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Interindividuální rozdíly v chování laboratorních potkanů

Inter-individual differences in behaviour of laboratory rats

Diplomová práce

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literature. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Abstrakt

Stále častěji se ukazuje, že ačkoliv jsou pokusná zvířata vystavena stejným podmínkám, jejich chování se mezi sebou liší. Pokud jsou tyto rozdíly stabilní v čase a napříč různými situacemi, můžeme mluvit o personalitě. Tato diplomová práce testuje interindividuální rozdíly v chování laboratorních potkanů (kmene Long Evans) v sérii experimentů prováděných v rané onotgenezi a v dospělosti. Kromě stanovení interindividuálních rozdílů v chování a personality jedinců si tato práce ukládá dva hlavní cíle. (1) Stanovit stabilitu personality napříč ontogenezí. (2) Zjistit, zda interindividuiální rozdíly v chování souvisí s výkonem v kognitivních testech.

Potvrdili jsme existenci interindividuálních rozdílů v chování u laboratorních potkanů. V této sérii experimentů jsme však nebyli schopni určit personalitu jednotlivých pokusných jedinců. Rozdíly v chování jedinců byly nejlépe vyjádřené chováním v open field testu a ve vyvýšeném křížovém bludišti (Elevated plus maze test). Naše výsledky také ukazují, že v jednotlivých opakováních těchto testů se zvířata chovají rozdílně. Dále jsme zjistili, že chování v testu aktivního vyhýbání se místu na rotující arénce a v Morrisově vodním bludišti spolu nesouvisí, ačkoliv oba tyto experimenty testují kognitivní schopnosti. Korelace parametrů z testů aktivního vyhýbání se místu na rotující arénce, jumping testu, open fieldu a vyvýšeného křížového bludiště možná ukazuje na odvahu či ochotu riskovat (boldness). Korelace chování v Morrisově vodním bludišti a latence udržení se na hrazdě (bar holding test) může vyjadřovat motivaci nebo flexibititu daného jedince.

Klíčová slova: personalita, interindividuální rozdíly, potkan, kognice, behaviorální testy, ontogeneze

Abstract

Number of studies report that even when experimental animals are subjected to the exact same conditions, they differ in their behaviour. If these differences were stable in time and across several experimental procedures, we could talk about personality. This diploma thesis studies inter-individual differences in behaviour of laboratory rats (Long Evans strain) in a series of experiments conducted in early ontogeny and in adult age. Apart from analysing inter-individual differences in behaviour and personality of experimental animals, this thesis has two main aims. The first aim is assessing stability of inter-individual differences in behaviour throughout ontogeny. The second aim is to explore possible link between inter-individual differences in behaviour and performance in cognitive tests.

We confirmed the existence of inter-individual difference in behaviour in laboratory rats. In this thesis we were, however, not able to assess personality of experimental animals. The differences in behaviour were best described by behaviour in Open field test and Elevated plus maze test. Our results also show marked differences between successive trials of these experiments. We also report that performance in Active allothetic place avoidance is not linked to performance in Morris water maze, even though both of these experiments test cognition. Correlation among performance in Active allothetic place avoidance, Jumipng test, Open field test and Elevated plus maze test might represent boldness of our experimental animals. Correlation among performance in Morris water maze test and latency to hold on to the bar in Bar holding test might represent motivation or behavioural flexibility of an individual.

Key words: personality, inter-individual differences, rat, cognition, behavioural tests, ontogeny

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1 Introduction

The aim of this thesis was to test inter-individual differences in behaviour in laboratory rats (Long Evans strain) in a series of experiments. Concerning this topic we had two main goals in our research. The first one was to test stability of inter-individual differences in behaviour throughout ontogeny. The second goal was to explore whether there is a link between inter-individual differences in behaviour and performance in cognitive tests.

We subjected our experimental animals to a series of experiments. We concentrated on two timeframes within ontogeny. The first period we concentrated on was juvenile age, when we performed a series of sensorymotor tests. In the second testing period, adult age (from three months of age) we subjected the animals to another series of experiments.

1.1 Personality

For a long time people have noticed that individuals of the same species do not behave in the same way, that they are somehow different. This variation can be detected even in laboratory animals, where the different strains are a product of selection and they are therefore as uniform as possible.

Inter-individual differences which are consistent in time and throughout contexts, are called personality. Personality is a “higher-order construct”, which refers to how an individual behaves in response to its environment (Capitanio *et al.*, 2008). Because behaviour of an animal can be influenced by many factors and on the other hand it also influences many factors in the life of the animal, personality studies connect various research areas. Personality research comprises of rather longitudinal studies, focusing on several experimental paradigms and several behaviours. Many of these studies also aim to integrate differences in behaviour with proximate (genetic background, maternal care and others) and ultimate mechanisms (ecology and evolution) (Carere and Eens, 2005).

There are, however, more terms which describe the same phenomenon. In physiology, it is called coping style and this point of view focuses on differences in physiology regarding coping with stress and environmental demands (Koolhaas, De Boer and Bohus, 1997; Koolhaas *et al.*, 1999). Temperament is a label used in psychology and sometimes overlapping to behavioural sciences (Réale *et al.*, 2007).

Behavioural profile is a term which includes both behaviour and physiology of the animals (Groothuis and Carere, 2005).

There is vast theoretical framework regarding development and preserving inter-individual differences in behaviour. For example Stamps (2003) suggests quite simple and intuitive concept which has four basic assumptions. When (1) the environment is heterogenous, (2) each individual prefers certain niche within the environment, (3) living in the environment and experiences influence subsequent performance in the niche and (4) individuals prefer the niche where they are most successful, therefore the initial choice of niche within an environment will not only allow but enhance development and maintenance of different personalities. This view is consistent with so called "preference induction" which describes tendency of adult individuals to select the same type of habitat for breeding as was the one they grew up in (Stamps, 2003).

Carere and colleagues (2010) summarized four most frequent explanations of maintenance of personalities in populations. (1) Differences in personality do not correspond to differences in fitness. This notion was, however, contradicted by long-term personality studies. (2) Selection is not constant but rather it fluctuates unpredictably. Therefore even when individuals behave rather consistently, selection could preserve differences in behaviour and personalities. (3) Personality is linked to "underlying state differences" which influence trade-offs of different behaviours. (4) Personalities and differences in behaviour might be preserved by frequency-dependent selection. In this scenario, certain personality type is favoured when it is rare and evolution can therefore lead to more or less stable mixture of personality types.

1.2 Axes

Personality studies claim that individuals are not identical in their behaviour. Therefore scientists had to come up with a framework of how to describe the behaviour and discriminate between individuals. First it was done in humans. Several theoretical frameworks and schemes were introduced in regard to human personality research. Here I will describe only one of them, which is highly relevant to animal studies because it was used in a very interesting cross-species review. The Five factor model (or Big Five) consists of five bipolar axes or factors. Each axis summarizes several characteristics, which include many behavioural traits. Axes included in the Five factor model are: N (neuroticism, nervousness, negative affectivity), A (agreeableness, altruism, affection)

E (extraversion, energy, enthusiasm), O (openness, originality, open-mindedness), C (conscientiousness, control, constraint). For example the axis Extraversion would include characteristic such as sociability which could be specified as talkative or outgoing personality (Gosling and John, 1999).

In a cross-species review, Gosling and John (1999) reported that dimensions N (neuroticism), A (agreeableness) and E (extraversion) are general across species. Dimension A was in animal studies described as affinity and affection representing one extreme of this dimension and aggression or hostility representing the opposite extreme. Another often identified dimension was O (openness), related to behaviours described as curiosity and playfulness. Dimension C (conscientiousness) was found only in humans and chimpanzees. These authors added two additional axes, described in many animal studies – activity and dominance. It turned out that activity is ambiguous factor, as only two studies supported it as a distinct factor. Dominance, on the other hand, appeared as a separate factor in many studies (Gosling and John, 1999).

Cross-species reviews are not the only way how to link animal studies with human studies. Comparative psychology uses two main approaches of relating human and animal research. In the bottom-up approach, scientists generate hypotheses about humans, basing them on findings from animal models. In the top-down approach, animal models are adopted to experimentally test the details of our knowledge from human research (Gosling, 2001).

For animal studies, five axis paradigm was also introduced (Réale *et al.*, 2007):

- Boldness – shyness: reaction to risky situation, which is not new (also called docility, tameness, fearfulness)
- Exploration – avoidance: reaction to a new situation (new habitat, novel food or object)
- Activity: general level of activity
- Aggressiveness: agonistic interactions with conspecifics
- Sociability: tendency to associate with conspecifics

Each axis represents a continuum of a trait. Therefore for example bold and shy individuals represent extreme cases of the trait and many individuals would score somewhere along the continuum instead. Some researchers also consider dominance axis in their experiments (Gosling, 2001).

In personality research, there are, however, many unresolved questions and issues, mostly regarding definition of axes, construct and discriminant validity of experiments. Consensus about the definition of each axis was not found yet. The most problematic in this regard is boldness. All researchers agree that it is a propensity to take risks. However some consider boldness as a reaction to novel stimuli and situation and others acknowledge propensity to take risks only in familiar environment.

Construct validity describes whether two tests measure the same trait. This is a common problem in personality studies because researchers use different tests to refer to the same trait, but when they try to correlate results of these experiments, they often find out that the correlations are low or non-existent (Walsh and Cummins, 1976; Belzung and Le Pape, 1994). For example to measure boldness, researchers use paradigms such as Novel object recognition, Novel environment test or response to predation risk. However results for these experiments do not correlate and seem to represent different traits (Carter, Marshall, *et al.*, 2012). Discriminant validity, on the other hand, refers to a question whether two experiments measure different traits.

Even though these issues exist and we have to acknowledge them, there is still unequivocal evidence that differences between individuals exist. When we ask to arrange individual on a trait relative to other individuals, results from independent observers agree across a range of species. The easiest and most straightforward explanation of this phenomenon is that observers base their decisions on existing differences between individuals in the characteristic they are rating. Moreover personality studies using rating techniques give the same results as studies which analyse behavioural parameters from video recordings or direct observation (Gosling, 2001). One study even showed agreement in personality characterization even though observers could choose freely on which axis and how to describe the individuals (Wemelsfelder *et al.*, 2000).

1.3 Behavioural syndromes

Generally, we talk about behavioural syndrome when two axes correlate together. There are, however, two prevailing opinions regarding the definition of behavioural syndromes. The first one, domain specific correlation, is a link between two different behavioural traits. The second one, domain general correlation, is a correlation between the same behavioural traits in different environmental contexts (Sih *et al.*, 2004; Reale *et al.*, 2010).

Physiologists distinguish two different behavioural syndromes, which are based on differences in coping with stress. Individuals adopting proactive strategy are aggressive, bold and show fight or flight behavioural response to danger. Proactive animals dominate in stable environment. Reactive animals, on the other hand, are less aggressive, show freezing behaviour when exposed to risky situation and they perform better in changing environment (Koolhaas *et al.*, 1999).

The existence of behavioural syndromes implies limited plasticity of behaviour or so called "behavioural carryover" (Sih *et al.*, 2004). It can explain behaviour that would be described as suboptimal, when judged alone. But when we put the behaviour in a larger context, we might find out that several traits are correlated in the animal and therefore perfect adaptation to one situation might limit adaptation to another situation or context.

Sih and colleagues (2004) therefore argue, that behavioural syndromes "might have important ecological implications". They can generate trade-offs which influence the ability to cope with changing or limiting environmental factors. Moreover, they might influence population and community ecology by linking personality to birth, death and dispersal.

1.4 Proximate causes of personality

Personality research is biologically valid, because it revealed several links between behavioural traits and underlying factors such as genetics and physiology (Réale *et al.*, 2007). Some of the proximate factors influencing personality are for example genetic background of the animal, maternal care, level of hormones and differential activity of brain. The exact links and their strength are in most cases, however, still unclear. Detailed description of proximate studies of personality are reviewed in Carere and Maestripieri, 2013.

1.5 Ultimate causes of personality

Behavioural traits are linked to ecologically important traits. Personality research is therefore ecologically valid and it offers a means to link personality with ecological factors and selection (Réale *et al.*, 2007). Moreover, behavioural syndromes were reported to affect factors related to birth, death and dispersal, which happen to be major factors influencing dynamics of populations (Sih *et al.*, 2004).

It has been shown, that personality can influence the probability of trapping an individual, therefore creating sampling bias (*Gryllus campestris* – Niemelä, Lattenkamp and Dingemanse, 2015, *Agama planiceps* – Carter, Goldizen and Tromp, 2010; Carter, Heinsohn, *et al.*, 2012).

