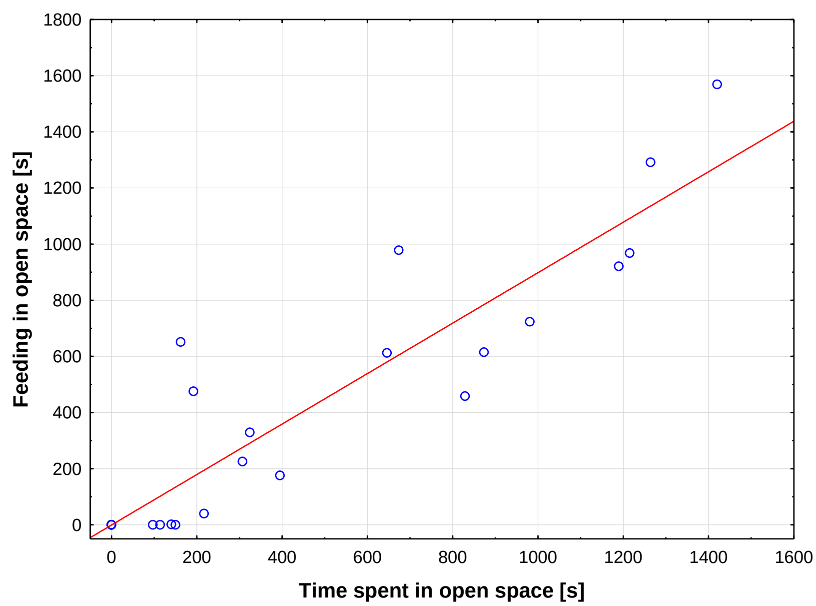

Figure 20: The correlation between the total time spent in the open area and the time spent feeding in the open space in the isolation test.

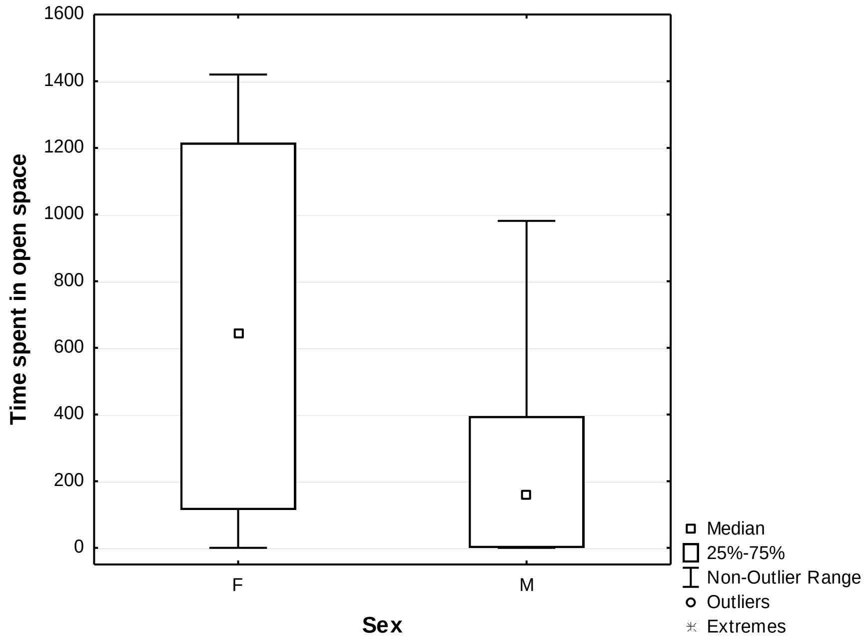

Figure 21: The influence of sex on the time spent in the open space in the isolation test. F-females, M-males.

4.2 Novel Environment Test

The novel environment test was recorded for a one hour period and its results were evaluated separately for time periods of 10, 30 and 60 minutes. The number of explored squares ranged from 1 to 436 in the maximum duration of the test (60 minutes), from 1 to 164 in 30 minutes, and rose from 1 to 112 in the first ten minutes. The numbers of total squares visited were positively correlated with the first 10-, 30- and 60-minute periods (ANNEX 1). We selected a data-set from the first 30 minutes of the whole video-recording for further analysis. The amount of visited squares varied considerably between guinea pigs. In 30 minutes, only one animal visited just one square (i.e., stayed immobile), 15 animals visited between 2 to 10 squares, and 4 out of the 24 animals were really active and visited more than 100 squares. Jumping occurred only in 3 animals during the experiment.

Guinea pigs that visited more squares also went more often to the central part of the arena (Spearman Correlations.: R_S=0.762, p<0,0001) (Figure 22).. Animals which explored more squares also made more rearings (Spearman Correlations: R_S=0.682, p=0.0002) (Figure 23). In the case of the more explorative animals we measured a higher amount of boluses (Spearman

Correlations: R_S=0.632, p= 0.0009), but the number of urines did not correlate with the number of visited squares (Spearman Correlations: R_S=0.303, p=0.150).

When comparing the first center-leaving latency and the total number of squares visited during the first ten minutes, the results were negatively correlated (Spearman Correlation: R_S= -0.462, p=0.023). The guinea pigs which left the center earlier also moved around more in the arena and visited more inner and outer squares. But this relationship disappeared when I compared the total number of visited squares in 30 minutes with the center-leaving latency (R_S= -0.283, p=0.197).

The number of visited squares (Mann-Whitney U Test: U=67.5, N1=11, N2=13, p=0.839) and also the center-leaving latency (U=50.0, N1=11, N2=13, p=0.213) were independent of sex. The number of rearings (U=64.5, N1=11, N2=13, p=0.706) or boluses (U=53.0, N1=11, N2=13, p=0.297) did not differ between sexes.

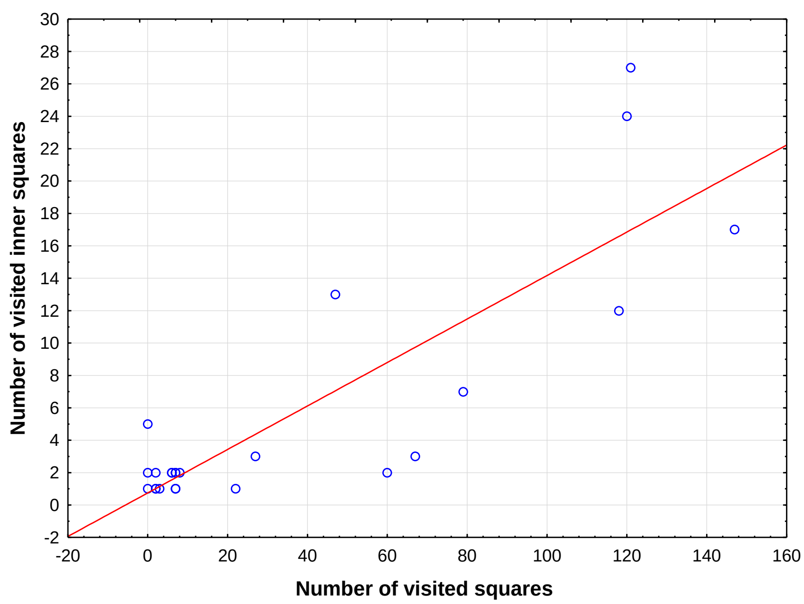

Figure 22: The correlation between the total number of visited squares and the number of inner visited squares in the novel environment test.

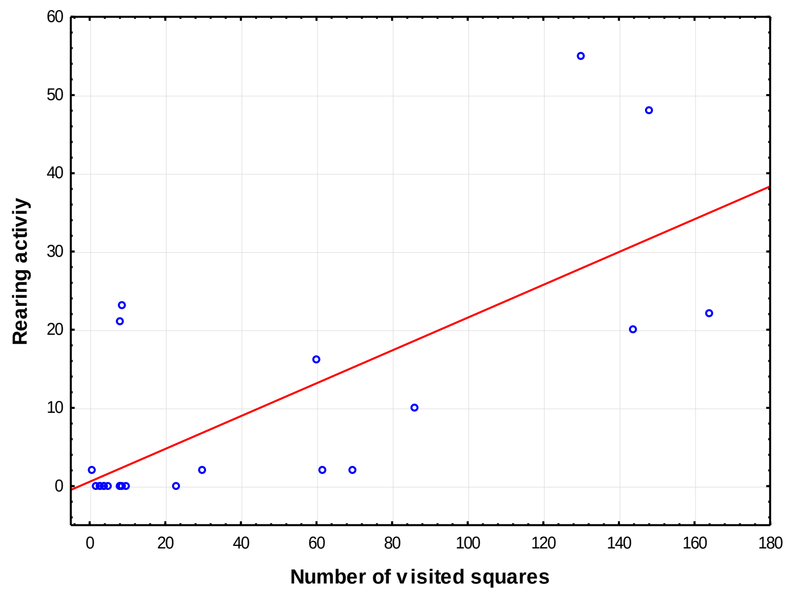

Figure 23: The correlation between the number of squares visited and the number of rearings in the novel environment test.

4.3 Novel object test

4.3.1 Novel Object Test 1 with a Pink Ball

This test was carried out on the second day of the personality test session. The animals had 60 minutes to approach an unknown object placed in their home box. This test was evaluated in detail for the first ten minutes. Four animals did not approach the pink ball during one hour and 4 out of the 24 individuals needed more than 30 minutes to come close to the novel object.

I found a negative relationship between the approach latency to the novel object (the pink ball) and the time spent showing interest in this new object (Spearman Correlations: R_S= -0.869, $p<0.00001$) (Figure 24). The animals which spent more time showing interest in the novel object (sniffing and biting) also had a higher rate of rearings (Spearman Correlations: R_S=0.482, p=0.018) (Figure 25).

