The influence of individual differences on animal behaviour

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Abstract

Already in 1966, Crowcroft described consistent individual differences in mice behaviour. Since then, individual differences in behaviour have been showed in many other animal species like rats, great tits, killifish, naked mole rats, etcetera (Dingemanse *et al.* 2003, Dingemanse and Goede 2004, Fraser *et al.* 2001, Nathaniel *et al.* 2007, Suarez-Jimenez *et al.* 2013). Research showed that these consistent individual differences in behaviour cause variation in test results. Variation which was first seen as errors. In some cases, individual differences are still not taken into account in experiments. Experiments which for example require the animals learning something, discard the slow-learning animals from the tests. This results in a group of animals which are 'selected' by their learning ability. To some extent, this is not surprising. Although individual differences were already noticed in 1966, a lot of further information is still unclear. There is for example indistinctness about the terms that are being used and, the origin(s) of personality and how to measure personality is not clear.

This review will explain more about these problems. The main question is if it is necessary to take individual differences into account while conducting a behavioural test? Especially the environment in which animals are tested seems of great influence on their personality. Considering this and other factors, the conclusion of this review is that it is important to take individual differences into account while conducting a behavioural test.

Introduction

In humans, it is widely accepted that every person is different; every person has its own personality. Some people are for example novelty-seekers while others avoid new situations (Kim *et al.* 2005). Even from monozygotic twins it is thought of as normal that they show individual differences in their behaviour (Freund *et al.* 2013). However, in animals, the existence of personality is not yet commonly accepted. Especially with genetically identical mice, we tend to think of them as one group showing the same behaviour.

In most research where behavioural tests are being used measures which lie too far away from the mean are called 'outliers' or variation in test results. This variation was seen as errors/noise (Nomakuchi *et al.* 2009, Réale *et al.* 2007, Uher 2011). However, research has shown that one of the causes of this variation is the individual differences in animal behaviour. Already in 1966, a researcher named Crowcroft noticed different individuals within a group of mice showing consistently different behaviour (Lathe 2004). Crowcroft investigated the behaviour of mice to improve pest control. He performed several tests in a large room. In this room, the behaviour of an individual mouse and groups of mice in a new environment, and the interactions between a group of mice and a newly introduced mouse were tested (Southern 1967). For one experiment, a group consisting of male and female mice was placed in a room with nesting boxes. The mice began exploring the room and spreading themselves over the nesting boxes. Most nesting boxes contained one male with one or two females. One box consisted of several males and females and one held only males. Then Crowcroft marked all the mice, wrote down which mice were in what box, and reintroduced them in the room. The dispersal of the mice among the nesting boxes was almost the same as the first time. This suggests that these mice behave the same way in this

situation. Crowcroft also noted some mice showing different behaviour from the others. Like a male mouse bringing pups back to the nesting box where they belong (Lathe 2004).

Crowcroft showed with his research that a few mice behave consistently different from the other mice. Although this was in 1966, further information about individual differences in behaviour of animals is largely unclear (Lathe 2004). The origin(s) of these individual differences or how exactly to measure them is not defined. Next to this, there is indistinctness about used terms concerning consistent individual differences in behaviour (Lathe 2004, Uher 2011). Although it is known that behavioural differences are present in animals, researchers still try to achieve results without variation by, for example, standardizing their tests and using animals which are genetically identical. However, as is shown by Lewejohann *et al.* (2011), even mice which are genetically identical and tested in a standardized environment show variation in their behaviour. Moreover, to assess welfare of animals, Ohl and van der Staay (2011) state that it is important to look at an individual. This information about genetically identical mice and assessing welfare in animals suggests that the behaviour of an individual might be important. Therefore, the research question of this review is: is it necessary to take individual differences into account while conducting a behavioural test? To answer this question, first will be discussed what personality exactly is. Then some background information about the five personality axes will be given followed by some methods which are being used for measuring personality. Further, we will focus on the influence of the environment on individual differences in behaviour and last the influence of individual differences on statistics will be discussed.

As said before, the concept of personality is accepted in humans and therefore studied in humans. While discussing the subject of animal personality, several of the reviewed articles refer to human psychology (Carter *et al.* 2013, Freund *et al.* 2013, Kim *et al.* 2005, Lathe 2004, Réale *et al.* 2007, Suarez-Jimenez *et al.* 2013, Uher 2011). Some of the knowledge about human psychology can be used in studying animal personality (Uher 2011). However, in some cases human psychology and behavioural ecology use different explanations for the same terms (Carter *et al.* 2013). Next to this, it is important to keep the risk of anthropomorphism in mind (Réale *et al.* 2007). In this review, human psychology will only be referred to when explaining the concept of the 'big five' of personality (Réale *et al.* 2007, Uher 2011).