Personality can also affect life-history strategies (*Parus major* - Cole and Quinn, 2012, *Tamias striatus* - Careau *et al.*, 2015), survival of individuals (*Gryllus campestris* - Niemelä, Lattenkamp and Dingemanse, 2015), foraging (*Dama dama* - Bergvall *et al.*, 2011), *Tringa tetanus* - Couchoux and Cresswell, 2012, *Parus major* – Aplin *et al.*, 2014), number and strength of social interactions (*Poecilia reticulata* - Croft *et al.*, 2009, *Parus major* - Aplin *et al.*, 2013), predator inspection (*Chlorocebus pygerythrus* - Blaszczyk, 2017), reproduction (*Tachycineta bicolor* - Betini and Norris, 2012), emergence of stereotypic behaviour (Ijichi, Collins and Elwood, 2013), health and progression of disease (Cavigelli, 2005; Capitanio *et al.*, 2008).

1.6 Repeatability

One of the premises behind personality research is stability of behaviour in time. It is, therefore, crucial to assess whether behaviour of individuals is repeatable during observational studies or experiments. First calculations of repeatability come from quantitative genetics. This measure was used for assessing variance among individuals. Later on repeatability started to appear also as a means for calculating reliability of repeated measurements on the same individual and to determine an upper limit to heritability (Lessells and Boag, 1987). In nowadays personality research it is customary to include repeatability values of observed behavioural elements. Two questions can be addressed by assessing repeatability – repeatability of the test and repeatability of the behaviour of individual animal in the test.

For data with Gaussian distribution, repeatability can be calculated from correlation coefficients, variance components from the Analysis of variance (ANOVA) or from Linear Mixed Models (LMM) (Nakagawa and Schielzeth, 2010). Most commonly used is probably the ANOVA based calculation of repeatability:

(1) $r = S^2_A/(S^2+S^2_A)$

where S^2_A represents variance among groups and S^2 the within-group variance (Lessells and Boag, 1987). In personality research, a group is represented by one individual. This formula can therefore be transcribed as inter-individual variability divided by all variability (both within individuals and among them). In ANOVA based repeatability calculations, the variance components are calculated from mean squares:

(2) $S^2 = MS_W$

(3) $S^2_A = (MS_A - MS_W)/n_o$

where n_o represents a coefficient related to the sample size per group (Lessells and Boag, 1987).

1.7 Ontogeny of personality

A few years ago an important question was introduced to personality studies. What exactly do we mean by stability or consistency of behaviour? Does behaviour have to be stable through whole life span (which was the original idea behind the concept of personality), or is it enough when certain behaviour is stable only over some period during life? Can we speak about personality in cases like this?

In their review focusing on stability of personality throughout ontogeny, Groothuis and Trillmich (2011) claim that the original notion of lifelong stability of behaviour is inconsistent with current theoretical frame in personality research. Findings from studies regarding ontogeny of personality do not agree with this premise as well. These authors also point out that most personality studies neglect the complexity of ontogeny and changes related to it. They also introduce four points or key issues that should be considered when discussing stability of personality traits: (1) Life history traits are not stable throughout life and an animal has to be well adapted to its ecological niche in order to survive and reproduce. Young individuals should therefore have different adaptations than adult individuals of the same species. (2) As correlations between behavioural traits are thought to pose a bias on development of optimal behaviour in evolution (Sih et al 04), the emphasis on stability may cause us to look away from changes in personalities. As a result of this, evolutionary explanations of personality could be misinterpreted. (3) Linking proximate mechanisms to particular changes in personality could help us understand the development of personalities and their plasticity. (4) Many researches are convinced that personalities are strongly influenced by genetics and therefore they underestimate interactions of genes with other factors and the fact that the expression of genes and their transmission to the next generation is realized through behaviour of the animal.

These authors (Groothuis and Trillmich, 2011) also argue that research regarding proximate mechanisms of personality does not suggest stability throughout entire life of animals. On the other hand, they suggest the existence of one or more specific sensitive phases later in life (for example sexual maturity or first reproduction), which may serve as a "consolidation phase" for emerging personality traits. They propose that in this sensitive period, an animal either confirms its previous experience or modifies its behaviour according to new situations end experiences.

Only a few studies addressed the issue of stability of personality throughout ontogeny. Studies with invertebrates as experimental subjects show quite consistent results. Field crickets *(Gryllus integer)* tested for boldness across metamorphosis (each individual was tested once in a nymphal stage and once as an adult) show repeatable latency to emerge from shelter. However, this repeatability was significant only for female crickets. Males seem to become more cautious after maturation (Hedrick and Körtet, 2017). Firebugs *(Pyrrhocoris apterus)* tested in the Open field test (activity and exploration) and latency to emerge from shelter (boldness) showed repeatable results and unchanged correlation structure of variables across ontogeny and final ecdysis (Gyuris, Feró and Barta, 2012). Experiments with dumpling squid *(Euprymna tasmanica)* revealed phenotype dependent plasticity in development of personality traits. This experiment explored stability of boldness in threat and feeding contexts during life span of the squid. In juvenile age and during sexual maturation, boldness remained constant, but after sexual maturation it changed. When exposed to threat, the behaviour of shy squid remained consistent even through maturation. The behaviour of bold squid, on the other hand, changed during ontogeny (Sinn, Gosling and Moltschaniwskyj, 2008).

Studies in fish revealed personality changes throughout ontogeny. In mangrove killifish *(Kryptolebias marmoratus)* both boldness and exploration changed during ontogeny. Scores of both of these traits increased until sexual maturity when they stabilized (Edenbrow and Croft, 2011). Bell and Stamps (2004) studied activity in unfamiliar environment, boldness under predation risk and aggressive behaviour in three-spined sticklebacks *(Gasterosteus aculeatus)* at three stages during ontogeny (juvenile, subadult and adult age). They found that neither of these traits was stable throughout ontogeny. However in one of the studied populations, positive correlation between boldness and aggression remained stable during the whole experiment.

Wilson and Krause (2012) tested whether inter-individual differences show consistency across ontogeny in lake frog *(Rana ridibunda)* even though niche occupied by tadpoles differ markedly from niche of metamorphosed frogs. They found that individual differences in exploration (open arena test) and activity (measured in a housing box) were positively correlated across metamorphosis and both of these traits were linked to refuge use.

Šimková and colleagues detected a phase in life of northern common boas (*Boa imperator*) when some behavioural traits may change. In this study, feeding behaviour of boas was modified during maturation. Defensive behaviour, on the other hand, remained constant throughout ontogeny (Šimková *et al.*, 2017).

Studies with birds as experimental animals, show changes in personality during ontogeny. In zebra finches (Taeniopygia guttata), fearlessness (derived from tonic immobility test) and exploration in novel environment were repeatable within life stages (subadult, young adult, mature adult), but not across life stages. In contrast, aggression against mirror was repeatable across life stages but not within them. During young adult stage, a behavioural syndrome consisting of aggression, activity in home cage and boldness towards novel object emerged (Wuerz and Krüger, 2015). Favati and colleagues (2016) reported low consistency in behaviour during ontogeny in red junglefowl *(Gallus gallus)*. Vigilance in predator test stabilized after the birds became independent, level of exploration in novel arena was stable only in adult animals. Authors of this study argue, that individuals of different phenotypes follow divergent developmental trajectories regarding their behaviour and that stable personality differences emerge in adulthood.

Rabbits *(Oryctolagus cuniculus)* showed consistent inter-individual differences in aggression. However, this correlation was quite low (0.32), even though it was estimated by Spearman correlation, which tends to overestimate repeatability (Nakagawa and Schielzeth, 2010; Eccard and Rödel, 2011).

In cavies *(Cavia aperea)*, distance travelled in the Open field test (described by authors as fearlessness) was the only personality trait that remained constant during ontogeny. This might be due to the fact that pups of this species are prekocial and from early age they engage in foraging with their mother. Boldness towards novel object and free exploration did not correlate across ontogeny. However in adult cavies, they are correlated and create a behavioural syndrome which is thought to indicate dispersal tendency in this species (Guenther, Finkemeier and Trillmich, 2014). In yellow-bellied marmots *(Marmota flaviventris)*, individual differences in reaction to trapping and handling remained consistent throughout life (Petelle *et al.*, 2013).

Ray and Hansen (2005) studied the ontogeny of personality in male Wistar rats between 6 weeks of age and 52 weeks of age (standard age when animals are tested is 16 weeks for laboratory rats). Experimental animals were subjected to Hole board test (nose pokes as a measure of exploration) and Canopy test (with locomotion as a proxy for anxiety). The authors reported "substantial behavioural constistency" throughout the whole experiment, however the correlation between these two traits was extremely strong between weeks 11 and 52. In a different study which focused on consistency in behaviour around weaning, Long Evans rats showed stability in latency to jump from a platform in Jumping test. Latency to jump also correlated with exploration in the Open field test and time spent on open arms in the Elevated plus maze test (Rödel and Meyer, 2011).

1.8 Personality, cognition and learning

The aim of many researchers in recent years is to find out whether there is any relationship between personality, cognition and learning. Theoretical framework behind these studies suggests that the way an animal acquires, processes and uses new information does not have to be identical. Neither for individuals of the same species nor for individuals belonging to the same species. And since cognitive abilities, such as attention or learning are reflected in behaviour, it is not surprising that questions about the existence of a link between personality and cognition have been raised. Griffin and colleagues (2015) suggested two possible scenarios of the relationship between personality and cognition: (1) Personality and cognition might correlate because of a common underlying process. (2) Certain personality traits (such as boldness, exploration or activity) might influence the possibility of learning. Either by facilitating or limiting opportunities in which an animal could learn. There are some experimental findings consistent with this second point. For example Amy and colleagues (2012) found that great tits from slow selection line exhibit shorter latencies to land on the experimental platform and Bouquet and colleagues (2015) reported that fast exploring mallards are slower to cross and experimental X maze for reward.

Majority of experimental studies focusing on the link between personality and cognition (or learning) test only single repeatable behavioural trait against single cognitive (or learning) task. Most papers report personality dependent performance in cognitive or learning tasks, thus supporting the notion of a link between them. Some researchers also found sex differences in the existence or strength of this link.

However whenever we measure cognitive abilities of animals, it is important to keep in mind that we actually do not measure cognition but performance in the test and probably also stress induced by handling and testing. Moreover, these experiments are carried out in laboratory conditions, which do not, in the least, resemble conditions in nature. There is also the issue that we cannot measure cognition per se, because we study it through behaviour. And behaviour of individuals can differ. Our perception of cognitive abilities can therefore be influenced by inter-individual differences and personality.

In guppies *(Poecilia reticulata*) only bold male guppies learned to associate a cue (plexiglass ring inserted into fish tank) with food reward (Dugatkin and Alfieri, 2003). Trompf and Brown (2014) reported that sex and personality influence the use of social cues for foraging. In their experiment, only bold female guppies learned to swim quickly and accurately to a foraging zone, which was rich in food during previous phase, when tested individual was observing foraging of other (demonstrator) guppies.

Great tits *(Parus major)* from slow selection line learn to avoid aposematic prey faster and they are also more cautious in subsequent experiment evaluating memory acquired during this test (Exnerová *et al.*, 2010). Amy and colleagues (2012) showed that previous experience affects performance in relearning task onlyin "slow" great tits. Another study on great tits found personality dependent influence on performance only in difficult learning task. This study also found a link between flexibility of learning, the sex of the individual and its personality (Titulaer, van Oers and Naguib, 2012). Bolder black-capped chickadees *(Poecile atricapillus)* learn an association in acoustic discrimination task faster than shy individuals (Guillette *et al.*, 2009). Gibelli and Dubois (2016) reported that less neophobic zebra finches *(Taeniopygia guttata)* learned significantly faster during a discriminant task than individuals which scored higher in neophobia.

In a study with rhesus macaques *(Macaca mulatta)* Coleman and colleagues (2005) showed that exploratory females can be trained to touch a target more easily than less exploratory females.

Even though it is nowadays customary to check for repeatabilities in personality studies, there is no such trend in research regarding cognition. However when scientists argue, that cognitive abilities of an individual and its personality are linked, one would assume that cognitive performance of this individual should also be stable. The first step of such analyses should therefore be identification of stability in cognitive abilities of individual animals. Only then should researchers look for repeatable behavioural effects correlated with cognition (Griffin, Guillette and Healy, 2015).

There is, however, one rather important confounding factor in the search for links between personality and cognition – motivation. Differences in motivation among experimental animals may be of critical importance when assessing individuals' performance in cognitive or learning tasks (Griffin, Guillette and Healy, 2015). The relevance of this issue was experimentally demonstrated in study on pheasant chicks, *Phasianus colchicus* (van Horik and Madden, 2016).