The novel object approach latency (Mann-Whitney U Test: U=29.0, N1=11, N2=13, p=0.487), the interest in the novel object (U=68.5, N1=11, N2=13, p=0.884), and also the rearing activity (U=52.5, N1=11, N2=13, p=0.284) were not affected by sex.

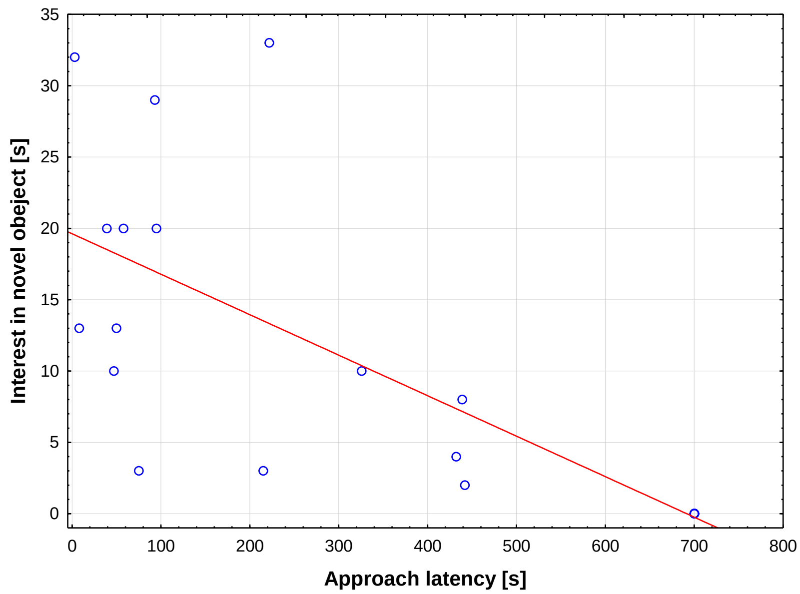

Figure 24: The correlation between the approach latency and the time spent showing interest in the novel object no.1 (the pink ball) in the novel object test 1.

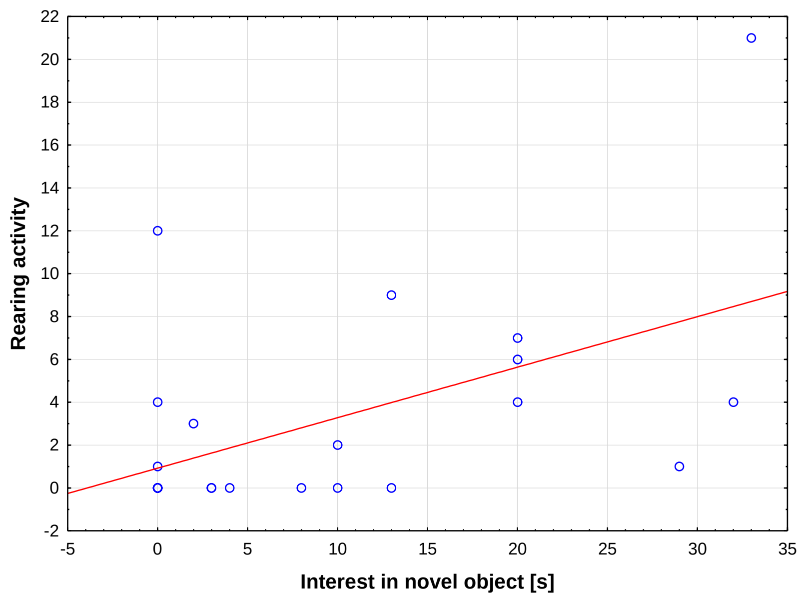

Figure 25: The correlation between the time spent showing interest in the novel object no. 1 (the pink ball) and the rearing activity.

4.3.2 Novel Object Test 2 with a Yellow Duck

The second novel object test, this time with a yellow plastic duck, followed the next day after the first object (the pink ball) was presented to the guinea pigs. The maximum duration of this test was 30 minutes. Only one female did not approach the yellow duck within the 30 minutes.

I found the same negative correlation between the approach latency and the time spent showing interest in the novel object no. 2 (the yellow duck) (Spearman Correlations: R_S= -0.547, p=0.0056) measured during the first ten minutes as well as for a time period of 30 minutes (Spearman Correlations: R_S= -0.561, p=0.0043). The animals which approached the novel object later also spent less time sniffing and biting this novel object. This relationship was also found in the case of the novel object no.1 – the pink ball.

Time spent showing interest in the novel object no.2 was significantly correlated with the rearing activity (Spearman Correlations: R_S=0.604, p=0.0018) (Figure 26). I measured a high number of rearings in the animals which spent more time interested in the yellow duck.

Males and females did not differ in their approach latencies (Mann-Whitney U Test: U=45.0, N1=11, N2=13, p=0.132), in the time spent showing their interest in the novel object (U=63.0, N1=11, N2=13, p=0.643), or in their rearing activity (U=66.0, N1=11, N2=13, p=0.772).

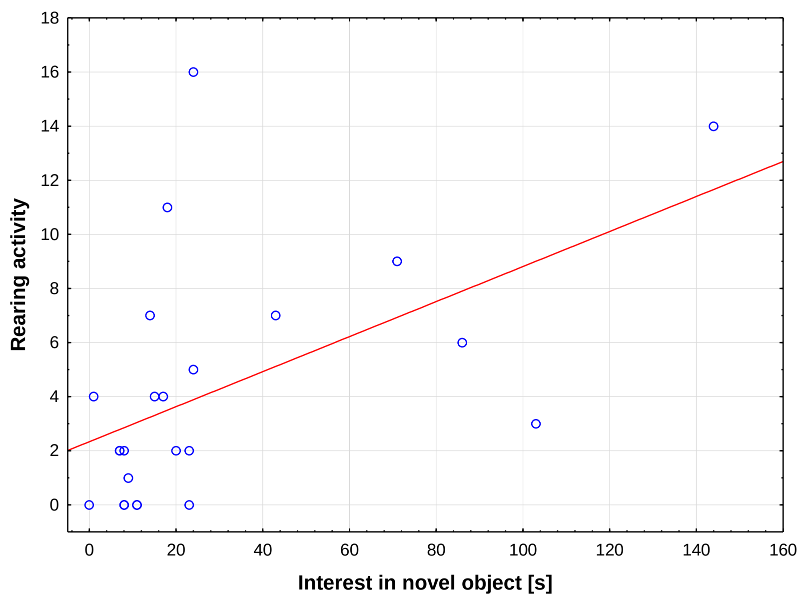

Figure 26: The correlation between the time spent showing interest in the novel object no. 2 (the yellow duck) and the rearing activity.

4.3.3 Novel Object Test 3 with an Orange Pig in a Group Context

The last unknown object – a plastic orange pig – was presented to a group of guinea pigs in their home box after all the individuals had gone through a complete personality-test set .

There was no evidence for any correlation between the approach latency to the orange pig and the time spent showing interest in this novel object (Spearman Correlations: R_S= -0.210, p=0.324). Eighteen out of the twenty four guinea pigs approached the orange pig in less than one minute. The number of interactions did not affect the total time spent showing interest in the novel object (R_S=0.202, p=0.345), or the approach latency (R_S = -0.016, p=0.940).

Males and females significantly differed in the proportion of time spent showing their interest in the novel object (the orange pig) (Mann-Whitney U Test: U=28.5, N1=11, N2=13, p=0.013). Females spent more time sniffing and biting the novel object (Figure 27). When comparing the approach latencies, there was no significant difference between the sexes (U=64.0, N1=11, N2=13, p=0.664).

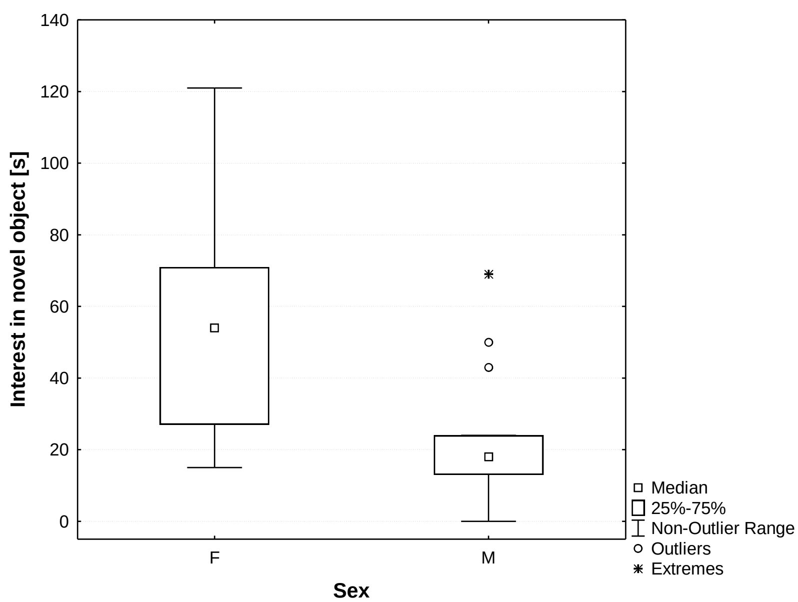

Figure 27: The influence of sex on the time spent showing interest in the novel object no. 3 in a group context. F-females, M-males.

4.3.4 Novel Objects – Comparison

The results obtained from the two novel object tests – the approach latencies and the time duration spent showing interest in those objects were compared to examine if there existed a relationship between them. The novel object test no.3 with the pink ball (in a group context) was used to compare the approach latencies.

The approach latency to the novel object no.1 (the pink ball) was positively associated with the approach latency to the novel object no.2 (the yellow duck) (Spearman Correlations: R_S=0.202, p=0.0002) (Figure 28) as well as the time spent showing interest in those two novel objects (Spearman Correlations: R_S = 0.673, p=0.0003) (Figure 29) over a period of 2 days. The animals which came earlier to the pink ball also hesitated less to approach the yellow duck the next day. I also found a positive relationship between time durations spent showing interest in those two novel objects.