Terminology

Réale *et al.* (2007) define personality in animals as 'the idea that individual behavioural differences are repeatable over time and across situations'. Personality is unique for an individual (Uher 2011). Different synonyms for personality, like temperament (Réale *et al.* 2007) and individuality (Lathe 2004, Réale *et al.* 2007), are being used. However, temperament is a more restrictive term than personality (Suarez-Jimenez *et al.* 2013, Uher 2011). Temperament only includes behaviours involving affection, activity and attention. Feeding behaviours for example are normally not scaled under the term temperament (Uher 2011). Réale *et al.* (2007) argue that the reason why temperament is seen as a more restrictive term is based on inconsistent arguments. This was concluded by comparing definitions for personality and temperament from different sources. Because of the vagueness about the meaning of the term temperament, this possible synonym for personality will not be used in this review. The other synonym, individuality, would be a correct synonym for personality. However, to be consistent, in this review only the term personality will be used.

In some articles another synonym for personality is used, the term behavioural syndrome (Dingemanse and Réale 2005, Lewejohann *et al.* 2011, Sih *et al.* 2004). However, according to Uher (2011), behavioural syndrome describes correlations in individual differences in behaviour on a population level. Personality describes behaviour on an individual level. Because behavioural syndromes describe behaviour on a population level, individual consistency of the behaviour is not necessary. In this review, the terminology according to Uher will be used (Uher 2011). In conclusion, personality describes the behaviour of one animal and behavioural syndrome describes the behaviour of a group or population of animals.

5 animal personality traits

In human psychology, personality is divided in five traits; boldness, exploration, activity, aggressiveness and sociability. These five traits are called the 'big five' of personality. The five traits and the term 'big five' are also used for animal personality (Réale *et al.* 2007, Uher 2011). Using this terminology has 2 advantages; the terms which are used are clear and there are no other traits involved with one of the five traits. For example, the trait boldness has a personality phenotype bold and shy (Réale *et al.* 2007*)*. This personality phenotype is present along a shy-bold continuum. A continuum means that an individual's personality can not only be shy or bold but everything in between (Nomakuchi *et al.* 2009).

The basis of personality traits can be separated into three parts: phenotypic variation, repeatability and a genetic basis (Réale *et al.* 2007). The first part, phenotypic variation, is caused by the interaction between variation in the environment and genetic variance. This genetic variance can for example be caused by epigenetic factors. Examples of epigenetic factors are DNA methylation and histone modification. These epigenetic factors influence the expression of genes without changing the DNA sequence (Ledón-Rettig *et al.* 2012). These kind of modifications to the DNA of an individual causes variation in its phenotype without changing the sequence of the DNA. Because of the influence of the environment on phenotypic variation, it is for example important to test the reaction towards something novel in the home cage. The two involved traits, boldness and exploration, are often correlated (Carter *et al.* 2013). In a test it is important that only one of these two traits is measured, not both. By testing animals in different situations with varying degrees of novelty, it can be checked whether the traits boldness and exploration are two different traits. This is the case when individuals differ in their reaction towards the novelty and show a different reaction to the varying degrees of novelty (Réale *et al.* 2007). Another test where this separation between boldness and exploration is important is the open field test. This test is designed to test the exploration behaviour of an animal in a novel environment. However, if the animal is forced to move, the animal might reflect fearfulness or activity instead of exploration behaviour. This is for example the case if an animal is placed in a novel environment where there is no opportunity to escape from this environment. By letting the animal move freely into a novel environment, only exploration behaviour will be measured without fearfulness or activity. Next to this, by checking if the animals differ in their reaction towards the environment, it can be made sure if the test measures boldness and exploration as two different traits (Carter *et al.* 2013, Réale *et al.* 2007).

The second part, repeatability, is important to confirm the consistency of the personality across different contexts, observers or through time. A synonym for repeatability is reliability. Repeatability gives an indication to which degree a test is reliable in being repeatable and yielding the same results (Carter *et al.* 2013, Réale *et al.* 2007).

Last, there is the genetic basis of personality. The difference between genetic variance and this genetic basis is that genetic variance (in combination with variation in the environment) causes variation in the phenotype of the animals and the genetic basis is an indication of the heritability of personality. The heritability gives an indication of the amount of phenotypic variation caused by genetic variation (Ledón-Rettig *et al.* 2012). That there is a genetic basis of personality has for example been shown by Dingemanse *et al.* (2002). This research showed that wild birds' exploratory behaviour is repeatable and heritable. Repeatability is one of the three parts of personality. Therefore, this indicates that this exploratory behaviour is part of the personality of the animals. The heritability of the behaviour, another of the three parts of personality, shows that the behaviour has a genetic basis. Réale *et al.* (2007) explain that, next to these three parts of personality, there are more variables which influence personality in animals. These are variables like maternal care and position of the foetus in the uterus etcetera. This will be explained later in this review.