Another intriguing issue in personality research is assessment of hierarchy. Some authors consider hierarchy as another axis which should be recognized in personality research (Gosling and John, 1999). However the question whether it should be considered as a separate axis is quite complicated. Hierarchy is not a behavioural trait characteristic for the individual, because it is influenced by social environment in which the individual lives. Level of dominance actually displayed by an animal combines inherent tendency in regard to interaction with conspecifics and the effect of social situation in which the animal is.

1.9 Personality research in laboratory animals

Even though there are hundreds of papers (for review see Carere and Maestripieri, 2013) focusing on personality of laboratory animals (rodents in particular), only a few of them study personality in complex series of experiments. Majority of studies subject animals to only one or two experimental procedures in order to test personality (Carere and Maestripieri, 2013). This might be influenced by the fact, that results from tests, which should measure the same trait usually do not correlate with one another (Walsh and Cummins, 1976; Belzung and Le Pape, 1994; but see also Žampachová *et al.*, 2017).

Most studies aim to unravel the link between personality and its proximate or ultimate mechanisms (Carere and Maestripieri, 2013). Number of researchers study physiology or activity of different brain regions as these seem to be important factors underlying inter-individual differences in behaviour (for example Capitanio *et al.*, 2008; Baugh *et al.*, 2012; Castro *et al.*, 2012). Other researchers focus on links between personality and ecology or evolution, for example foraging, various life-history traits or survival (Carter, Heinsohn, *et al.*, 2012; Aplin *et al.*, 2013, 2014; Niemelä, Lattenkamp and Dingemanse, 2015 and others). In recent years, the focus of personality research has somewhat shifted towards inspection of consistency in behaviour and ontogeny of personality.

Experiments commonly used in rodent personality research are: open field test, novel object recognition test, elevated plus maze test (or other experiments assessing anxiety of animals) and some form of social interaction (Žampachová, Landová and Frynta, 2017).

1.10 Selected behavioural tasks

In this section I would like to briefly introduce experimental methods, which are frequently used in the laboratory, where this thesis was performed (Institute of Physiology, Czech Academy of Sciences). We used these procedures because we wanted to focus on the influence of inter-individual differences in behaviour on performance in various tests and their interpretation.

1.10.1 Ultrasonic vocalization

When separated from their mother, rodent pups elicit ultrasonic calls (detected in all species in Cricetidae and Muridae where ultrasonic vocalization of pups has been studied). These calls are an expression of the pups' negative affective state. When heard by the mother, this type of vocalization should induce maternal behaviour towards the abandoned pup. In rat pups, four types of vocalization with distinct waveform were reported. Mean frequency of these calls is around 40 kHz (Hashimoto *et al.*, 2004; Portfors, 2007).

Recording juvenile vocalization is not usually a part of personality studies. It can, however, potentially influence behaviour of animals. Vocalization of pups is a signal of stressful situation and it induces maternal behaviour. And because maternal care is one of many parameters influencing behaviour of individuals (Carere and Maestripieri, 2013), differences in juvenile vocalization could be linked to differences in maternal care and thus to differences in behaviour. As S.A.Brunelli and her research group demonstrated, rats selected on the basis of juvenile vocalization show distinct personality (or coping styles) throughout ontogeny. Individuals with high juvenile vocalization scores can be described as “anxious or depressed”. Scarcely vocalizing animals, on the other hand, are more aggressive (Brunelli, 2005; Brunelli and Hofer, 2007).

1.10.2 Geotaxis

Experimental investigation of geotaxis is supposed to demonstrate “unlearned response to gravitational cues” (Motz and Alberts, 2005). When an animal (usually rodent pup) is placed on an inclined plane, it first carefully scans the environment and then slowly moves and starts to climb. This experiment tests sensory and proprioreceptive abilities of the pup (Motz and Alberts, 2005).

However at a closer look, this experiment has undergone serious discussion and design changes since the introduction of this paradigm. When it was created by J. Crozier and G. Pincus, the slopes diverged between 15° and 70° and the surface of the inclined plane was wire mesh. Pups about 13 days old were put on the plane horizontally to the slope. With this arrangement the pups displayed positive geotaxis (Crozier and Pincus, 1929). Later on, J. Altman and K. Sundarsham used 15°or 25° incline and plywood surface. They tested animals aged 1 – 9 days and put them facing downhill onto the plane (Altman and Sudarshan, 1975). Knieder and Blumberg tested several slopes (30, 35, 40 and 45°) with wire mesh surface. Again they used older pups (12-14 days) but in all four possible orientations (head up, head down, horizontally). They found out that pups insert their claws into the wire mesh to stabilize their position on the inclined plane and this is best done when the pup is oriented head up towards the slope. When they repeated the same experiment but with smooth high-friction material instead of wire mesh, the pups did not show negative geotaxis but moved rather randomly. These experiments made it very clear that the surface is a crucial factor influencing the behaviour of the rats (Kreider and Blumberg, 1999). Alberts and colleagues (1984 in Motz and Alberts 2005) reported positive geotaxis, when the incline was low (2°, 4°, 8°), but the pups first scanned the environment and crawled slowly until they touched a wall. Only then did they turn downhill and started to climb.

1.10.3 Bar holding test

Bar holding experiment evaluates motor abilities of rodent pups. The pup is hanged on the bar by its front paws and its behaviour (for example if it is able to pull up to the bar and put its hind paws on to it or even climb on top of the bar) and latency to hold on to the bar are recorded (Mikulecká and Mareš, 2002).

1.10.4 Jumping test

In the Jumping test, an animal is placed on a platform, which is located between two boxes – home cage (with the rest of the pups inside it) and an empty box filled with bedding but empty and free of odours. The individual has to decide in which box it is going to jump. The latency to jump into one of the boxes and its behaviour is recorded (Mikulecká and Mareš, 2002).

1.10.5 Spontaneous alternation

Spontaneous alternation was invented to study animal's tendency to explore novel environment. When put into Y or T shaped maze, the animal chooses one closed arm to explore or hide into. When the same animal is put in the maze again, it usually enters the other arm (the one which it did not enter in the first trial). This behaviour is called alternation. Both rats and mice alternate in this test, however alternation decreases with increasing number of trials (Lalonde, 2002).

Some researchers use this procedure to test exploratory behaviour, others use it to study working memory and perception and some scientists use spontaneous alternation paradigm in reinforcement training. Our initial plan, however, was to use this test for another purpose. We included this test in our study to check for laterality (if individual animals are right oriented, left oriented or if they have no side preference).

1.10.6 Open field test

Open field test was created by Calvin Hall with an aim to study emotional reactivity in rodents. For the concept of emotionality, two parameters of the test were crucial – distance travelled (sometimes referred to as activity) and defecation. Emotional individuals were the ones which did not move around the arena so much but defecated a lot (Hall, 1934 in Prut and Belzung, 2003). Due to this experiments' simplicity and straightforward analysis it became popular among scientists (Walsh and Cummins, 1976). Even though the Open field test was originally created for rodents, nowadays a wide spectre of species are tested in this paradigm and it represents a means for testing emotionality, activity and anxiety (Prut and Belzung, 2003).

Even though the original test was very simple, nowadays we can see quite a lot of variation in protocols concerning this experiment. Parameters which vary among different laboratories are: size of the arena, its shape (circular, square, rectangular), lighting of the arena (intensity and position of the light, colour – white/red), wall height, material from which the open field apparatus is made and position and visibility of the observer (Walsh and Cummins, 1976; Prut and Belzung, 2003). One trial in the Open field test lasts from two minutes to twenty minutes, but most laboratories use five minute long trials (Prut and Belzung, 2003).

These factors are crucial and strongly influence behaviour in the test. For example illumination of the arena interacts with many variables measured in the test. Bright lighting is (in most studies) associated with decreased locomotion, decreased rearing and alterations in thigmotactic behaviour (Valle, 1970 in Walsh and Cummins, 1976). An unexpected result is the lack of evidence for a link between illumination and defecation, since defecation is supposed to be related to emotionality and bright light is considered stressful, especially for animals that dig their burrows underground (Walsh and Cummins, 1976). Moreover behaviour is in fact an interaction of number of factors such as genetic background, maternal care, experience, prior testing, stimulation stemming from moving an animal into experimental room and stimulation by the test apparatus (Walsh and Cummins, 1976). It is therefore important to take these factors into consideration when planning the experiment and researches should publish detailed description of the experiment along with the important and interesting part – results.

Moreover, behaviour within a trial and in successive trials of this experiment changes. When analysed in Factor Analysis, parameters from different trials load on different factors (Denenberg, 1969; Walsh and Cummins, 1976 for more details see Discussion).

There are many parameters that are recorded in the Open field test. The most prevalent, which appear in almost every paper including this experiment are: distance travelled in the arena, number of rears, inactivity, grooming, defecation and urination. Since one of the aspects that could be studied in the Open field test is thigmotaxis (tendency to avoid open brightly lit spaces and staying close to walls), some authors divide the Open field arena into zones and score the behaviour also on the basis of where in the arena does it appear.

1.10.7 Elevated plus maze

The elevated plus maze is an X-shaped apparatus with two closed and two open arms (opposite arms are always the same). This paradigm is used in various areas of research, either for assessment of the combination of exploratory and avoidance behaviours or for assessing anxiety (Carobrez and Bertoglio, 2005). Among parameters which strongly influence behaviour in this experiment are: housing conditions, previous handling and stress, previous maze experience, duration of adaptation to experimental room, intensity of light in the testing room, time of day when testing takes place, material from which the maze is constructed, height of maze walls, presence or absence of rims on the open arms, presence of experimenter during testing and other parameters (Hogg, 1996; Rodgers and Dalvi, 1997). The height of the maze seems, interestingly, not to be an important factor influencing behaviour of animals tested in the Elevated plus maze (Rodgers and Dalvi, 1997).

Several parameters are usually measured in the Elevated plus maze test, originally only spatiotemporal parameters were used. Number of entrances and time spent in open and closed arms are recorded. Most researchers do not use these primary parameters, instead they use percentages or ration computed from them (Rodgers and Dalvi, 1997).

Later on, when Blanchard and Blanchard (Blanchard *et al.*, 1990 in Rodgers and Dalvi, 1997) described defensive reaction of rodents, some researchers started analysing behavioural parameters as well. After fleeding from potentially dangerous stimulus, rodents carefully scan the environment with only their head visible from a tunnel. This careful scanning behaviour is closely associated with specific postures and movements, which seem to represent some kind of vigilance behaviour (Rodgers and Dalvi, 1997). Parameters measured in the Elevated plus maze, which are related to vigilance or risk assessment: rearing, stretched attend postures, head-dips, freezing, defecation and urination. For example, experimental findings for head-dipping and stretched-attend postures clearly show a non-random spatial distribution of these behavioural elements in the maze. Mostly these elements are recorded when an animal is in closed arms and this behaviour is directed towards the center of the maze, the other most common possibility of direction of these behaviours is from the center square towards open arms (Fernandes and File, 1996; Rodgers and Dalvi, 1997). These behavioural or risk assessment measures are especially useful in pharmacological research, because these parameters are "consistent and highly sensitive" to various manipulations (Wall and Messier, 2001).

Many researchers use total arm entries score as a proxy for activity in the Elevated plus maze. It has, however been pointed out, that this parameter includes number of entries into open arms, therefore it contains a measure of anxiety as well and cannot be considered a separate measure for activity (Rodgers and Dalvi, 1997).

Because drug treatments often impair motor abilities of tested animals, some laboratories added small rims to open arms of their mazes to prevent animals from falling of the apparatus. However it seems that even very small rims on open arms act as a thigmotactic clue and therefore reduce anxiety (Fernandes and File, 1996) and influence exploration of the maze as animals tend to spend more time in open arms when there are rims present (Rodgers and Dalvi, 1997).

Several methods which should increase exploration of the Elevated maze have been introduced. For example shortly before subjecting an animal to the Elevated plus maze test, the animal is subjected to Open field test or Hole board test (Hogg, 1996).

The definition of an arm entry is also crucial for analysing behaviour in this test. Originally, an arm entry was calculated, when all four paws of tested individual crossed a "line" into the arm. But with the emergence of automated tracking, the definition of arm entry became neglected and less clear. Another issue related to the previous one are parameters related to behaviour and time spent in the central square. Usually, however not always, the central square is considered separately from open and closed arms of the maze. Moreover, central square-related parameters are highly dependent on the definition of an arm entry. Consider a scenario, where, an arm entry is counted only when all four paws are in the arm. Any other situation – less than four paws in an arms, only head in an arm, whole animal in the center square – therefore counts as time spent in the center square (Wall and Messier, 2001).