When comparing approach latencies to the 3 novel objects (the orange pig also being involved), the approach latencies decreased over time (Friedman ANOVA: N=24. Df=2, X^2 =15.763, p=0.0004) (Figure 30).

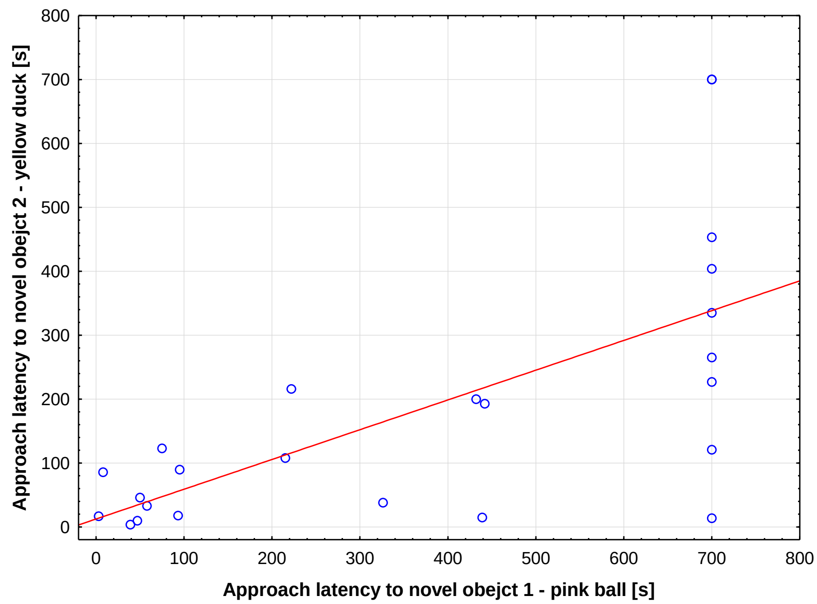

Figure 28: Correlation between the approach latencies to the novel object no.1 (the pink ball) and the novel object no.2 (the yellow duck) over two days.

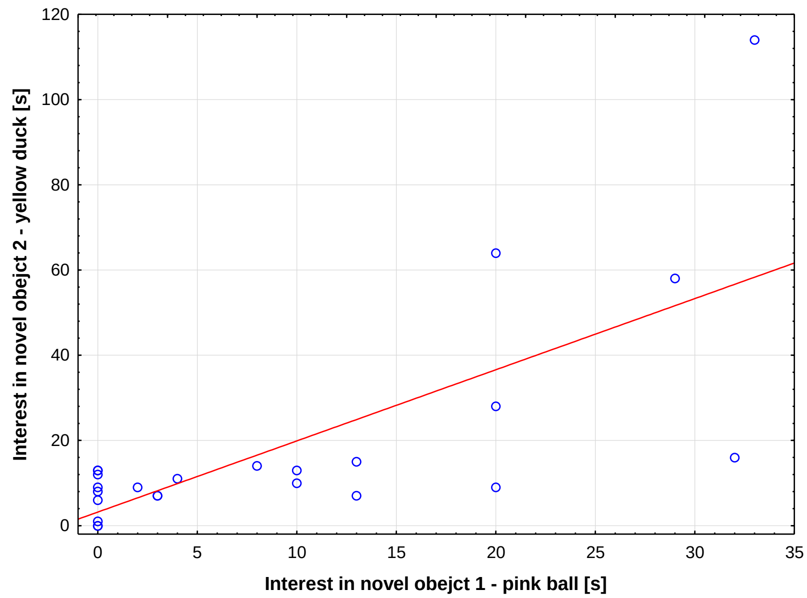

Figure 29: Correlation between the time spent showing interest in the novel object no.1 (the pink ball) and the novel object no.2 (the yellow duck) over two days.

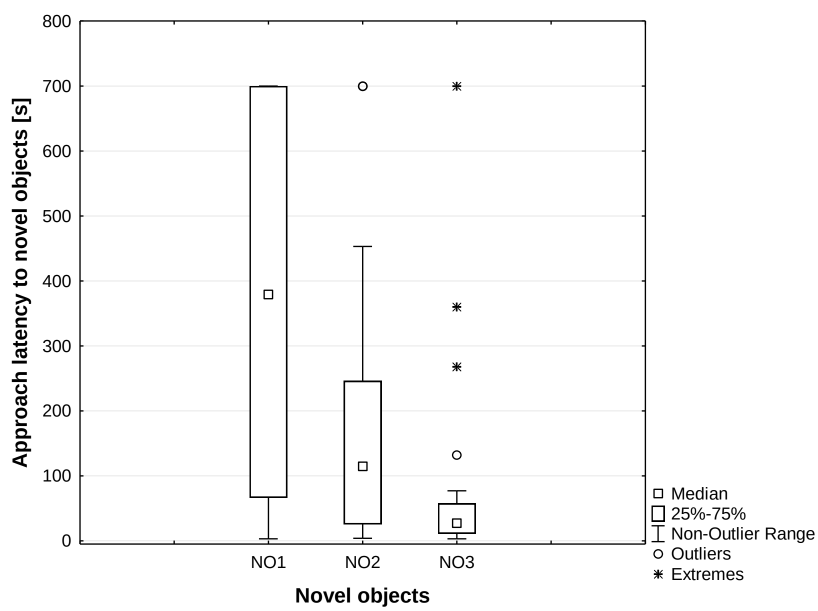

Figure 30: The comparison of approach latencies to three novel objects no.1-no.3. NO1 – the pink ball, NO2 – the yellow duck, NO3 – the orange pig.

4.4 Elevated Plus-Maze

A good parameter for evaluating this task is whether individuals cross the center of the elevated plus-maze (i.e., whether they moved from one arm to the other arm through the center). Ten guinea pigs from all the 24 individuals crossed the central part of the EPM apparatus at least once. The guinea pigs which passed through the central square of the elevated plus-maze at least once were also more explorative and visited more arms (open and closed arms in total) (Mann-Whitney U Test: $U=7.0$, $N1=10$, $N2=14$, $p=0.0003$) (Figure 31). The more explorative animals which visited more arms also entered the open arms more often (Spearman Correlations: $R_S=0.809$, $p<0.0001$) (Figure 32) and also spent more time there ($R_S=0.735$, $p<0.0001$). There were no significant differences between the center-leaving latency and the number of arms visited ($R_S= -0.235$, $p=0.268$) and also the time spent on the central square did not correlate with the total number of visited arms ($R_S=0.288$, $p=0.172$). We found a positive correlation between the overall number of visited ($U =41.0$, $N1=11$, $N2=13$, $p=0.082$) and the rearing activity ($R_S=0.861$, $p<0.0001$). The guinea pigs which were more explorative in visiting the arms also made more rears.

The total number of visited arms (Mann-Whitney U Test: $U =62.5$, $N1=11$, $N2=13$, $p=0.622$), the number of entries to open arms ($U =65.0$, $N1=11$, $N2=13$, $p=0.728$), the number of rears ($U =49.0$, $N1=11$, $N2=13$, $p=0.202$), the center leaving latency ($U =54.0$, $N1=11$, $N2=13$, $p=0.325$), or the time spent in the centre ($U =41.0$, $N1=11$, $N2=13$, $p=0.082$) did not differ between males and females.

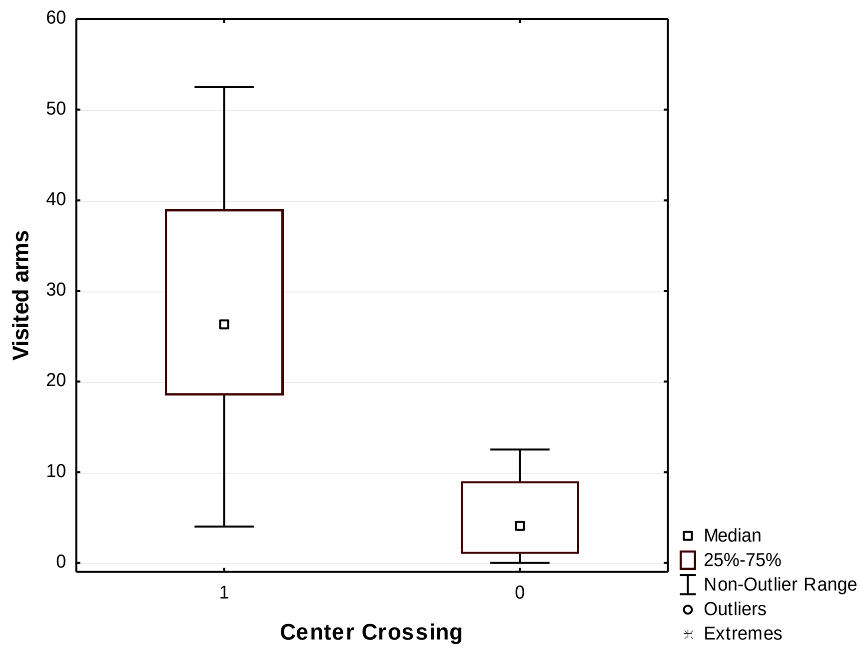

Figure 31: The comparison of total number of visited arms; animals that crossed the central part of the elevated plus-maze at least once (1) and those that did not do so in the same maze (0) were compared.