Uher (2011) explains the basis of personality in another way. He describes personality using three different characteristics. These are 1) probabilistic, 2) specific for the individual and 3) differential. The first, probabilistic, means that although animals of one species will be partly similar in their behaviour, there are differences in the probability of the animals displaying these behaviours specific for their species. The individual differences animals show in their behaviour are therefore probabilistic behavioural patterns. The second characteristic is that an individual shows behaviours which are specific for that individual and stable over time. The last characteristic involves the relation of the behaviour of one individual in relation with other individuals. This behaviour has to be unique from the behaviour of the other individuals because personality is unique for an animal (Uher 2011).

Methods of measuring personality

There are several possible ways to define personality; by experimentation, behavioural coding and subjective personality ratings (Carter *et al.* 2013, Uher 2011). The first, experimentation, is used to test whether different traits (of the 'big five' of traits mentioned before) are variable. This way of testing uses an ethogram to score the behaviour of the animals. In the second way of defining personality, behavioural coding, an ethogram is also used. The difference with experimentation is that during behavioural coding only natural behaviour is examined without using a test set up. The last, subjective personality ratings are the least precise way of describing personality. For this, observers which are familiar with the animals describe the personality of them along the five different trait continuums of the 'big five' mentioned before (Carter *et al.* 2013).

To test personality traits, researchers usually test animals in new and challenging settings because personality of animals is mostly shown in these kinds of situations (Réale *et al.* 2007). For example the open field test (involving a new environment) and the novel object test are often used to test boldness and exploration behaviour. Especially the novel object test triggers a conflict between approaching and avoidance behaviour (Kim *et al.* 2005). However, while designing a behavioural test it is important to keep the situational strength and relevance of the test in mind. A good test situation should contain relevant cues for the animal. The situational strength should be weak to moderate to avoid floor or ceiling effects. For example, a situation testing boldness of animals where a strong predator is used, will make a lot of animals look "shy" while using a weak predator will make a lot of animals look "bold" (Carter *et al.* 2013, Uher 2011).

Réale *et al.* (2007) describe four steps to conduct good behavioural research. The first step is to design a test which is appropriate to measure the wanted trait. Most behavioural tests measure one or more traits. However, Réale *et al.* (2007) argue that it is important that every test should only measure one trait from the 'big five' (Réale *et al.* 2007). For example, it is very important that in a boldness test only the boldness behaviour of the animal is measured and not the reaction towards novelty as explained before (Carter *et al.* 2013, Réale *et al.* 2007).

The second step of Réale's 'guide' is to check if the test is valid. By checking validity, it is made sure if a test measures the wanted trait (Carter *et al.* 2013). This is important when testing personality because it has to be certain that a test designed to measure boldness for example, actually measures boldness and not another trait. Testing validity can be done by measuring different traits with different tests and then examine the relationship between the traits (Carter *et al.* 2013, Réale *et al.* 2007). There are several types of validity like convergent validity, discriminant validity, face validity, construct validity and ecological validity (Carter *et al.* 2013, Réale *et al.* 2007, van der Staay *et al.* 2009). Convergent validity describes the degree in which two tests measure the same trait. The opposite is the case for discriminant validity. These two validities can be measured by using correlations. For a test to be convergent valid, the correlation has to be high and vice versa for the discriminant validity. Another validity is face validity. This validity describes to what degree the test looks like it measures what it is supposed to measure (Carter *et al.* 2013). It can also be used to check whether an animal model is accurate for a human disease. For an animal to be used as a model for a human disease, the symptoms of the disease in humans have to be similar to the symptoms in animals (van der Staay *et al.* 2009). Next there is the construct validity. This validity is the most important one for animal models because it tells to what extent the test measures what it is supposed to measure (van der Staay *et al.* 2009). Two tests can for example have convergent validity, this does not mean that they measure the wanted trait (Carter *et al.* 2013). The last, ecological validity describes to what extent results from tests performed in the lab can be true for a more natural situation. In the lab, the ecological validity is usually compromised (Uher 2011). To ecologically validate a behavioural test done in the lab, results of this test have to be compared with results from tests performed in the wild or at least in a more natural situation (Réale *et al.* 2007). Réale *et al.* (2007) name two examples in which a personality measure is linked to a trait used in the wild; Dingemanse *et al.* (2003) and Fraser *et al.* (2001) tested the relation between exploratory behaviour tested in the lab with dispersal in the wild of respectively great tits (*Parus major*) and killifish (*Rivulus hartii*). The great tits were first tested in an open field test in a laboratory. Then they were released in the wild and their dispersal behaviour was measured. In the experiment of Fraser *et al.* (2001), the boldness of killifish was first tested in a laboratory set-up. Data from this test was compared with data from dispersal behaviour in the wild of the same killifish (Fraser *et al.* 2003). The results from these experiments show that exploratory behaviour tested in the lab is related to the dispersal in the wild of great tits (*Parus major*) and killifish (*Rivulus hartii*) (Dingemanse *et al.* 2003, Fraser *et al.* 2011). Another way of defining the ecological validity is by comparing for example aggressiveness in a laboratory test with aggressiveness in a situation which resembles the natural habitat of the animal (Réale *et al.* 2007).