1.10.8 Active allothetic place avoidance

This task was first described by Bureš and colleagues (1997) as a modification of place preference and place avoidance tasks. This procedure tests cognition, spatial navigation and memory. It is especially sophisticated because it enables researchers to control sources of spatial navigation available to experimental animals. In this task animals are put onto rotating arena and they have to differentiate between two spatial reference frames. The room frame is defined by spatial relationship between cues in the experimental room, the arena frame can be defined by scent marks and idiothetic information. Experimental animals have to learn to discriminate between relevant and irrelevant spatial cues (Stuchlík *et al.*, 2013). In the active allothetic place avoidance variant of avoidance tasks, the animal has to use cues within the room frame to navigate itself in the task. This aspect of cognitive coordination is especially useful in schizophrenia research, where selective activation and inhibition of different cognitive representations is studied.

Parameters usually recorded in this experiment are: number of entrances into the sector, maximum time an animal avoided the sector, number of shocks per entrance, number of shocks per trial and distance travelled.

Rats subjected to this test learn fast to avoid the sector. They were able to learn to avoid the sector in one twenty minutes long trial and when the shock was turned off, they still avoided the aversive sector for about 30 minutes (Bureš, Fenton, Yu Kaminsky, *et al.*, 1997).

1.10.9 Morris water maze

Morris water maze is one of the most often used methods for studying spatial cognition and learning in rodent behavioural research. Because experimental animals are placed into water tank and they have to find their way to a hidden platform, this test is quite immune to individual differences in motivation (Vorhees and Williams, 2006).

The maze is standardly divided into four quadrants, therefore creating four starting points (where an animal is released into the water maze) which are arbitrarily assigned as South, North, East and West. During the test, the experimental animal is in turns released from these starting points. Transparent platform is then placed in the middle of one of the quadrants. The platform has to be submerged a little under the surface of the water (usually about 2 cm) so the animal cannot see it. Some researchers also use colouring or paint to make sure animals cannot see the platform through the water. Experimental animals must learn to use distal cues to help them navigate in the maze and to find the platform.

There are many possibilities for modification of this task. Many researchers include probe trial as the last sail in their protocols. In these trials, the platform is removed from the maze and swimming in the area, where the platform was previously located, is recorded. Probe trials are used to assess reference memory at the end of the experimental block. Another common modification is spatial reversal task. In this task, the platform is moved to another quadrant (usually the opposite one) and learning for a new position is studied (Vorhees and Williams, 2006).

Usually, latency to reach the platform, path length and several other parameters are measured. Most parameters measured in this test are highly correlated with one another. In probe trials, the number of crossings of the location, where the platform is supposed to be, is measured.

1.10.10 Social interaction

Social interaction test is an experiment focusing on anxiety and social or aggressive behaviour between a pair of experimental animals. It is also used to study neural basis of anxiety and the effect of drugs on social behaviour (File and Seth, 2003). In this test, a pair of males is placed in a novel environment, where neither of the males has a territory and their interaction is observed and recorded. As the inventors of this test emphasize, scores for each individual are dependent on the behaviour of the other animal, therefore only scores for the pair should be used in analyses of behaviour in this test (File and Hyde, 1978).

Parameters recorded in this test are active and passive interaction and also nature of the interaction. Mostly, the interaction consists of sniffing or following the other individual (File and Hyde, 1978).

Behaviour in this experiment changes with successive trials. Distance travelled, number of rears and active social interaction decrease over trials (File and Seth, 2003).

1.10.11 Novel object recognition

Novel object recognition is a procedure designed to test working memory and learning. Researchers also use it to test boldness, neophobia, involvement of different brain regions in recognition and to test effect of various drugs (Antunes and Biala, 2012).

In this experiment, an animal is placed into an arena, which contains two objects. The animal has encountered one of the objects before and it is, therefore, familiar with it. The other object, on the other hand, is novel. The behaviour of the animal is measured and the time spent in close exploration of both objects is measured. From these parameters, differences in exploration of novel and familiar object are assessed.

Novel object or novel environment tests are common procedures to test boldness of experimental animals. Behavioural syndrome consisting of boldness and exploration is probably the best studied behavioural syndrome in personality research. However different experiments testing boldness often yield uncorrelated results. Novel object test, novel environment test, predator inspection test and other procedures assessing boldness therefore probably measure different aspects of boldness or risk-taking (see for example

Carter, Marshall, *et al.*, 2012). Nevertheless, results from these experiments are usually correlated to other aspects of personality.

2 Methods

For the purposes of this thesis we obtained 36 male laboratory rats of the Long Evans strain from the breeding facilities within the Czech Academy of Sciences. The pups were sexed on the second day after birth and female pups were removed from the litters. Thereafter each litter consisted only of male pups and their mother. In the same step the litters were also reduced to add up to 36 pups from six females.

Because of time consuming nature of some of the planed tests we had to divide experimental animals into two groups, each consisting of three litters and 18 pups. Rats in the second group were born when the litters form the first group reached four weeks of age.

During the first three weeks the pups were housed in a box with their mother. At the age of twenty one days, they were separated from their mother and all siblings were housed in a box together. At the age of approximately two months, the pups were moved from the breeding facilities to the Institute of Physiology. After moving the animals, we took each litter and within the litter we randomly divided the males into new boxes. Therefore the animals were now housed in sibling pairs or groups of three.

Experimental animals were housed in standard laboratory conditions. They were housed in a box (25 × 25 × 50 cm) in groups consisting of two or three individuals. In the room there was stable temperature (21°C) and standard 12 hour light-dark-cycle. All animals had ad libitum access to food pellets (Altromin diet 1314) and water.

Testing of experimental animals was divided into two blocks (figure 1). The first block of tests started when the rats were five days old and it consisted mainly of experiments evaluating sensory and motor abilities of the pup. We also included laterality tests and shorter versions of open field test and elevated plus maze test into this experimental block (figure 2). The second part of tests started when experimental animals reached the age of three months – the age when a rat is considered to be adult and also standard age for testing in our laboratory (figure 3).

Before each individual was subjected to an experiment, the apparatus or arena was thoroughly cleaned with Sterilium to eliminate odour cues (with the exception of those juvenile tests, where apparatus contained bedding and Morris water maze).

Fig.1: Experimental design was divided into two blocks. From five to twenty five days of age, the rats were tested in series of procedures evaluating mainly sensory and motor abilities of the pups. From three months of age, the rats were tested in series of experiments which focused mainly on inter-individual differences and personality.

All experiments were conducted in the Czech Academy of Sciences. Juvenile tests in the breeding facilities, adult tests in the Institute of Physiology (Neurophysiology of Memory department).

All of the procedures complied with the Animal Protection Code of the Czech Republic and with the European Council directives (2010/63/EC; 86/609/EEC). The study protocol was approved by the Animal Care Committee of the Institute of Physiology of Academy of Sciences of the Czech Republic.

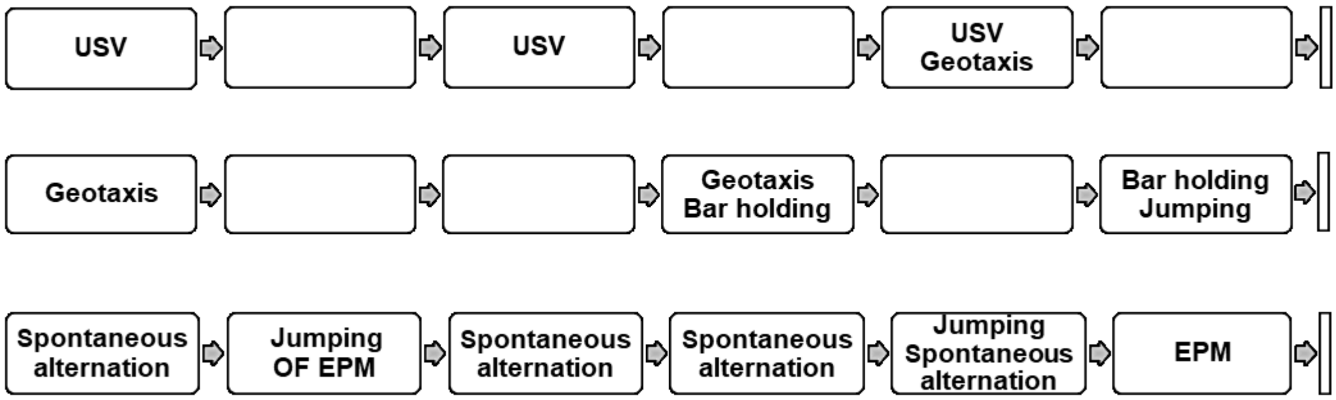

Fig.2: Experimental design of juvenile tests. First experiment was conducted when the animals were 5 days old. Each line of the scheme represents one week (one box represents one day). USV = Ultrasonic vocalization, OF = Open field test, EPM = Elevated plus maze test

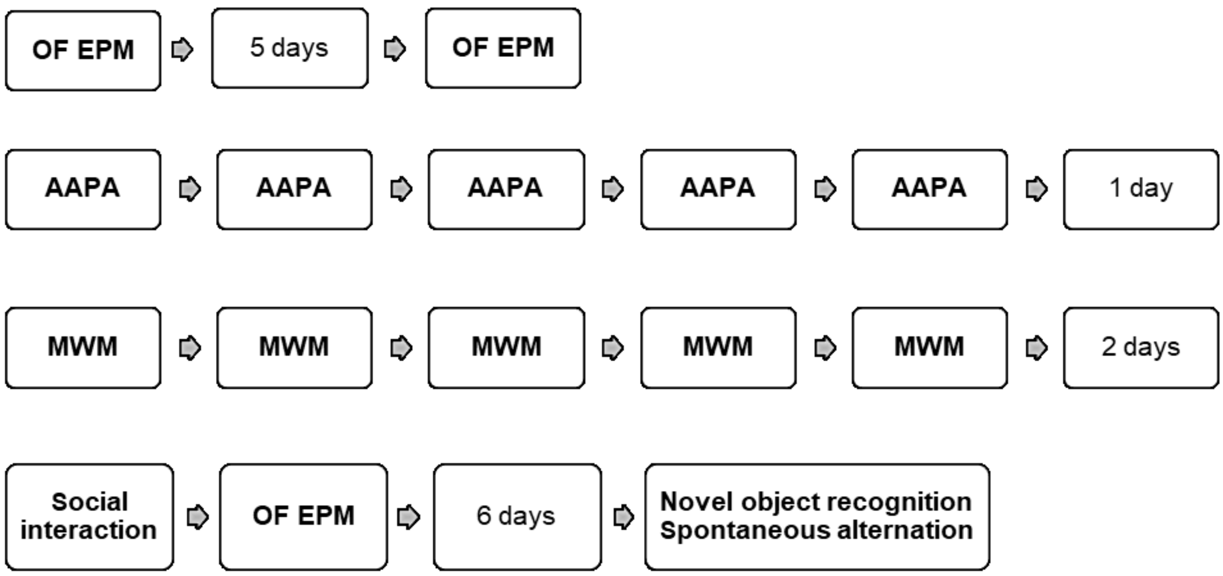

Fig.3: Experimental design of adult tests. Experiments in adult age started when the animals were three months old – the age of early adulthood for laboratory rats. OF = Open field test, EPM = Elevated plus maze test, AAPA = Active allothetic place avoidance test, MWM = Morris water maze test

2.1 Procedures

2.1.1 Ultrasonic vocalization

For this test the whole litter was separated from their mother and brought into adjacent experimental room. Each pup was then individually placed in an empty housing box with sawdust bedding. The behaviour of the tested pup and its calls were recorded with a videocamera and bat sonar.

Ultrasonic vocalization test was conducted on days 5, 7 and 9 of age of the pups. Each test lasted one and a half minute.

<u>Parameters recorded in the test and calculated parameters:</u>

CALL = number of calls

CALLsq = number of calls, square root transformation

SER = number of series of calls

SERsq = number of series of calls, square root transformation

DUR C = mean duration of calls

DUR S = mean duration of series of calls

2.1.2 Geotaxis

The geotaxis test consisted of a plastic board covered with rubber, which was inserted into an empty housing box to create an inclined plane with approximately 30° incline. The pup was placed head up to the middle of the plane and its behaviour was recorded for one minute.

Geotaxis test was performed on days 9, 12 and 15 of age.

<u>Parameters recorded in the test and calculated parameters:</u>

STAY = whether the individual was able to stay on the inclined place or fell down

GEO = positive/negative geotaxis

ANGLE = how much did the individual rotate before climbing

SIDE = to which side did the individual rotate

LAT = latency to start climbing (s)

LATln = latency to start climbing (s), logarithmic (ln) transformation

2.1.3 Bar holding test

The apparatus for this test consisted of an empty housing box with bedding and a wooden bar with diameter approximately one centimeter, which was held in place by a retort stand. The pup was hanged by its front paws on the bar and its behaviour was recorded for 30 seconds or until it fell down from the bar.

Bar holding was tested on days 15 and 17 of age (2 trials each day).