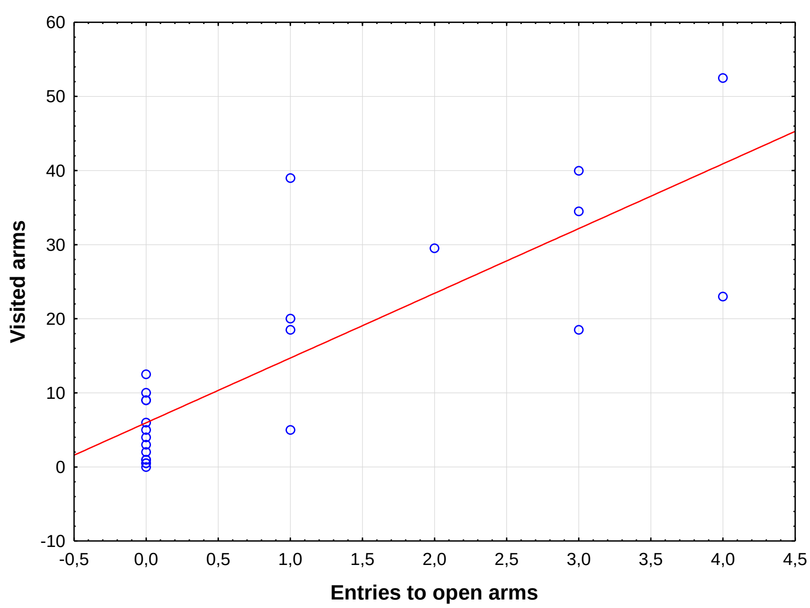

Figure 32: The correlation between the number of entries to open arms and the total number of all visited arms in the elevated plus-maze.

4.5 Predator Test

Only one out of the 24 animals responded vocally to the predator. So it was impossible to involve vocal reactions to the predator in the overall analysis of behavioural traits obtained from different personality tests. One call made by a juvenile female was analyzed by Ing. Aneta Baklová in Avisoft programme The spectrogram (Figure 33) displayed a low frequency sound (max. 1700 Hz). This sound is called a “drrr” sound. It is generally a sound which animals produce when being disturbed (Arvola, 1974; Berryman, 1976; Coulon, 1982). This alarm call reflected the guinea pig’s reaction to the predator.

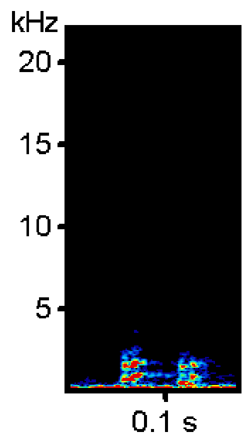

Figure 33: The spectogram of the call (the “drrr” sound) reflecting the vocal reaction to the predator.

The number of squares visited in the presence of the predator was negatively correlated with the animals’ center-leaving latency (Spearman Correlations: R_S= -0.696, p=0.0016). The animals which had escaped from the inner squares earlier (i.e., from the center of the arena) also visited more squares when the dog was circulating around the glass aquarium and barking.

The number of visited squares (Mann-Whitney U Test: U=50.5, N1=11, N2=13 p=0.224) and also the center-leaving latency (U=44.0, N1=11, N2=13 p=0,111) were independent of sex.

4.6 Principal Component Analysis

4.6.1 Principal Component Analysis

16 different parameters from the series of the personality tests were included to the first principal component analysis. The first axis explained 27.8 % of total variation. It was most saturated with the total number of visited arms and the center crossing in the EPM, with the time spent showing interest in the novel object no. 1 in the novel object test 1, and slightly less with the total number of squares visited in the NE test and with the time spent showing interest in the novel object no. 2 in the novel object test 2 on one side, and with the center- leaving latency in the NE test and all approach latencies to 3 novel objects on the other side.

The second axis explained 15.5 % of total variation. It was best described by the house-leaving latency in the isolation test and by the number of squares visited in the presence of the predator on one side and by the presence of urinations in the NE test and by the center-leaving latency in the predator test and also by the house-leaving latency in the isolation test on the second side (Figure 34).

The third axis explained 13.8 % of total variation and the fourth axis 11.3 % of total variation. Total explained variation was 68.5 % (Figure 34).

Strong positive correlations were found between the number of visited squares in the NE test, the number of visited arms in the EPM test and also the center crossing in the EPM test. These parameters also correlated with the time spent showing interest in all three novel objects (no. 1 – no. 3). Weaker correlations with the time spent in the open space in the isolation test and the number of boluses measured in the NE test were detected. These parameters (with exception of the number of boluses from NE test) were negatively correlated with the approach latency to novel object no. 3 (Figure 34).

All approach latencies to novel objects were positively associated, but the approach latency to the novel object no. 2 was only weakly correlated with others. Positive correlations were also found between the approach latency to novel object no. 3, house-leaving latency in isolation test and the number of squares visited in the presence of the predator (Figure 34).

Approach latencies to novel objects no. 2 and no. 3, center-leaving latencies in the NE test and in the predator test and the presence of urination in the NE test were positively correlated. These results were negatively correlated with the presence of boluses in the NE test (Figure 34).

For more detail see ANNEX2 and ANNE3 (Species scores for each behavioural trait –adjusted for species variance and sample scores for each individual and Eingenvalues).

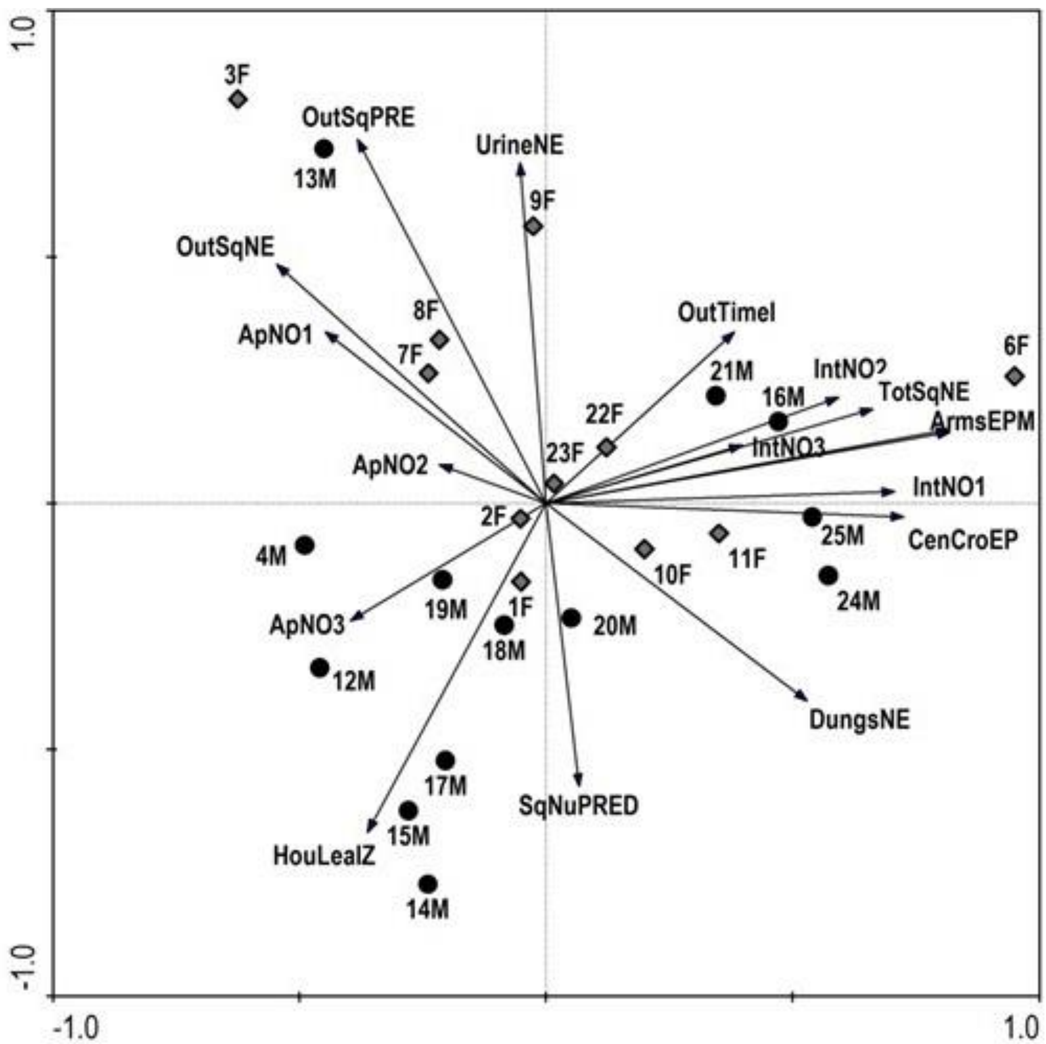

Figure 34: The PCA analysis for selected behavioural parameters from all personality tests – the isolation test (HouLeaIZ – house-leaving latency, OutTimeI – time spent in open space), the novel environment test (TotSqNE – total number of visited squares, OutSqNE –center-leaving latency, UrineNE- presence of urination, DungsNE –presence of boluses), the novel object test 1 (ApNO1- approach latency to novel object no. 1 , IntNO1 – time spent interesting in novel object no. 1), the novel object test 2 (ApNO2- approach latency to novel object no. 2 , IntNO2 – time spent interesting in novel object no. 2), the novel object test 3 (ApNO3- approach latency to novel object no. 3 , IntNO3 – time spent interesting in novel object no. 3), the elevated plus-maze (ArmsEPM – number of visited arms, CenCroEP – center crossing), the predator test (SqNuPRED – number of squares visited, OutSqPRE –center-leaving latency). F-females, M - males. **The first axis explained 27.8 % of total variation, the second axis explained 15.5 % of total variation.**

4.6.2 Principal Component Analysis - Limited Parameters Selection

7 parameters were involved in the second PCA analysis (1 parameter from each personality test). The first axis explained 31.2 % of total variability. It was mainly loaded by the house leaving latency (the isolation test) and the approach latency to the novel object no. 3 on one side and by the number of squares visited in the NE test on the second side (Figure 35)

The second axis explained 21.7 % of total variation. It was best described by the latency of center-leaving in the presence of the predator on one side and by the approach latency to the novel object no. 2 on the second side (Figure 35).