<u>Parameters recorded in the test and calculated parameters:</u>

LAT = latency (s) to hold on to the bar

LATln = latency (s) to hold on to the bar, logarithmic (ln) transformation

BACK = whether the pup managed to put back feet on the bar

PULL = if the pup pulled himself up on the bar

SIDE = to which side did the individual move

BOX = whether the pup managed to climb on the side of the box

2.1.4 Jumping test

The pup was placed on a see-through square platform, which was positioned in between two housing boxes. One box was empty, only filled with bedding. The other box contained siblings of the tested individual. The behaviour of tested rat was recorded.

Jumping test was conducted on days 17, 20 and 23 of age and it lasted for one and a half minute or until the rat jumped into one two the two boxes.

<u>Parameters recorded in the test and calculated parameters:</u>

LAT = latency (s) to jump from the platform

LATln = latency (s) to jump from the platform, logarithmic (ln) transformation

HOME = whether the pup jumped into homecage (with siblings)/ to clean box

2.1.5 Spontaneous alternation

For spontaneous alternation test we used a T-shaped maze. The pup was placed at the end of the open arm with its head facing the middle of the maze. Its behaviour was recorded until the pup chose one of the closed arms and went inside – until its body was in the closed arm at least up to hind feet. The pup was then quickly retracted form the maze.

Spontaneous alternation was tested on days 19, 21, 22 and 23 of age and once in adult age. Adult spontaneous alternation consisted of four consecutive trials.

Parameters recorded in the test and calculated parameters:

SIDE = whether the rat chose the right or the left closed arm

LAT = latency (s) to choose a side and go into closed arm

LATln = latency (s) to choose a side and go into closed arm, logarithmic (ln) transformation

2.1.6 Open field test

The open field apparatus was brightly illuminated (standard indoor working space illumination about 250-500 lx) white square box with high walls. At the beginning of the experiment we put the animal into the middle of the apparatus. The person who conducted these experiments was not present in the room during the trial.

The open field test was conducted once in juvenile age (20 days of age) and three times in adult age (see figure 3 – scheme of the adult experiments). For juveniles the base of the box measured 47,5 x 47,5 cm and the test lasted one and a half minute. In adult tests the base of the box measured 100 x 100 cm and the test lasted 10 minutes (standard length in our laboratory). Behaviour of the animal was recorded during the whole test.

For analysis purposes we divided the apparatus into three zones (see figure 4). Center zone, which covered 50% of the base of the arena. The rest of the arena was divided into wall zone corner zone (all four corners were put together to create one zone called “corner zone”, the same for walls).

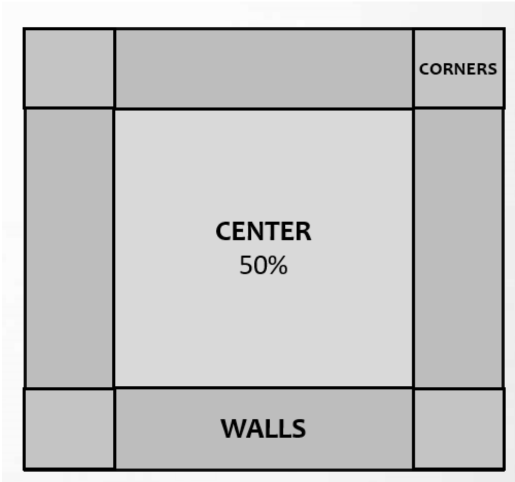

Fig.4: Zones defined in the Open field test. For more thorough analysis of behaviour in the experiment, we divided the apparatus into three zones. Center zone covered 50% of the base of the arena. All four corners were considered as one “corner zone”. Zones adjacent to walls were put to together to create “wall zone”.

Parameters standardly recorded in this experiment are: distance travelled, number of rears, inactivity (time spent without movement), grooming, defecation and urination. Some researchers divide the arena into center zone and zone adjacent to walls and score behaviour occurring in these zones separately.

We recorded and calculated number of parameters that could be of potential interest for us (for complete list see Appendix 1).

2.1.7 Elevated plus maze test

We used elevated plus maze twice in the first (juvenile) block of experiments – on days 20 and 24 of age (see figure 2). For juvenile test, we used smaller maze, which is usually used for murine experiments. Both mazes (juvenile and adult) had small rims on the edges of open arms. The arm length of the juvenile elevated plus maze was 29 centimeters. Juvenile test was one and a half minute long. In adult age we repated the elevated plus maze three times (see figure 3 - scheme of adult experiments) and the arm of the maze measured 50 centimeters. Adult elevated plus maze test lasted for 10 minutes.

Parameters normally recorded in this test: entrances to open arms and time spent there, entrances to closed arms and time spent there. Sometimes additional behavioural parameters (such as rearing, stretched attend postures and head-dipping) are measured.

Parameters recorded in the test and calculated parameters:

TIME L = time spent in closed arms

TIMEar L = time spent in closed arms, arcsin transformation

TIME O = time spent in open arms

TIMEar O = time spent in open arms, arcsin transformation

TIME C = time spent in center

TIMEar C = time spent in center, arcsin transformation

ENTR L = number of entrances to closed arms

ENTRsq L = number of entrances to closed arms, arcsin transformation

ENTR O = number of entrances to open arms

ENTRsq O = number of entrances to open arms, arcsin transformation

TIME LO = relative time spent in closed/open arms

ENTR LO = relative number of entrances to closed/open arms

ENTRsq LO = relative number of entrances to closed/open arms, arcsin transformation

HDIP = number of head-dips

HDIPsq = number of head-dips, square root transformation

SATT = number of stretched attend postures

SATTsq = number of stretched attend postures, square root transformation

DEF = defecation

DEFsq = defecation, square root transformation

Head-dips and stretched attend postures were recorded only in Elevated plus maze tests conducted in juvenile age. All three adult Elevated plus maze test were conducted in experimental room with automated trecking program, so we were not able to record these parameters.

2.1.8 Active allothetic place avoidance test

In this task, an animal was put on a rotating arena with plexiglass walls that prevent it from escaping. In our experiment, the aversive sector was defined within the room frame. It means that its position was stable and did not rotate along with the arena. The electric shocks was set at 0,4 mA.

Active allothetic place avoidance task was conducted in a block of five consecutive days, each day an animal was subjected to one 20 minute trial.

Parameters recorded in the test and calculated parameters:
ENTER = entrances into sector
ENTERsq = entrances into sector, square root transformation
SHOCK = number of shocks
SHOCKsq = number of shocks, square root transformation
DIST = distance (m)
DISTln = distance, logarithmic transformation
DISTsqrt = distance, square root transformation
+ entrances, shocks and distance travelled for each 5 minute part of the trial

2.1.9 Morris water maze test

Morris water maze test was conducted in five consecutive days. Water temperature was 22°C and it was coloured with white paint to ensure the animals canot see through it. Each day experimental animals were subjected to eight sails from different positions in the water tank. On days 4 and 5 we changed the position of the platform so we could study relearning for a new target position. On days 3 and 5 we added one probe trial where we removed the target platform from the tank and one subsequent sail with a platform back in its usual place (see figure 5).

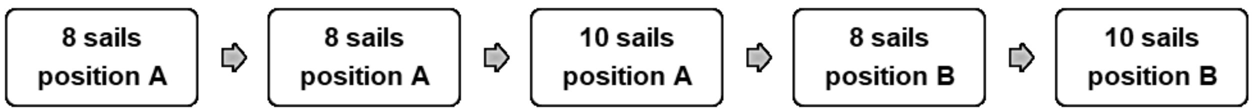

Fig.5: Scheme of the Morris water maze test

Parameters recorded in the test and calculated parameters:
TIME = time to reach the platform
TIMEln = time to reach the platform, logarithmic (ln) transformation
DIST = distance (m) travelled
DISTln = distance travelled, logarithmic transformation
DISTsq = distance travelled, square root transformation
SPE = speed
SPEln = speed, logarithmic transformation
SPEsq = speed, square root transformation

2.1.10 Social interaction test

For social interaction test we used previously mentioned Open field apparatus (100 x 100 cm). We put two males into the arena for 10 minutes and recorded their behaviour with a videocamera.

Parameters recorded in the test and calculated parameters:

AN INV = time spent in anogenital investigation of the other individual

AN INVar = time spent in anogenital investigation of the other individual, arcsin transformation

NAN INV = time spent in non-anogenital investigation of the other individual

NAN INVar = time spent in non-anogenital investigation of the other individual, arcsin transformation

NPIN = number of pinnings (climbing on and over the other individual)

NPINsq = number of pinnings square root tranformation

PIN = time spent in pinning

PINar = time spent in pinning, arcsin transformation

NFOL = number of followings

NFOLsq = number of followings, square root transformation

FOL = time spent following the other individual

FOLar = time spent following the other individual,l arcsin transformation

NESC = number of escapes from the other individual

NESCsq = number of escapes from the other individual, square root transformation

PASS = time spent in passive contact (initiated by the other individual) with the other individual

PASSar = time spent in passive contact (initiated by the other individual) with the other individual, arcsin transformation

GROOM = time spent grooming

GROOMar = time spent grooming, arcsin transformation

AGROOM = time spent in allogrooming (groomed by the other individual)

AGROOMar = time spent in allogrooming (groomed by the other individual), arcsin transformation

2.1.11 Novel object recognition test

In novel object recognition test we put the experimental animal into open field arena (100 x 100 cm). In the first 3 minutes long session we put two novel objects into the arena (plastic sandbox forms) so the animal could freely explore and inspect them. The second session lasted 3 minutes as well. Before we put the animal into the open field apparatus, we put there one novel and one familiar object. The second session of the test was recorded.

<u>Parameters recorded in the test and calculated parameters:</u>
TIME N = time spent inspecting novel object
TIMEar N = time spent inspecting novel object arcsin transformation
TIME F = time spent inspecting familiar object
TIMEar F = time spent inspecting familiar object arcsin transformation
DI = discrimination index = (time spent inspecting novel object + time spent inspecting familiar object/ time spent inspecting novel object - time spent inspecting familiar object)
DEF = defecation
DEFsq = defecation square root transformation
URI = urination
URIsq = urination square root transformation

2.2 Analysis and statistics

Video recordings were analysed in EthoVision software platform (Noldus). Sound recordings from the ultrasonic vocalization test were analysed in Audacity software. Morris water maze and Active allothetic place avoidance task were analysed in Track Analysis program (Biosignal Group US), adult Elevated plus maze tests were automatically analysed by tracking software (Tracker, Biosignal Group US).

The inner structure of the tests was explored with the use of Principal Component Analysis and Factor Analysis in Statistica (StatSoft). Statistica was also used to create correlation matrices for overall analyses of juvenile and adult tests. Parallel analyses were computed in SPSS program (IBM Analytics). Repeatabilites, ANOVAs and Linear models were computed in R (R-Project) and RStudio (open source software).

For the overall analysis we used parameters that best suited these four rules: (1) The variable should be repeatable. (2) The variable should represent other parameters from the test – it should comprise of as much variability from the first axis of PCA as possible. (3) The variable should be either easily measurable or standard parameter in analysis of the test.

2.3 Limitations of the study

First limitation of this study is the fact that we had to divide experimental animals into two cohorts. Therefore prior to analyses we had to check for differences between the cohorts. Moreover an error was made in setting up the room, where the animals were kept during juvenile tests. Because of this mistake, some of the animals (18 males) were not subjected to standard 12 hour light-dark cycle during the first three weeks of age. Instead they were kept in the light 24 hours a day. We see this as an important problem that should be thoroughly inspected and discussed.

When tested for a difference between animals exposed to light and animals housed under standard laboratory conditions, we found that there are significant differences between them. However, the effect cohort and identity of individual explained approximately the same amount of variability in most parameters. Therefore we cannot attribute the differences in behaviour to cohort (or housing conditions). However because we are not interested in proximate aspects of personality in this study, we decided to analyse results from all animals together. By doing this we argue that we are more interested in intra-individual variability and consistency in behaviour.

Second limitation of this study is the fact, that it was designed as a part of a bigger project in the Institute of Physiology of Academy of Sciences of the Czech Republic. Therefore we had to adapt planned experiments according to procedures used in the laboratory. For testing in juvenile age we used experiments standardly used in the Institute of Physiology to assess pharmacological effects of various drugs. Regarding experiments conducted in adult age, we had to simplify the experimental design. Some tests, which should have been repeated several times (so they could be included in personality study) were performed only once. These tests are therefore insufficient for analysing personality of experimental animals.

Another limitation related to the previous one is the time frame of experiments we used. We tested rats when they were very young (5 to 25 days of age) and when they were adult (3 months of age). However, it would be better to test individuals in a bigger and differently set time frame. Testing rats from 2 months of age in a sequence of tests with several trials would probably be better for testing ontogeny of personality.

Another notion I would like to discuss here is manipulation with litters. In the Institute of Physiology, it is customary to sex newborn pups and to keep only males in the litters. Also, the pups are often given to another female to take care of them. Our males were reared by their own mother but females and some male pups were removed from the litter. This manipulation probably reduces inter-individual variability among individuals from the same litter. Not only because of absence of females, but the reduction of male pups might also contribute to reduction of variability. We can only assume how employees of the breeding facilities choose which males to keep in the litter and which ones to remove. However, their choice is probably influenced either by physical attributes of the pup or the order in which they catch individual pups.

Another limitation of this thesis is a common problem whenever researchers use a series of experiments rather than only one trial of one experiment. Handling and manipulation of animals influence their level of stress and behaviour in experiments. The more experiments are performed, the more the individual is handled and therefore has bigger influence on behaviour in the test. Many research questions, however, cannot be answered by performance in one experiment. Researchers therefore have to employ complex experimental paradigm, even though animals habituate to handling and exposure to experimental procedures.

3 Results

For our understanding of the behaviour and looking for personalities it is crucial to inspect the inner structure of each test. In the first step of statistical computing we explored our data using Principal component analysis and Factor analysis (Principal component extraction, Varimax normalized rotation).

Subsequently we used Parallel analysis to assess the number of factors that are meaningful and should therefore be used in further analyses. Results from Parallel analysis suggested that only one factor computed from exploratory analyses should be considered significant.

For each repeated test we computed repeatability of each variable.

3.1 Bar holding test

Latency for which the individual was able to hold on to the bar was repeatable ($r = 0.14$, $p = 0.035$).

3.2 Jumping test

One variable was repeatable in the jumping test – latency to jump from the platform ($r = 0.31$, $p = 0.002$).

3.3 Juvenile Open field test

In the Open field test conducted in juvenile age, the first component (PC1) explained 49.1% of variability in the data. PC1 best correlated with distance travelled in the arena (-0.86), relative time spent in the center of the arena (-0.76) and number of unsupported rears (-0.72). The second component (PC2) explained 25.8% of variability and was correlated with number of supported rears (0.91, see Appendix 2).

3.4 *Adult Open field tests*

In the first Open field test conducted in adult age, the first factor (F1) of Factor analysis (Principal components extraction) explained 44.8% of variability. F1 best correlated with number of unsupported rears (-0.87) and distance travelled (-0.85). 25.1% was explained by the second factor (F2). F2 was correlated to number of supported rears (0.85, see Appendix 3).

After rotation (Varimax normalized), the first factor (F1) was best correlated with distance travelled (0.91), number of supported rears (0.85) and time spent sitting (-0.70). The second factor (F2) best correlated with relative time spent in the center (0.88), relative time spent in corners (-0.79) and number of unsupported rears (0.74, see Appendix 3).

In the second adult Open field test, the first factor (F1) explained 65.1% of variability in the data. The first factor correlated with number of unsupported rears (-0.93), distance travelled (-0.93), time spent sitting (0.84), number of supported rears (-0.79), relative time in the "corner zone" (0.77) and relative time in the center (-0.76). The second factor explained 14.5 % percent of variability and its only variable with correlation higher or equal to 0.6 was time spent grooming (-0.60, see Appendix 3).

When rotated (Varimax normalized), the first factor (F1) was correlated with time spent sitting (-0.91), number of supported rears (0.90), distance travelled (0.83) and number of unsupported rears (0.72). The second factor (F2) correlated best with time spent grooming (0.84), relative time spent in the "corner zone" (0.77) and relative time spent in the center (-0.73, see Appendix 3).

In the third adult Open field test, the first factor from Factor analysis (F1) explained 54.6% of variability. The first factor correlated best with distance travelled in the 10 minute test (-0.94), relative time spent in the "corner zone" (0.92), number of unsupported rears (-0.81), relative time spent in the center of the apparatus (-0.73) and number of supported rears (-0.71). The second factor (F2) explained 17.5% of variability and correlated best with time spent sitting during the Open field test (0.93, see Appendix 3).

After the rotation (Varimax normalized), the first factor (F1) correlated with relative time spent in the "corner zone" of the arena (-0.92), distance travelled (0.92), number of unsupported rears (0.79) and relative time spent in the center (0.76). The second factor (F2) correlated with time spent sitting in the Open field arena (-0.94, see Appendix 3).

Only four variables from adult Open field tests had higher repeatability scores than 0.30. These were: speed ($r = 0.35$, $p = 0.001$), distance travelled ($r = 0.34$, $p = 0.001$), number of supported rears in the “corner zone” ($r = 0.31$, $p = 0.001$) and time spent in active movement ($r = 0.31$, $p = 0.002$). Other variables with significant repeatability are listed in table 1 below.

Other repeatable parameters in Open field tests conducted in adult age

	r	p
REARunsq	0.18	0.047
REARssq	0.24	0.012
VISIT W	0.21	0.023
REARun L	0.27	0.006
GROOM L	0.22	0.021
SIT L	0.18	0.049
GROOMar	0.23	0.014
SITar	0.24	0.012

Tab.1: (REARunsq = number of unsupported rears, REARssq = number of supported rears, VISIT W = number of visits to the “wall zone”, REARun L = number of unsupported rears in the “corner zone”, GROOM L = time spent grooming in the “corner zone”, SIT L = time spent sitting in the “corner zone”, GROOMar = time spent grooming, SITar = time spent sitting)

3.5 Juvenile Elevated plus maze tests

In the first juvenile Elevated plus maze, the first factor (F1) from Factor analysis (Principal components extraction) explained 46.3% of variability in the data. The first factor correlated with number of entrances into open arms of the maze (-0.86) and time spent in closed arms (0.82). The second factor (F2) explained 22.2% of variability and we found no variable with correlation coefficient exceeding 0.7 (see Appendix 4).

When rotated (Varimax normalized), the first factor (F1) correlated with time spent in the open arms of the maze (0.86) and number of entrances into open arms (0.78).

The second factor (F2) best correlated with entrances into closed arms (0.86) and number of stretched attend postures (0.85, see Appendix 4).

In the second juvenile Elevated plus maze, the fist factor (F1) explained 54.6% of variability. It correlated with the number of entrances to open arms of the maze (-0.90), time spent in closed arms (0.84) and time spent in the open arms (-0.74). The second factor (F2) explained 18.9% of variability and correlated with the number of stretched attend postures (0.75, see Appendix 4).

When rotated (Varimax normalized), the first factor (F1) best correlated with time spent in the open arms (0.87), number of entrances to open arms (0.81) and number of head-dips (0.77). The second factor (F2) correlated with the number of stretched attend postures (0.91) and number of entrances into closed arms (0.77, see Appendix 3).

We did not find any repeatable variable in juvenile Elevated plus maze tests.

3.6 Adult Elevated plus maze tests

In the first adult Elevated plus maze test, Principal component analysis revealed only one significant axis. This axis (PC1) explained 62.0% of variability in the data and was correlated with all factors in the analysis – number of entrances to open arms (-0.85), time spent in closed arms (0.78), time spent in open arms (-0.77) and number of entrances to closed arms (-0.74, see Appendix 5).

Principal component analysis of the second adult Elevated plus maze revealed also only one component (PC1) which explained 61.3% of variability. This component (PC1) correlated with time spent in closed arms (-0.94), time spent in open arms (0.93) and number of entrances into open arms (0.81, see Appendix 5).

In the third Elevated plus maze conducted in adult age, the first component (PC1) of Principal component analysis explained 47.9% of variability. The first component (PC1) correlated with number of entrances into open arms (-0.83), time spent in open arms (-0.79) and time spent in closed arms (0.77). The second component (PC2) explained 26.4% of variability and best correlated with number of entrances to closed arms (-0.98, see Appendix 5).

When checked for repeatability, only three variables were repeatable above significance level in adult Elevated plus maze tests - relative time spent in closed/open arms (r = 0.29, p = 0.003), time spent in open arms (r = 0.26, p = 0.007) and time spent in closed arms (r = 0.24, p = 0.012).

3.7 Spontaneous alternation

Latency to choose one closed arm to explore or hide in was repeatable across four trials of adult test of spontaneous alternation (r = 0.20, p = 0.006).

All tests with at least one repeatable variable were considered as personality tests. One variable from each personality test was selected for more overall analyses of behaviour in juvenile and adult age. We also included one variable from Open field test and Elevated plus maze tests conducted in juvenile age, even though Open field was conducted only once in juvenile age and Elevated plus maze test in juvenile age did not show repeatability. These tests, however, represent standard experiments often used in personality research.

The variable, which is supposed to represent each test, was selected following the same criteria stated above (1) The variable should be repeatable. (2) The variable should represent other parameters from the test – it should comprise of as much variability from the first axis of PCA or FA as possible. (3) The variable should be either easily measurable or standard parameter in analysis of the test.

In the next step of statistical analysis, we conducted two overall Factor analyses. The first including personality tests conducted in juvenile age – Bar holding test, Jumping test, Open field test and Elevated plus maze test. The second Factor analysis comprised personality tests conducted in adult age – Open field test, Elevated plus maze test and Spontaneous alternation.

To investigate possible link between personality and cognitive tests, we correlated all variables entering into both overall Factor analyses with factors from Factor analyses of both cognitive tests – Active allothetic place avoidance task and Morris water maze.

In the overall Factor analysis (Maximum likelihood factors extraction) of personality tests conducted in juvenile age, there was only one significant factor (F1) which explained 14% of variability in our data. This factor correlated over 0.7 with only one variable – latency to jump from the platform in the second Jumping test (-0.88, see table 2).

Overall factor analysis of juvenile personality tests

Factor Loadings Extraction: Maximum likelihood factors	
Variable	Factor 1
bar1_LATln	-0.094301
bar2_LATln	-0.249677
bar3_LATln	-0.154966
bar4_LATln	0.045034
jump1_LATln	-0.604767
jump2_LATln	-0.876113
jump3_LATln	-0.322589
juvOF_DIST	-0.107444
juvEPM1_TIMEar O	-0.119011
juvEPM2_TIMEar O	0.200535
Expl.Var	1.400576
Prp.Totl	0.140058

Tab.2: (bar1_LATln = latency to hold on to the bar in the Barholding test, jump1_Latln = latency to jump from the platform in the Jumping test, juvOF_DIST = distance travelled in the Open field test conducted in juvenile age, juv_EPM1_TIMEar O = time spent in open arms in Elevated plus maze conducted in juvenile age; the number represents number of a trial)

Results from overall Factor analysis (Maximum likelihood factors extraction) of personality tests conducted in adult age were identical before and after rotation, except that after rotation, the first factor changed from positive to negative. Therefore we present here only the results of the rotated Factor analysis.

The first factor (F1) explained 20% of variability and best correlated with time spent in open arms in the first (-0.98) and second (-0.77) Elevated plus maze conducted in adult age. The second axis explained 15.5% of variability in the data and correlated with distance travelled in the second adult Open field test (-0.93, figure 6).

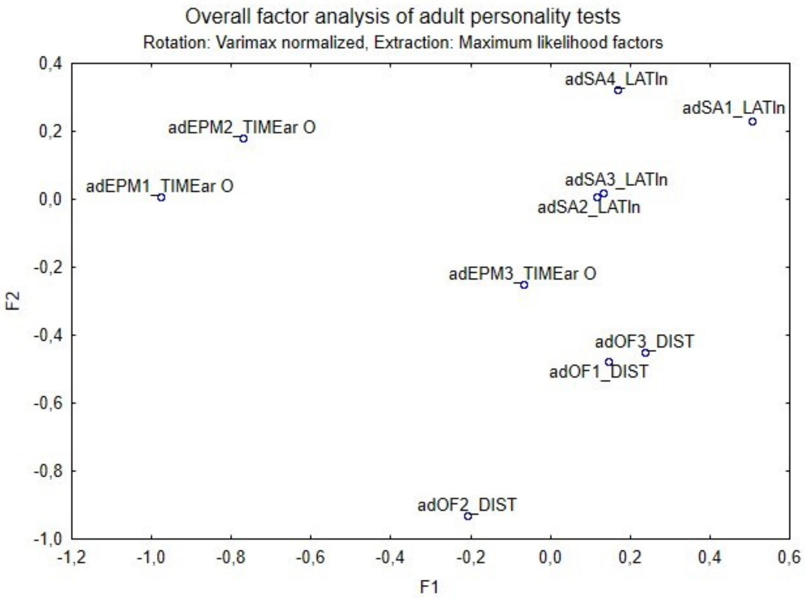

Fig.6: Factor analysis of personality tests conducted in adult age

Factor loadings of overall factor analysis of personality tests conducted in adult age

Factor Loadings Extraction: Maximum likelihood factors Varimax normalized rotation		
Variable	Factor 1	Factor 2
adOF1_DIST	0.144043	-0.475314
adOF2_DIST	-0.207386	-0.930621
adOF3_DIST	0.238064	-0.450868
adEPM1_TIMEar O	-0.978410	0.007751
adEPM2_TIMEar O	-0.772030	0.182652
adEPM3_TIMEar O	-0.067738	-0.251741
adSA1_LATln	0.504343	0.230311
adSA2_LATln	0.115685	0.005955
adSA3_LATln	0.132074	0.018286
adSA4_LATln	0.166690	0.324535
Expl.Var	1.991309	1.550793
Prp.Totl	0.199131	0.155079

Tab.3: (adOF1_DIST = distance travelled in Open field test conducted in adult age, adEPM1_TIMEar O = time spent in open arms in Elevated plus maze conducted in adult age, adSA1_LAtln = latency to enter one of the closed arms in Spontaneous alternation conducted in adult age; the number represents number of a trial)

To inspect the consistence of personality over ontogeny, we computed Factor analysis for all personality tests, including both tests conducted in juvenile age and tests conducted in adult age. The result, however, showed no significant contribution of juvenile tests to the factors extracted by this method.