The third axis explained 19 % of total variation and the fourth axis 13.5 % of total variation. Total cumulative percentage variance of species data was 85.5 %.

All approach latencies to 3 novel objects and also the house- leaving latency in isolation were positively correlated. Between approach latency to the first novel object (the pink ball) and second novel object (the yellow duck) was found strong relationship, but the approach latency to novel object no. 3 (the orange pig) was only weakly associated with others (Figure 35).

There was a negative relationship between the center leaving latency in the predator test, and two parameters: the number of squares visited in NE test and the number of arms visited in the EPM (and the center crossing in the EPM) (Figure 35).

There was found a positive relationship between the exploration activity in the NE test and in the EPM test. The guinea pigs which visited more squares in the NE test also visited more arms in the EPM test. More explorative animals left the central part of a glass aquarium later in the presence of the predator and also approach the novel object no.3 (presented in a group context) later. Parameters obtained from the NE test and the EPM test did not correlate with approach latencies to novel object no. 1 and no. 2 and with the house-leaving latency after isolation (Figure 35).

According the results from the second PCA analysis animals created 2(or 3) groups - a bigger group consisted of 15 guinea pigs, and the second group of 6 individuals (3 males and 3 females) Three females (no. 1, 2 and 8) were separated from these two groups (Figure 35)..

For more detail see ANNEX4 and ANNE5 (Species scores for each behavioural trait –adjusted for species variance and sample scores for each individual and Eingenvalues).

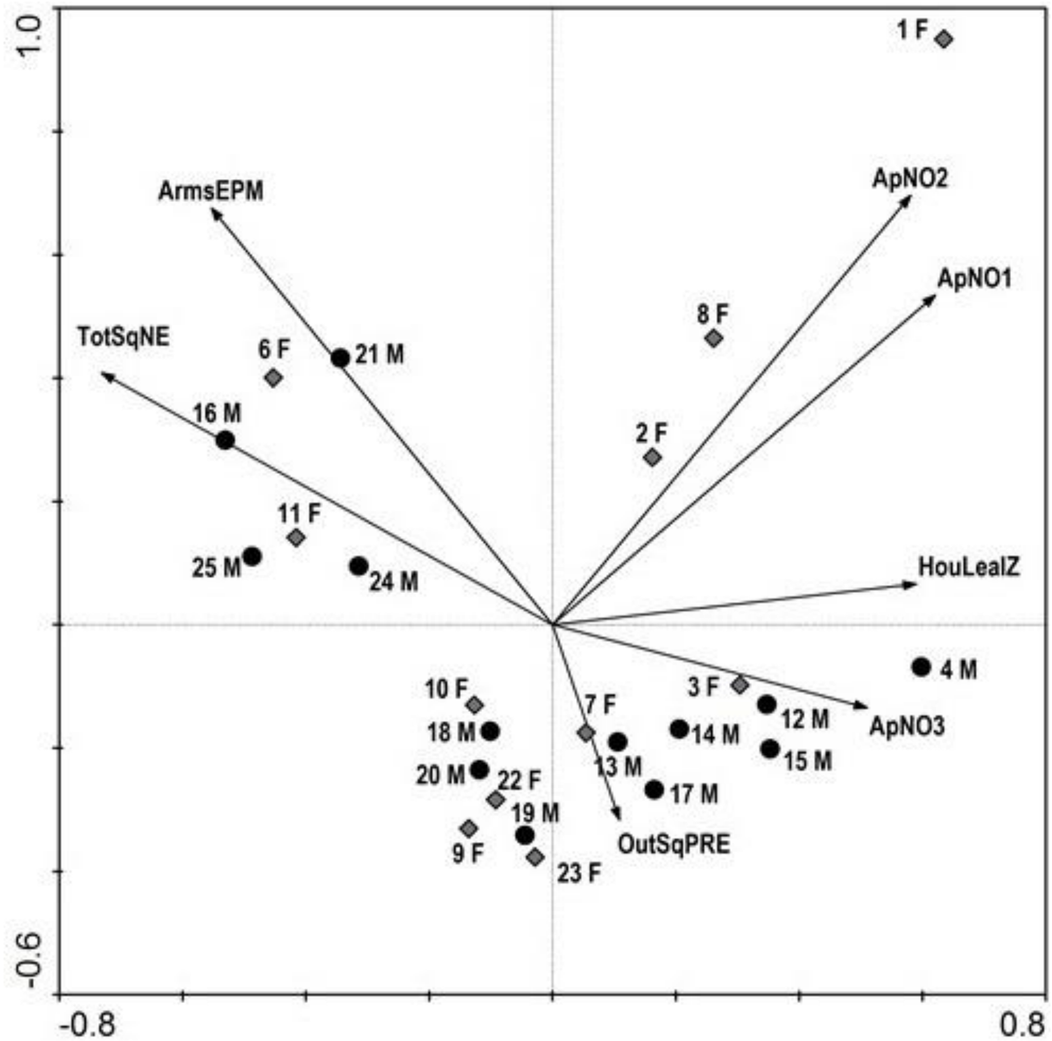

Figure 35: The PCA analysis for selected behavioural parameters - 1 from each personality test – the isolation test (HouLeaIZ – house-leaving latency), the novel environment test (TotSqNE – total number of visited squares, the novel object test 1 (ApNO1- approach latency to novel object no. 1), the novel object test 2 (ApNO2- approach latency to novel object no. 2), the novel object test 3 (ApNO3- approach latency to novel object no. 3), the elevated plus-maze (ArmsEPM – number of visited arms), the predator test (OutSqPRE –center leaving latency). F-females, M - males.**The first axis explained 31.2 % of total variation, the second axis explained 21.7 % of total variation.**

in

4.6.3 Principal Component Analysis - Limited Parameters Selection for 15 Individuals

9 parameters were used for the third PCA analysis (8 parameters – 1 parameter for each personality test and the body mass of animals was also included). The data set for 15 individulas in a comparison to the previous analysis included also the number of visited squares in the repeated novel environment test (TotSqNE2) and the body mass (Weight).The first axis explained 31.7 % of total variability. It was loaded most by the number of squares visited in the repeated novel environment test (NE2) and also by the number of squares visited in the first novel environment test (NE) and with the number of arms visited in EPM test (Figure 36).

The second axis explained 22.2 % of total variation and it contributed the most to the body mass, the center-leaving latency in the presence of the predator and the approach latency to novel object no. 1 (Figure 36).

The third axis explained 17.7 % of total variation and the fourth axis 10.9 % of total variation. Total cumulative percentage variance of species data was 82.6 %.

Approach latencies to the novel object no. 1 (the pink ball) and the novel object no. 2 (the yellow duck) and the the latency of center leaving in the presence of the predator were positively correlated. The guinea pigs that approach novel objects later, also stayed for a longer time in central squares before leaving when the predator was barking and circulating around the glass aquarium. The heaviest animals needed more time to come close to novel objects and to leave the center in the predator test (Figure36).

The number of squares visited in NE test was associated with the number of squares visited in repeated novel environment test 2 and with the number of visited arms in EPM. These results were negatively correlated with the approach latency to novel object no. 3 and the house leaving latency in the isolation test (Figure 36).

The house-leaving latency positively correlated with the approach latency to novel object no. 3, but did not correlate with the approach latency to the novel object no.1 and only weakly correlated with approach latency to novel object no. 2 (Figure 36).

For more detail see ANNEX6 and ANNE7 (Species scores for each behavioural trait –adjusted for species variance and sample scores for each individual and Eingenvalues).

According the results from PCA analysis animals created 2 groups - a bigger group consisted of 7 males and a second group of 4 males, 2 females (no. 22 and 23) were found on the borders between these two groups. 2 individuals were found outside of these groups (Figure 36).

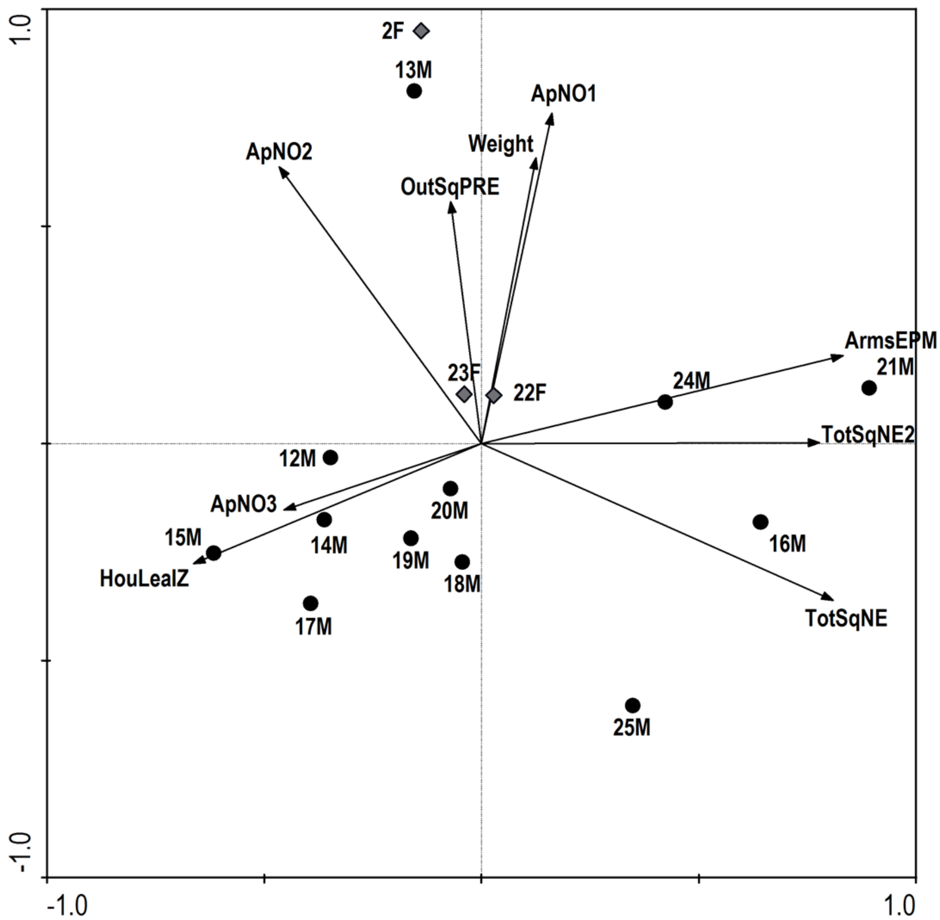

Figure 36: The PCA analysis for selected behavioural parameters for 15 individuals – the novel environment test (TotSqNE – total number of squares visited), the novel environment test 2 (TotSqNE2 – total number of squares visited) the novel object test 1 (ApNO1- approach latency to novel object no. 1), the novel object test 2 (ApNO2- approach latency to novel object no. 2), the novel object test 3 (ApNO3- approach latency to novel object no. 3), the isolation test (HouLeaIZ – house-leaving latency), the predator test (OutSqPRE – center-leaving latency), the elevated plus-maze (ARMExpl – number of arms visited) and weight of animals (Weight). F-females, M - males. **The first axis explained 31.7 % of total variation, the second axis explained 22.2 % of total variation.**

5 DISCUSSION

5.1 Isolation Test

In the isolation test animal's behaviour after a stressful situation represented by the isolation from its group was observed. The plastic box with a shelter (a plastic house) used in this test could also represent a novel enriched environment. Guenther and Trillmich (2013) pointed to the fact that separation creates a stressful event for the guinea pig. They tried to reduce the stress due to social isolation by keeping other individuals of the same species in the room during habituation and test time.

The guinea pigs which spent more time out of protected area made more rears –actively explored the arena and also fed more in the open space. Feeding in such a stressful situation could be used as a good measurement for evaluating boldness. More bold (or explorative) animals left the shelter earlier, spent more time in the open space of the plastic box and also fed there more. The latency of first feeding on a mealworm (*Tenebrio molitor*) placed in the novel environment was used as a one criterion for personality determination in great and blue tits (Fuchsová, 2007, Jěžová, 2008, Šimánková, 2011). More explorative and bolder tits flew earlier on a glass bowl with a mealworm and fed there. The higher number of rearings is connected with habituation to the new environment (Vadasz et al., 1992) but it will be discussed more in the next chapter 5.2. Novel environment test.

There were also animals that did not leave the shelter during one hour period in the presented study, but it was only five out of 24 guinea pigs, mainly males. These animals coped hardest with an acting stress caused by the isolation from their group and showed no interest in the exploration of a new home box, or the offered food. But the majority, 18 out of 24 guinea pigs firstly visited open parts of the enclosure in less than 10 minutes. Females in comparison to males spent more time out of protected area, but these results were just above significance.

It seems that this test is appropriate for personality determination. Relationship with novel object tests were also revealed.

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5.2 Novel Environment Test

Guinea pigs differed in their exploration activity in an open space – in the plastic arena. Animals which did not move during the whole experiment (max. 60 minutes) were scored with 1 square. The most explorative guinea pig male visited 436 squares during the whole experiment. Also in the first ten minutes big variability (1 -112 squares) was measured. Guinea pigs tend to be immobile in the open field situation and they are likely to remain in the centre of the enclosure (Tobach and Gold, 1966). I did not find such results with long center-leaving latency. In our experiment, only 3 out of 24 guinea pigs remained in the centre for 10 minutes and only one animal stayed there for whole experiment (60 minutes).

The rate of defecation in a novel environment is typically used as a measure of emotionality (Hall, 1934 ex Gosling 2001; Maier et al. 1988). But Tobach and Gold (1966) , however, pointed out in their study that the persistence of defecation is not – in the case of guinea pigs – a good measure of describing the individuals' "emotional temperament". .My results are in agreement with this study (Tobach and Gold, 1966). The number of boluses was positively correlated with the number of visited squares in the novel environment test. More explorative animals defecated more. The total rate of defecation did not reflected emotional reaction of animals to stressful situation – placing into the novel environment. One explanation could be found in the length of the test, because boluses were counted after the end of the experiment (a time period of one hour). So the number of boluses did not reflect a direct reaction to an unknown environment. The defecation could be affected also by different amounts of ingested food before the experiment. The locomotive activity of animals probably stimulated their defecation more than in those animals that were less active. No relationship between urination and exploration activity was measured.

Animals that show a relatively low total ambulation in an open-field test have a low central ambulation as well (Hall, 1934). We found the same relationship in the novel environment test. The guinea pigs that visited more squares also went more often to the central part of the arena. The aversive character of the open arena is demonstrated by the higher proportion of time spent in the peripheral sections of the system in relation to the more exposed central parts (Gerano & Schmidek, 2000).

The tested guinea pigs that explored more squares also made more rearings. As Vadasz et al. (1992) found, a higher rearing activity is measured in animals when they are habituated to the new environment. The higher rearing activity should thus reflect weaker stress acting on animals. In contrast, Gerano and Schmidek, (2000) summarized that the high number of rearings and also the higher locomotion in the poor and frightening environment of the open-field situation probably indicates escape reactions and not exploratory behaviour itself. In a rich environment the occurrence of rearings was not reduced. That probably denotes the expression of a real exploratory motivation.

It was proved that obtained results are comparable with other rodents open field test with typical duration of 10 minutes, because the number of total squares visited were positively correlated with the first 10-, 30- and 60-minute periods. Animals which were a little active in first half (30 minutes) did not change their exploration activity in second half. Only in three animals which were almost inactive in the first 30-minute period, the higher exploration activity was measured in the second part of the NE test.

5.3 Novel Object Tests

Three different novel plastic objects (the pink ball, the yellow duck and the orange pig) were presented to the guinea pigs in their home boxes (one of them in a group context). Placing a novel object into a home enclosure causes the animal to experience a conflict between exploration and the fear of novelty (Montgomery, 1955; Berlyne, 1960; Powell et al., 2004). Animals that approached the pink ball (the first novel object) earlier also spent more time investigating this novel object. The same relationship was also found in the case of the second novel object (the yellow duck). This indicates that the novel object test is sufficient for personality research in guinea pigs.

That indicates that animals that feared novel objects less came to them earlier and also spent more time sitting close to novel objects, sniffing and biting them. Verbeek et al. (1994) found that great tits belonging to slow explorers had longer latencies than the fast explorers in approaching novel objects placed in their home cages. Slow explorers also approached an unknown object less closely and spent less time interacting and sitting close to this object.

In the case of the yellow duck, the more explorative animals made more rearings. As has been mentioned above, a higher rearing activity could reflect a habituation to the novel situation and thus, the animals that were less stressed by the unknown object also explored the area around the novel object and the plastic box itself more (i.e., they moved more and made more rearings). The guinea pigs which approached novel objects later spent more time sitting and resting.

The third novel object (the orange pig) was presented to a whole group of guinea pigs. Any significant influence of interactions between guinea pigs on the approach latency or the time spent interesting in the novel object no. 3 was observed. Only one male did not approach the orange pig. Generally, they less hesitated to investigate the novel object in a group context but it could be caused by the fact that it was repeatable presentation of unknown toy. Aggressive behaviour is connected with personality types, fast explorers started and won more fights (Verbeek et atl, 1996). But we did not find relationship between exploration of novel object and the number of agonistic interaction (chasing and biting).

When comparing the approach latencies between the three novel object tests, these decreased in time.The guinea pigs hesitated longer to approach to the pink ball which was presented the first day and needed less time to investigate the yellow duck next day. In a group context the measured latencies were the shortest. The same decreasing approach latencies were found in great tits. Juveniles approached the second unknown object significantly faster than the first object, but their reactions towards novel objects were consistent (Verbeek et al, 1994).

5.4 Elevated Plus-Maze

The elevated plus maze test was designed to evaluate the relationship between the fear of heights and open space, and exploration.(Lister, 1987). The standard length of this test is usually not more than 15 minutes, but I allow the guinea pigs to explore the EPM apparatus for 30 minutes to give them enough time. In comparison with other rodents, the guinea pigs in our study did not visit open arms often, maximum number of entries to open arms was 4 in 30 minutes. In rats, 6.6 ± 0.6 and 7 ± 0.6 entries to open arms were measured in 10 minutes (Pawlak and Schwarting, 2002) or 2.8 ± 0.4 entries to open arms in laboratory mice measured in 5 minutes (Espejo, 1997). The guinea pigs stayed more in closed arms than in open ones. The preference for closed protected arms could be caused by the fear of open and elevated spaces (File, 2001) or by the fact that an open arm did not allow the animals to stay close to the walls (Treit et al., 1993).

In studied animals that visited more arms the higher rearing activity was measured. This is in agreement with results from study of Espejo (1997) in laboratory mice in the EPM test that confirmed that the occurrence of rearings is an exploratory motor activity pattern. They also found that the higher motor activity was connected with the higher number of closed arm entries and total arm entries and the higher rate of sniffing.

The central part of the EPM apparatus is connected with decision making and also risk-taking behaviour (Rodgers and Johnson, 1995; Trullas et al., 1991). Not only visiting the open arm, but sitting in the central part as well forces animals to face their fear of heights; thus, I expected that animals that had at least once passed through the central square (i.,e. moved from one arm to another through the central part) would be bolder than the animals that stayed immobile in the center, or that moved from the center to one arm and stayed there for the whole experiment. 10 out of 24 guinea pigs that crossed the central part (considered as bolder) were also more explorative because they visited more arms and more often entered open arm.