As a measure of performance in cognitive tests, we decided to use factor scores of significant factors from Factory analyses of both cognitive tests – Active allothetic place avoidance and Morris water maze.

In active allothetic place avoidance task, the first factor (F1) of rotated Factor analysis (Varimax normalized) explained 63.5% of variability in the data. It correlated with performance in the two last days of the test and number of shocks received on the third day of the test - number of shocks on the third day (0.75), distance travelled on the fourth day (-0.82), number of entrances to the aversive sector on the fourth day (0.71), number of shocks on the fourth day (0.81), distance travelled on the fifth day (-0.88), number of entrances to the aversive sector on the fifth day (0.75), number of shocks on the fifth day (0.87). The second factor (F2) explained 11.4% of variability and correlated with performance in the first two days of the task – distance travelled on the first day (-0.70), number of entrances to the aversive sector on the first day (0.70), number of shocks on the first day (0.83), number of entrances to the aversive sector on the second day (0.83), number of shocks on the second day (0.80, see Appendix 6).

Factor analysis of Morris water maze test revealed that five factors were significant, but they each explained only small proportion of variability. The first factor (F1) explained 12.8% of variability and correlated with performance on the second sail on the fourth day of the experiment – distance travelled (0.75) and latency to reach the platform (0.80). The second sail on the fourth day was the second sail of relearning for a new position of the platform. The second factor (F2) explained 10.9% of variability and correlated with performance on the first sail on the fifth day - distance travelled (0.75) and latency to reach the platform (0.72) and with performance on the third sail on the fifth day - latency to reach the platform (0.71). These sails represent the beginning of the second day of relearning for a new position. The third factor (F3) explained 9.1% of variability and correlated with the fourth sail on the first day of experiment - distance travelled (0.75) and latency to reach the platform (0.80, see Appendix 7).

To inspect a link between personality tests and cognition, we then correlated parameters from personality tests with factors extracted from cognitive tests. We found only four significant correlations.

The first factor extracted from Active allothetic place avoidance task (performance at the end of the experiment) correlated with latency to jump from the platform in the third Jumping test conducted in juvenile age (0.37), distance travelled in the third Open field test conducted in adult age (-0.45) and time spent in open arms in the first Elevated plus maze conducted in adult age (0.39, see table 4).

Correlations among factors extracted from Active allothetic place avoidance and personality tests

Spearman Rank Order Correlations				
Pair of Variables	Valid	Spearman	t(N-2)	p-value
AAPA_F1 & bar1_LATln	36	-0.238189	-1.43002	0.161836
AAPA_F1 & bar2_LATln	36	0.280293	1.70263	0.097765
AAPA_F1 & bar3_LATln	36	-0.041653	-0.24309	0.809397
AAPA_F1 & bar4_LATln	36	0.145363	0.85671	0.397605
AAPA_F1 & jump1_LATln	36	0.027949	0.16303	0.871458
AAPA_F1 & jump2_LATln	36	-0.032594	-0.19016	0.850317
AAPA_F1 & jump3_LATln	36	0.368684	2.31270	0.026923
AAPA_F1 & juvOF_DIST	36	-0.201802	-1.20141	0.237893
AAPA_F1 & adOF1_DIST	36	0.024710	0.14413	0.886249
AAPA_F1 & adOF2_DIST	36	-0.163707	-0.96762	0.340068
AAPA_F1 & adOF3_DIST	36	-0.452252	-2.95671	0.005619
AAPA_F1 & juvEPM1_TIMEar O	36	-0.264127	-1.59682	0.119561
AAPA_F1 & juvEPM2_TIMEar O	36	-0.140421	-0.82698	0.414014
AAPA_F1 & adEPM1_TIMEar O	36	0.392793	2.49053	0.017802
AAPA_F1 & adEPM2_TIMEar O	36	0.196911	1.17111	0.249698
AAPA_F1 & adEPM3_TIMEar O	36	-0.204904	-1.22068	0.230604
AAPA_F1 & adSA1_LATln	36	0.022139	0.12912	0.898020
AAPA_F1 & adSA2_LATln	36	0.032690	0.19071	0.849883
AAPA_F1 & adSA3_LATln	36	0.106186	0.62268	0.537647
AAPA_F1 & adSA4_LATln	36	-0.075392	-0.44086	0.662106
AAPA_F2 & bar1_LATln	36	-0.030209	-0.17623	0.861159
AAPA_F2 & bar2_LATln	36	0.129366	0.76072	0.452068
AAPA_F2 & bar3_LATln	36	0.008253	0.04813	0.961897
AAPA_F2 & bar4_LATln	36	-0.224559	-1.34371	0.187942
AAPA_F2 & jump1_LATln	36	-0.020056	-0.11697	0.907573
AAPA_F2 & jump2_LATln	36	-0.040872	-0.23852	0.812907
AAPA_F2 & jump3_LATln	36	-0.068409	-0.39983	0.691786
AAPA_F2 & juvOF_DIST	36	0.168597	0.99736	0.325636
AAPA_F2 & adOF1_DIST	36	-0.226770	-1.35765	0.183517
AAPA_F2 & adOF2_DIST	36	-0.013127	-0.07655	0.939429
AAPA_F2 & adOF3_DIST	36	0.010039	0.05854	0.953663
AAPA_F2 & juvEPM1_TIMEar O	36	-0.025100	-0.14640	0.884469
AAPA_F2 & juvEPM2_TIMEar O	36	0.246348	1.48212	0.147518
AAPA_F2 & adEPM1_TIMEar O	36	-0.122780	-0.72138	0.475606
AAPA_F2 & adEPM2_TIMEar O	36	-0.047619	-0.27798	0.782711
AAPA_F2 & adEPM3_TIMEar O	36	0.028959	0.16893	0.866851
AAPA_F2 & adSA1_LATln	36	-0.055863	-0.32624	0.746239
AAPA_F2 & adSA2_LATln	36	-0.104505	-0.61272	0.544141
AAPA_F2 & adSA3_LATln	36	-0.172681	-1.02225	0.313882
AAPA_F2 & adSA4_LATln	36	0.162382	0.95958	0.344043

Tab.4: (AAPA_F1 and AAPA_F2 = factors extracted from Factor analysis of Active allothetic place avoidance, bar_LATln = latency to hold on to the bar in the Bar holding test, jump_LATln = latency to jump from the platform in the Jumping test, juvOF_DIST =

distance travelled in the juvenile Open field test, adOF_DIST = distance travelled in adult Open field test, juvEPM_TIMEar O = time spent in open arms of the Elevated plus maze conducted in juvenile age, adEPM_TIMEar O = time spent in open arms of the Elevated plus maze conducted in adult age, adSA_LATln = latency to enter one of the closed arms in Spontaneous alternation conducted in adult age; the number represents number of trial)

The first factor extracted from Morris water maze (performance at the beginning of relearning) correlated with latency to hold on to the bar in the second Bar holding test conducted in juvenile age (0.36, see table 5).

Correlations among factors extracted from Morris water maze and personality tests

Spearman Rank Order Correlations				
Pair of Variables	Valid	Spearman	t(N-2)	p-value
MWM_F1 & bar1_LATln	36	-0.007100	-0.04140	0.967216
MWM_F1 & bar2_LATln	36	0.356467	2.22468	0.032844
MWM_F1 & bar3_LATln	36	0.080212	0.46922	0.641906
MWM_F1 & bar4_LATln	36	0.191668	1.13872	0.262780
MWM_F1 & jump1_LATln	36	0.057709	0.33706	0.738142
MWM_F1 & jump2_LATln	36	0.067517	0.39459	0.695611
MWM_F1 & jump3_LATln	36	0.050563	0.29521	0.769630
MWM_F1 & juvOF_DIST	36	0.101673	0.59594	0.555163
MWM_F1 & adOF1_DIST	36	0.010553	0.06154	0.951290
MWM_F1 & adOF2_DIST	36	0.175290	1.03818	0.306514
MWM_F1 & adOF3_DIST	36	0.246589	1.48367	0.147109
MWM_F1 & juvEPM1_TIMEar O	36	0.184194	1.09272	0.282199
MWM_F1 & juvEPM2_TIMEar O	36	-0.120857	-0.70992	0.482597
MWM_F1 & adEPM1_TIMEar O	36	0.006950	0.04052	0.967912
MWM_F1 & adEPM2_TIMEar O	36	-0.047362	-0.27647	0.783858
MWM_F1 & adEPM3_TIMEar O	36	0.121372	0.71299	0.480720
MWM_F1 & adSA1_LATln	36	0.094864	0.55565	0.582085
MWM_F1 & adSA2_LATln	36	0.057400	0.33525	0.739496
MWM_F1 & adSA3_LATln	36	0.029381	0.17140	0.864928
MWM_F1 & adSA4_LATln	36	-0.113538	-0.66635	0.509687
MWM_F2 & bar1_LATln	36	-0.197135	-1.17249	0.249149
MWM_F2 & bar2_LATln	36	0.155188	0.91599	0.366124
MWM_F2 & bar3_LATln	36	-0.242827	-1.45960	0.153578
MWM_F2 & bar4_LATln	36	-0.254612	-1.53522	0.133983
MWM_F2 & jump1_LATln	36	-0.158377	-0.93529	0.356233
MWM_F2 & jump2_LATln	36	0.220270	1.31673	0.196737
MWM_F2 & jump3_LATln	36	0.006595	0.03846	0.969548

MWM_F2 & juvOF_DIST	36	-0.061261	-0.35788	0.722643
MWM_F2 & adOF1_DIST	36	-0.028571	-0.16667	0.868620
MWM_F2 & adOF2_DIST	36	0.027542	0.16066	0.873315
MWM_F2 & adOF3_DIST	36	-0.271300	-1.64358	0.109480
MWM_F2 & juvEPM1_TIMEar O	36	0.189214	1.12359	0.269057
MWM_F2 & juvEPM2_TIMEar O	36	-0.009524	-0.05554	0.956034
MWM_F2 & adEPM1_TIMEar O	36	0.300386	1.83634	0.075064
MWM_F2 & adEPM2_TIMEar O	36	0.200515	1.19343	0.240962
MWM_F2 & adEPM3_TIMEar O	36	0.216488	1.29299	0.204731
MWM_F2 & adSA1_LATln	36	-0.054061	-0.31569	0.754168
MWM_F2 & adSA2_LATln	36	0.051995	0.30359	0.763290
MWM_F2 & adSA3_LATln	36	-0.203608	-1.21263	0.233629
MWM_F2 & adSA4_LATln	36	-0.087763	-0.51373	0.610766
MWM_F3 & bar1_LATln	36	0.095275	0.55809	0.580443
MWM_F3 & bar2_LATln	36	0.199601	1.18776	0.243158
MWM_F3 & bar3_LATln	36	-0.005416	-0.03158	0.974990
MWM_F3 & bar4_LATln	36	-0.168193	-0.99490	0.326813
MWM_F3 & jump1_LATln	36	-0.136768	-0.80506	0.426382
MWM_F3 & jump2_LATln	36	-0.154952	-0.91457	0.366860
MWM_F3 & jump3_LATln	36	0.251781	1.51699	0.138512
MWM_F3 & juvOF_DIST	36	0.176577	1.04605	0.302920
MWM_F3 & adOF1_DIST	36	0.002317	0.01351	0.989301
MWM_F3 & adOF2_DIST	36	0.012613	0.07355	0.941800
MWM_F3 & adOF3_DIST	36	-0.042471	-0.24787	0.805726
MWM_F3 & juvEPM1_TIMEar O	36	0.044793	0.26145	0.795322
MWM_F3 & juvEPM2_TIMEar O	36	-0.022009	-0.12837	0.898616
MWM_F3 & adEPM1_TIMEar O	36	-0.010039	-0.05854	0.953663
MWM_F3 & adEPM2_TIMEar O	36	-0.127671	-0.75058	0.458067
MWM_F3 & adEPM3_TIMEar O	36	-0.114937	-0.67466	0.504453
MWM_F3 & adSA1_LATln	36	-0.221007	-1.32135	0.195207
MWM_F3 & adSA2_LATln	36	-0.158559	-0.93639	0.355675
MWM_F3 & adSA3_LATln	36	-0.054381	-0.31757	0.752756
MWM_F3 & adSA4_LATln	36	-0.225659	-1.35065	0.185731

Tab.5: (MWM_F1, MWM_F2 and MWM_F3 = factors extracted from Factor analysis of Morris water maze, bar_LATln = latency to hold on to the bar in the Bar holding test, jump_LATln = latency to jump from the platform in the Jumping test, juvOF_DIST = distance travelled in the juvenile Open field test, adOF_DIST = distance travelled in adult Open field test, juvEPM_TIMEar O = time spent in open arms of the Elevated plus maze conducted in juvenile age, adEPM_TIMEar O = time spent in open arms of the Elevated plus maze conducted in adult age, adSA_LATln = latency to enter one of the closed arms in Spontaneous alternation conducted in adult age; the number represents number of trial)

When inspecting correlations between the two cognitive tests, we found that performance in the Active allothetic place avoidance task and Morris water maze do not correlate with one another.