No relationships between the center-leaving latency and the number of arms visited and also between the time spent on the central square and the total number of arms visited were found. The most stressed animals stayed immobile in the central square and did not move during the whole experiment.

5.5 Predator Test

The results obtained from the predator test, when the guinea pigs had to face a real predator – a dog, showed that this test is not suitable for personality determination, because this test was mainly focused on direct vocal reaction to a dog's presence (involved barking and circulating around the glass aquarium). But only one of 24 animals answered vocally. Also no reactions of spiny mice (*Acomys cahirinus*) to the playback of recorded calls of the tawny owl were found (Hendrie et al., 1998). The measured alarm call – ”drrr“ sound of low intensity typical for short distance is connected usually with immobility and reflected changes in the environment or general environmental disturbance (Arvola, 1974; Berryman, 1976; Coulon, 1982; Eisenberg, 1974). In this case, the presence of the dog represented the disturbance. This ”drrr“ sound is produced through the nose, the mouth is closed in that moment. The structure of this alarm call does not differ between males and females nor is it influenced by age or body size (Arvola, 1974).

The guinea pigs’ reactions were generally uniform, almost no vocal reactions occurred (only in one case), most of them left the central part quickly, made no rears and also no defecation was recorded. A negative relationship between the center-leaving latency and the number of squares visited in the presence of the predator was found. The animals which had earlier left the central squares also moved more. This could be interpreted to mean that individuals which dared to move from the central part earlier on also showed less immobility and were bolder in the presence of a predator. But I found no big differences between the amount of visited squares (the maximum number of visited squares was 11 in two minutes, the minimum was 1 for those who stayed in the center). An additional reduction in the animals’ activity in the centre of the apparatus was also measured. The guinea pigs placed in the glass aquarium quickly left the central part. 15 out of 24 individuals escaped from the center in less than 8 seconds, and only 3 animals stayed immobile in the center for a total of 2 minutes. So it could be concluded that guinea pigs were not very active in the presence of the predator. Reduced levels of activity were also found in voles (*Microtus socialis*), dormice (*Eliomys melanurus*) and jerboa (*Jaculus jaculus*) which answered to the playback of predator. Voles had lower rearing activity and dormice travelled for shorter distances and sp ent more time in or near the burrow (Hendrie et al., 1998). Decreasing patterns in foraging ime (known as behavioural resource depression) and also increasing number of visits to protected sites in gerbils (*Gerbillus allenbyi* and *Gerbillus pyramidum*) in the presence of predators - barn owls (*Tyto alba*) were also detected (Kotler et al., 1992). Feeding behaviour was also inhibited in laboratory mice after exposure to owl calls (Hendrie & Neill, 1991). Typical behavioural reaction for small rodents, such as woodmice, is freezing or leaping when exposed to predators - stoats (Erlinge, Bergsten & Kristansson, 1974) and usually bolting for a hole when exposed to other predators (King, 1985).

No identified vocal differences could be caused by many factors. One explanation could be the fact that no vocal reaction is typical answer for animals in the presence of a predator, not to attract the predator’s attention and reduce the probability of a detection. Blanchard & Blanchard (1989) described the most adaptive strategies of animals upon detecting the presence of a predator to remove themselves from the immediate location, try to shelter in strategically defensible places or to alter their patterns of movement. Animals must either constantly move to avoid their location being predicted, or minimize their movements to avoid being detected. Other factor that could affect the vocal and behavioural response could be the presence of 3 people in the tested room which could stress tested animals a lot. The Demonstrational and Experimental Stable where the experiments were hold are located in the area where people regularly walk their dogs. Therefore, guinea pigs may have previous experiences with dog barking, they could get used to it and thus I did not measured almost any reactions to the predator. The effect of domestication could also affected reaction of guinea pigs towards predators (Price, 1984). Behaviour towards predators could also differ between animals of various age (Trillmich and Hudson, 2011).

Based on the results obtained from the predator test (mainly due to almost any recorded calls) I do not find that the evaluation reflects the differences between individuals sufficiently, and thus I consider this test insufficient for the purposes of personality research in guinea pigs.

5.6 Principal Component Analysis and Personality

. Positive correlations between approach latencies to all new objects (the pink ball, the yellow duck, and the orange pig) were found. The relationship with the last presented object – the orange pig was not so strong as between the first two novel objects, but it could be caused by the fact that the third novel object test was presented in a group context meaning that the animals had to react to other individuals, face aggressive interactions such as biting or chasing, so it definitely affected their attitude to the novel object. The dominance status of individuals also might play an important role. The dominance status in guinea pigs is stable over time (Zipser et al., 2013).

Approach latencies between two different objects (the pink ball and the yellow duck) presented to a single individual were strongly positively correlated. This is consistent with Verbeek et al. (1994), who found that the approach time to the penlight battery (the first presented novel object) was positively correlated with the approach time to the rubber toy (the second presented novel object). It revealed that the reactions to a novel object were similar for different presented objects. So it could be concluded that this test is suitable for guinea pigs. It is better to observe animal’s behaviour for a longer period than ten minutes, at least the first day.

The second test, which was repeated, but only with limited number of animals (15 guinea pigs) was the novel environment test (NE2). This test was re-tested at the end of all experiments from the personality set. The total number of visited squares positively correlated over time.

I found a positive relationship between the number of squares visited in the open filed test and the number of arms visited in the elevated plus maze. These results show that animals which were more explorative in one new environment were also less fearful in other type of unknown environments and were exploring more. The individual specific responses to the novel environment test corresponded with the behavioural parameters in the elevated plus maze. The same relationship between performances in the novel environment test (a glass enclosed arena) and the eight-arm radial maze were found in common voles (*Microtus arvalis*) (Lantová et al., 2011) The guinea pigs that were more active in the plastic arena in the novel environment test, visited more arms in the EPM test during 30-minute period than the less active individuals.

Almost none of the parameters investigated in the set of personality tests were influenced by sex. It could be caused by the fact that I worked with young individuals and I did not have enough information about conditions in their breed stock. Guenther and Trillmich (2013) pointed out that personality traits changed over different ontogenetic stages and could be strongly affected by environment or prenatal conditions. They found different personality types only in heavy females, all males and females with lower weight did not differ in their personality types.

The behaviour of tested guinea pigs was not uniform. The animals behaved differently in the investigated personality set of tests, but according the results from this Master thesis it is not easy to divide them into 2 personality types - such as fast and slow explorers in great tits. Personality in great tits is evaluated based on scores obtained from the novel environment test and the novel object test. Fast explorers explored novel environment quickly and also hesitated less to approach novel objects. Slow explorers needed more time to start exploration and also to investigate novel objects (Verbeek et al., 1994; Drent et al., 2003). I did not find such relationship between parameters from NE test and NO tests.

Similar results reflecting correlations between two different environments were found in the wild Cavies (Guenther and Trillmich, 2013). The number of trips made in the long field test correlated with the distance explored in the open field test in juveniles. Also no relationship with the novel object test was found. These two behavioural tests did not correlate with the novel object test. Both the latency to start exploration in the long field test and the distance explored in the open field test were consistent over six months. The latency to interact with novel object was not repeatable

Zipser et al. (2013) did not found find any correlations among emotionality in domestic guinea pigs during two testing phases, but the male dominance status did not change over 8 weeks The cortisol reactivity and social behaviour were stable over time. Although no identified stability of behaviour and stress reactivity across different context was found, it did not necessarily mean that correlations across different contexts do not exist. The correlations of behavioural traits across context can be presented only during some phases in ontogeny.

PCA analysis revealed relationships between different tests. Based on the results, animals created group with similar characteristics. Two (or 3) groups could be found. The smaller group could be characterized by high exploration score. The animals that belong to this group visited more than 100 squares in the NE test and also in the EPM test was active. For the 3 females (a possible third group – no. 1, 2 and 8) was typical the long approach latencies to novel objects, so they were more slow exploring.

The data obtained from this research indicate the presence of the personality in domestic guinea pigs. The results from the novel objet tests were repeatable and also the exploration activity in the novel environment tests was correlated between two trials over time. But the data-set is limited. In this diploma thesis it was not possible to test behavioural stability over time in all behavioural tests because this research was time limited and the animals were not allowed to stay in the Demonstrational and Experimental Stable for a longer period. Also due to time possibilities only 24 individuals (13 males and 11 females, 2 females died shortly after transportation to the Stable probably due to bad health conditions in combination with the applied stress) were tested. The research was authorized only for non-invasive methods, so we could not measure physiological data (such as cortisol level from blood samples). Zipser et al. (2013) highlighted in their personality study in domestic guinea pigs that it is important to also involve physiological parameters that could help better understand differences in animal behaviour.

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6 CONCLUSION

The guinea pigs significantly differed in their exploratory behaviour. The more explorative animals that visited more squares in the novel environment also visited more arms of the elevated plus-maze. Positive correlations were found between their approach latencies to novel objects and between the proportions of time spent showing interest in these objects. That indicates that this test is repeatable. The time needed to leave the protected area after isolation was related to the animals' approach latencies to novel objects. The animals that hesitated longer to leave the shelter after stressful isolation from their group also needed more time to investigate novel objects. Almost any vocal reaction of the guinea pigs to the predator was measured and the behavioural reactions were mainly uniform (i.e., the animals generally displayed low activity), so it could be concluded that this test is not sufficient for determining personality in guinea pigs. Females and males did not differ in their performance almost in any test from the personality set, possibly due to their low age.