Correlations between factors extracted from cognitive tests

Spearman Rank Order Correlations					
Variable	AAPA_F1	AAPA_F2	MWM_F1	MWM_F2	MWM_F3
AAPA_F1	1.000000	-0.011326	0.177606	0.277735	0.034234
AAPA_F2	-0.011326	1.000000	-0.202059	0.031403	-0.201802
MWM_F1	0.177606	-0.202059	1.000000	0.096268	0.056113
MWM_F2	0.277735	0.031403	0.096268	1.000000	-0.018790
MWM_F3	0.034234	-0.201802	0.056113	-0.018790	1.000000

Tab.6: (AAPA_F1 = performance at the end of the test, AAPA_F2 = performance at the beginning of the test, MWM_F1 = performance at the beginning of relearning, MWM_F2 = performance on the fifth day, MWM_F3 = performance on the first day of the test)

4 Discussion

To sum up the most important results of this thesis, we confirmed the existence of inter-individual differences in behaviour of laboratory rats. In several experiments, we found parameters with significant repeatability. We also report differences in behaviour between successive trials of the same experiment. Regarding cognitive tests, we found no correlation between performance in Active allothetic place avoidance and Morris water maze. We therefore argue that these experiments test different aspects of cognition.

In juvenile animals we found significant repeatability in the Bar holding test (latency to hold on to the bar) and in the Jumping test (latency to jump from the platform). These parameters might represent personality of individual animals. However they might also represent differences in strength, motor abilities or motivation.

Behaviour in juvenile tests did not correlate with behaviour in tests conducted in adult age. We therefore argue that the procedures we used to study inter-individual differences in behaviour in juvenile age are not suitable for personality research.

We found several repeatable parameters in the tests conducted in adult age. We can therefore claim that we were able to detect inter-individual differences in behaviour. These differences were best described by behaviour in the Open field test and Elevated plus maze test. Even though it turned out that Open field test and Elevated plus maze tests held the highest predictive value for assessing differences in behaviour, different trials of these experiments correlated rather poorly. This finding is in accordance with results of previous studies (Denenberg, 1969; Walsh and Cummins, 1976; Fernandes and File, 1996; File *et al.*, 1993 in Carobrez and Bertoglio, 2005 an others).

In several papers, it has been demonstrated that animals do not behave in the same manner throughout the whole experiment. Not to mention across several trials of the experiment. Therefore thorough analyses and statistics should be used to study repeated measures of Open field test and Elevated plus maze test.

In the Open field tests, there is a clear pattern general for many species – decreasing distance travelled in the arena within a trial (Walsh and Cummins, 1976). At first the animal runs around the apparatus, trying to find a way to escape. Towards the end of the trial, the individual rather engages in grooming, sitting or rearing. Across trials, decrease in distance travelled in the Open field arena is still detectable (this phenomenon is more consistent in mice than in rats) and defecation rate also changes (in rats it decreases, in mice it increases) (Archer, 1973; Guenther, Finkemeier and Trillmich, 2014).When explored with Principal Component Analysis or Factor Analysis, it is clear that distance travelled on the first day loads on a different factor than distance travelled on other days (Denenberg, 1969; Walsh and Cummins, 1976). The first Open field test therefore seems to measure something else than subsequent trials. It is, however, important to note that interpretation of these changes in behaviour vary among studies (Denenberg, 1969; Walsh and Cummins, 1976).

Even though we detected several repeatable parameters in Open field tests conducted in adult age, there were almost non-existent correlations among the three trials. This results corresponds with the notion, that the first exposure to the Open field test apparatus is somehow different than the second exposure to the same apparatus. In the first trial it is a novel stressful situation and the animal is trying to find a way to escape from it (high scores of both distance travelled and number of rears). The second time the animal is put into the Open field test, the situation is no longer novel, however it may still be stressful. The fact that the third trial of Open field test correlated as poorly with the second trial as the first trial, was a bit of a surprise. It means that novelty of the situation cannot be the only factor which explains these low correlations between trials. There has to be some other factor influencing the behaviour as strongly.

Regarding the Elevated plus maze test, we found that the first and the second trial were closely correlated and also represented the behaviour of the animals well. The third trial of the Elevated plus maze did not correlate with the previous trials.

This finding is, again, consistent with previously published papers regarding changes in behaviour in successive trials of Elevated plus maze test. A fair amount of papers report that parameters from the first exposure to Elevated plus maze procedure load on a different factor than parameters from subsequent Elevated plus maze tests (Fernandes and File, 1996; File *et al.*, 1993 in Carobrez and Bertoglio, 2005). Time spent in open arms of the maze decreases between first two trials. Number of entries into arms remains stable from the first to the second trial of Elevated plus maze test, however from the second to the third trial it decreases (Fernandes and File, 1996). When three or more Elevated plus maze trials are put together in one analysis, several factors are revealed. The first factor represents anxiety in the second and the third trial and the second factor represents anxiety in the first Elevated plus maze test. Activity or exploration of protected areas create a third factor (trials 2 and 3) and a fourth factor (first trial) (Fernandes and File, 1996). In an apparatus with rims, second and third trial come apart and create separate factors (Cruz, Frei and Graeff, 1994 in Rodgers and Dalvi, 1997; Handley *et al.*, 1995 in Fernandes and File, 1996).

These results are consistent with so called test-retest phenomenon in pharmacology. When administered a drug, experimental animals respond to it only in the first exposure to the Elevated plus maze (Carobrez and Bertoglio, 2005).

Different authors provide different explanations for these marked differences in subsequent trials. Carobrez and Bertoglio (2005) suggest that exposure to Elevated plus maze reduces motivation to explore the maze in the subsequent trials, because it is no longer novel for the animal. They point out that this trend can be observed even within the first exposure to the maze. At the beginning of the trial, experimental animals approach and inspect both open and close arms equally, however later in the trial a marked tendency to avoid open arms arises. File and Zangrossi (1993 in Carobrez and Bertoglio, 2005) propose a notion that the first trial might measure different form of anxiety or fear than subsequent elevated plus maze trials.

One striking difference between our results and the results listed above is the fact, that in our study, the first two trials of this test were strongly correlated, however the third trial was not. The maze we used in this study is usually used for physiological and pharmacological research and therefore it has rims (few milimeters high) on the edges of open arms. Therefore we should probably compare the results from this thesis to studies where researchers used Elevated plus maze with rims as well. These studies, however, report that each trial loads on separate axis, which was not the case in this thesis.

We also found repeatability in latency to enter one of the closed arms in Spontaneous alternation conducted in adult age. We therefore suggest that even though Spontaneous alternation is not usually a part of personality tests, it is linked to inter-individual differences in behaviour. This test does not measure only alternation but it can also be an indicator of the drive to explore unknown parts of the apparatus (its closed arms). Latency to enter one of the closed arms might therefore represent exploration or boldness of experimental animals.

We did not find consistency in behaviour throughout ontogeny. When analysing juvenile and adult test together, we did not find any significant correlation between them.

However the fact that experiments conducted in juvenile age were pronouncedly shorter than experiments conducted in adult age shifts the weight of the parameters in favour of longer experiments. Moreover the experiments we used to test animals in juvenile age are probably not suitable for personality research. Also it would be better to test animals in between the two periods when we tested them. We tested only very small pups and mature rats. A timeframe around maturation (from two months of age) would be more suited to answer questions about ontogeny of personality (Groothuis and Trillmich, 2011, for more details see Ultimate causes of personality). Therefore a link between behaviour in juvenile and adult age may exist and we just were not able to find it.

We found a correlation between performance at the end of Active allothetic place avoidance task, latency to jump from the platform in the third Jumping test, distance travelled in the third Open field test conducted in adult age and time spent in open arms in the first Elevated plus maze conducted in adult age. There is a commonly accepted assumption that both Open field test and Elevated plus maze test can reliably measure anxiety of animals. The latency to jump from the platform in the Jumping test can also be a measure of anxiety. Active allothetic place avoidance task is considered to measure congition. However, interpretation of behaviour in this task is probably more complex. In this task, an animal has to differentiate between cognitive frames to avoid aversive sector, where it gets mild electric shocks. It is therefore possible that Active allothetic place avoidance task can reflect anxiety of animals. This correlation might therefore represent tendency to take risks - boldness.

Performance at the beginning of relearning in the Morris water maze test correlated with latency to hold on to the bar in the second Bar holding test conducted in juvenile age. Latency to hold on to the bar might very well represent strength or motivation of the individual, but we are not certain, what is the role these factors play in relearning task in Morris water maze. In Morris water maze, relearning is usually considered as a measure of flexibility. To find the platform in a new position, the animal has to abandon the solution it has learned in previous sails. The animal has to realize that the task has changed and it has to find the new position of the target platform. When we look at this correlation this way, we can argue that relearning in Morris water maze and performance in Bar holding test might be linked to coping styles. It is usually thought that proactive animals are dominant and successful in stable environment. But when the environment changes unpredictably, reactive animals are more successful, because they are able to adapt to it (Koolhaas *et al.*, 1999). Relearning in Morris water maze might simulate environmental changes.

When correlating both cognitive tests together, we found out that they are not correlated. This is not the first report of different performance in these two procedures. Kubík and Fenton (2005) reported differences in results from these two tests after unilateral inactivation of the hippocampus. We argue that Active allothetic place avoidance task might be more difficult to solve because experimental animals have to differentiate between two cognitive frames to solve this task. When an animal uses more than one cognitive frames, it has to inhibit the frames it does not need to use and activate only the one that is relevant at the moment (Kelemen and Fenton, 2010). There might, however, be more factors creating the difference in performance in these two experiments.

Both physiological and pharmacological research operate on the basis that all experimental individuals behave in the same way. These researchers suppose that inter-individual differences in behaviour can be statistically eliminated. They assume that differences between individuals are rather low and they do not interact with performance in experiments and therefore do not influence the effects of drugs.

In this thesis we found small but significant inter-individual differences in behaviour. However we did not find stability in time and across contexts. It however depends on the definition of personality (see Introduction), whether we can say that we were able to detect it in our experimental animals.

Repeatability scores we detected in our experimental animals were rather low. It means that if we want to use some procedure to preselect animals according to their behaviour, one trial of one test is not enough. We would have to subject experimental animals to several successive trials of the same experiment. Moreover, it would have to be the same experiment in which we wanted to test the animals in the first place, because behaviour in different tests does not correlate. But as we demonstrated earlier, successive trials of the same experiment do not have to correlate as well. This poses a big issue on physiological and pharmacological research because the existence of inter-individual differences in behaviour causes problems when analysing and interpreting data. Even more so, when there is no reasonable procedure which would enable researchers to eliminate these differences.

5 Conlusions

To conclude, we detected repeatable inter-individual differences in both juvenile and adult age. We were, however, not able to assess personality of experimental animals in this study. The differences between individuals were best demonstrated in behaviour in Open field tests conducted in adult age (specifically in the second trial) and in the Elevated plus maze tests conducted in adult age (first and second trial). Our results were in accord with previous studies, reporting that successive trials of the same test often do not correlate together.

We also report that performance in Active allothetic place avoidance does not correlate with performance in Morris water maze. Even though both of these test focus on cognition and learning, animals might employ different mechanisms to cope with these tests.

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