Not all the tested parameters were correlated between different contexts, but relationships between some of them were found (e.g., the data from the isolation tests and the novel object test or the data from the novel environment test and the elevated plus-maze test). Behavioural traits obtained from the novel object tests were repeatable, and the data from the novel environment tests (valid for 15 individuals) positively correlated over time as well. It was not possible to repeat all the tests from the personality set or to measure the physiological data, but we plan to continue guinea pig research in the future. To conclude, nevertheless, the measured data indicate the existence of personality in guinea pigs. According to the data-set obtained from the PCA analysis, the individuals formed mainly two (or three) groups (one group included 3 females) with similar characteristics. It was not possible to repeat all the tests from the personality set or to measure the physiological data, but we plan to continue guinea pigs research in the future.

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ANNEX1 The correlation matrices between squares visited in periods of 10-, 30-, 60- minutes in the novel environment test. Highlighted data with red colour show correlations.

Variable	Squares visited in 30 minutes	Squares visited in 10 minutes	Squares visited in 60 minutes
Squares visited in 30 minutes	1.000000	0.847333	0.880276
Squares visited in 10 minutes	0.847333	1.000000	0.616173
Squares visited in 60 minutes	0.880276	0.616173	1.000000

ANNEX 2 The species scores for each behavioural trait –adjusted for species variance for the first PCA analysis with 16 evaluated parameters. Eingenvalues included.

N	Name	Axis 1	Axis 2	Axis 3	Axis 4
	Eigenvalues	0.2782	0.1554	0.1377	0.1135
4	TotSqNE	0.6634	0.1918	0.0631	-0.4704
5	OutSqNE	-0.5458	0.4846	-0.0147	0.0149
6	HouLeaIZ	-0.3618	-0.6675	0.2971	0.2085
7	OutTimeIZ	0.3819	0.3476	-0.0676	-0.3068
8	OutSqPRE	-0.3821	0.7387	-0.2291	0.2407
9	SqNuPRED	0.0678	-0.5751	0.0713	-0.0212
10	ApNO1	-0.4472	0.3477	0.6954	-0.1687
11	IntNO1	0.7072	0.0232	-0.4313	0.4079
12	ApNO2	-0.2157	0.0786	0.8585	0.2817
13	IntNO2	0.5939	0.2149	-0.3193	0.4601
14	ApNO3	-0.3961	-0.2382	-0.0047	-0.3787
15	IntNO3	0.3982	0.1177	0.3186	0.7598
16	ArmsEPM	0.8197	0.1444	0.3879	-0.0594
17	CenCroEPM	0.7257	-0.028	0.4632	-0.2105
18	DungsNE	0.5298	-0.4011	-0.2333	-0.3586
19	UrineNE	-0.0529	0.6912	-0.0542	-0.3538

ANNEX3 The sample scores for each individual for the first PCA analysis with 16 evaluated parameters for 24 individuals. Eingenvalues included.¨

N	Name	Axis 1	Axis 2	Axis 3	Axis 4
	Eigenvalues	0.2782	0.1554	0.1377	0.1135
1	1 F	-0.1344	-0.4218	3.6936	1.925
2	2 F	-0.1362	-0.0832	1.3456	0.1674
3	3 F	-1.654	2.172	0.0026	0.1053
4	4 M	-1.2948	-0.2255	0.0399	-1.8867
5	6 F	2.5159	0.6829	0.0571	0.527
6	7 F	-0.6307	0.6989	-0.4101	-0.5607
7	8 F	-0.5711	0.8786	1.1963	-0.349
8	9 F	-0.0675	1.4892	-1.0551	0.886
9	10 F	0.5299	-0.2469	-0.1768	-0.3652
10	11 F	0.9287	-0.1626	0.1371	-1.2541
11	12 M	-1.2142	-0.8845	0.2416	0.1945
12	13 M	-1.1921	1.9063	-0.2252	0.1694
13	14 M	-0.6335	-2.0474	-0.2117	0.0943
14	15 M	-0.7381	-1.6529	-0.2877	-0.2819
15	16 M	1.2479	0.4388	0.2695	-1.2566
16	17 M	-0.5396	-1.3835	-0.729	0.3905
17	18 M	-0.2239	-0.6542	-0.0858	-0.8994
18	19 M	-0.5556	-0.4112	-0.856	0.2965
19	20 M	0.1359	-0.6159	-0.9162	0.1106
20	21 M	0.9127	0.5776	0.7739	-1.9355
21	22 F	0.3254	0.301	-1.2117	0.969
22	23 F	0.0439	0.1066	-1.058	2.3536
23	24 M	1.5156	-0.3882	-0.1453	0.6812
24	25 M	1.4298	-0.074	-0.3886	-0.0811
25	ORIGIN	-0.8435	-0.3545	-0.7781	-0.0346

ANNEX4 The species scores for each behavioural trait –adjusted for species variance for the second PCA analysis with 7 evaluated parameters. Eingenvalues included.¨

N	Name	Axis 1	Axis 2	Axis 3	Axis 4
	Eigenvalues	0.312	0.2169	0.1904	0.1354
4	TotSqNE	-0.7312	0.4088	-0.0413	0.2851
5	HouLeaIZ	0.59	0.0661	-0.5573	-0.3969
6	OutSqPRE	0.1085	-0.3167	0.845	-0.0464
7	ApNO1	0.6209	0.535	0.3867	0.3257
8	ApNO2	0.5808	0.6969	0.1732	-0.2412
9	ApNO3	0.5104	-0.1349	-0.3473	0.7366
10	ArmsEPM	-0.5533	0.6756	-0.0786	-0.0175

ANNEX5 The sample scores for each individual for the second PCA analysis with 7 evaluated parameters for 24 individuals. Eingenvalues included.¨

N	Name	Axis 1	Axis 2	Axis 3	Axis 4
	Eigenvalues	0.312	0.2169	0.1904	0.1354
1	1 F	1.982	2.9669	0.0748	-1.7791
2	2 F	0.5065	0.849	0.345	-0.0146
3	3 F	0.9493	-0.3065	2.639	0.0469
4	4 M	1.8676	-0.2122	-0.6507	3.6303
5	6 F	-1.4137	1.2516	-0.4028	-0.0512
6	7 F	0.1696	-0.5469	0.3479	-0.4128
7	8 F	0.8159	1.4522	0.7395	0.5268
8	9 F	-0.4245	-1.0326	1.6612	-0.1026
9	10 F	-0.3955	-0.4065	-0.1011	-0.1775
10	11 F	-1.2982	0.4417	-0.3234	0.194
11	12 M	1.0865	-0.402	-1.3479	0.0362
12	13 M	0.3308	-0.5924	2.1542	-0.1985
13	14 M	0.642	-0.527	-1.1263	-1.5004
14	15 M	1.1009	-0.6294	-1.6738	0.2913
15	16 M	-1.6584	0.9357	-0.1983	0.3025
16	17 M	0.5162	-0.8347	-1.2208	-1.5037
17	18 M	-0.3161	-0.5399	-0.4132	0.4745
18	19 M	-0.1385	-1.0664	-0.1099	-0.2051
19	20 M	-0.3691	-0.7336	-0.2325	-0.3948
20	21 M	-1.0744	1.3527	0.1154	1.2367
21	22 F	-0.2874	-0.8863	0.125	-0.259
22	23 F	-0.088	-1.1776	0.3868	-0.3407
23	24 M	-0.9811	0.2986	-0.4025	-0.2444
24	25 M	-1.5224	0.3456	-0.3855	0.4452
25	ORIGIN	-0.1902	-1.0631	-0.2175	-0.5051

ANNEX6 The species scores for each behavioural trait –adjusted for species variance for the third PCA analysis with 9 evaluated parameters. Eingenvalues included.¨

N	Name	Axis 1	Axis 2	Axis 3	Axis 4
	Eigenvalues	0.3174	0.2217	0.1774	0.1095
4	TotSqNE	0.8085	-0.361	0.2446	0.3488
5	HouLeaIZ	-0.6624	-0.2765	0.522	-0.0968
6	OutS qPRE	-0.0707	0.5565	-0.3806	0.3617
7	ApNO1	0.1618	0.7596	0.5459	0.1686
8	ApNO2	-0.465	0.6364	0.4181	0.2723
9	ApNO3	-0.4541	-0.1538	0.5722	-0.3113
10	ArmsEPM	0.8312	0.2019	0.2666	-0.0738
11	TotSqNE2	0.7773	0.0019	0.3909	-0.2811
12	Weight	0.1248	0.6574	-0.3086	-0.6631

ANNEX7 The sample scores for each individual for the third PCA analysis with 9 evaluated parameters for 15 individulas. Eingenvalues included.¨

N	Name	Axis 1	Axis 2	Axis 3	Axis 4
	Eigenvalues	0.3174	0.2217	0.1774	0.1095
1	2F	-0.3492	2.3882	1.0038	0.2923
2	12M	-0.8742	-0.0819	1.4458	-1.1536
3	13M	-0.3907	2.041	-0.8337	1.9183
4	14M	-0.9096	-0.4419	0.4366	0.4746
5	15M	-1.5505	-0.6349	1.5474	-0.3961
6	16M	1.6139	-0.4546	0.1967	0.4711
7	17M	-0.9894	-0.9267	-0.1082	0.4012
8	18M	-0.1115	-0.6867	0.0652	0.3268
9	19M	-0.4079	-0.5488	-1.0602	0.0633
10	20M	-0.18	-0.2614	-1.1443	-0.3038
11	21M	2.2425	0.3221	1.5927	-0.2432
12	22F	0.0706	0.2787	-1.2992	-1.2767
13	23F	-0.0997	0.2847	-1.2873	-0.7863
14	24M	1.0622	0.2389	-0.4988	-1.7114
15	25M	0.8734	-1.5166	-0.0566	1.9234
16	ORIGIN	-0.6641	-2.6831	-0.0689	4.2151