Van der Staay *et al.* (2009) explain that it is questionable whether a situation in the lab can resemble a situation in the wild and vice versa. As van der Staay *et al.* (2009) state in their article, "no animal model can be valid in all situations, for all purposes". This article

explains that validating an animal model consists of several validities which have to be checked. These are construct validity, as explained above, and internal validity, predictive validity and external validity. Internal validity includes the reliability (meaning that the used instrument produces consistent results) and the replicability (the degree in which the same results are yielded if the test is repeated) of a test. The predictive validity says to what extent behaviour in one situation predicts the behaviour in another situation. Last, there is the external validity. One condition for external validity is that internal validity has to be high. External validity is very important when building a model. It shows to what degree the results are generally true for populations (perhaps even species) and environments (van der Staay *et al.* 2009).

The third step of Réale's 'guide' to conducting good behavioural research is important when measuring personality. This step involves checking if found variation in personality is linked to differences in fitness (ability to survive and reproduce). If this is the case, this could provide information on the role of personality in ecology and evolution. It is known that personality has an effect on fitness and ecological processes. The effect on fitness is a result of the natural selection personality is subject to (Dingemanse *et al*. 2004). Personality can for example affect an individual's reaction towards a predator or its position in the dominance hierarchy. For example, more dominant animals usually have a higher reproductive success and therefore a higher fitness (Dingemanse *et al.* 2004). This relation between dominance and the personality of an animal is mostly present due to the aggressiveness of the animal (Nathaniel *et al.* 2007). Another behaviour which can affect fitness is joining behaviour. Nomakuchi *et al.* (2009) said that joining behaviour can have a fitness benefit because it protects individuals from predators and increases a chance for a mate and foraging efficiency. The link between personality and fitness is interesting for researchers which are interested in evolution of personality. Is one trait for example better for fitness than another trait? (Réale *et al.* 2007). The effect of personality on ecological processes can become visible while researching social organisations or the spreading of animals to another habitat (Réale *et al.* 2007).

The last (optional) step is to test personality between and within species. In this way, a broader image of personality can be obtained (Réale *et al.* 2007).

To test if behavioural differences are repeatable over different situations, it is needed to test one trait multiple times in different ways (Réale *et al.* 2007, Uher 2011). However, this repetition of testing might cause the animals to adapt to the tests. For example, by repeating tests including novelty (open field or novel objects), the animal habituates to the novel environment or object and its behaviour changes. Uher (2011) recommends testing animals in slightly different tests to prevent this habituation. However, Réale *et al.* (2007) state that even if the environment or object is changed in every test, a form of habituation might occur (Réale *et al.* 2007). A test which can be used repeatedly without habituation is the social defeat test. This test is used as an animal model of depression (Duclot *et al.* 2011).

Role of environment

To avoid variation in results of behavioural experiments, as much factors as possible are standardized. This is done by keeping known factors which influence individual differences under strict control. These are factors like the environment, health, birth weight, genotype, lighting, testing schedule and observer bias (Lewejohann *et al.* 2011)**.** However, many test results still show variability. Lewejohann *et al.* (2011) say this is because of individual differences in behaviour.

One factor which is controlled to minimize the influence of it on the behaviour of the animals is the environment. While testing the animals, they have to be housed preferably in such a way that it does not influence the test results. It is therefore important to house (or even more ideally test) animals in a standardized environment and in a social context which resembles the context in which they live in the wild as good as possible. Mice for example are social animals and will probably behave in a different way as they would in the wild if they are kept individually (Lathe 2004). As Duclot *et al.* (2011) state, housing rats socially or individually has an influence on for example their body mass, activity, social avoidance and anxiety.

In labs, mice are usually housed by gender. In males, this can result in territorial behaviour and aggression and therefore stress (Arndt *et al.* 2009, Nathaniel *et al.* 2007). To prevent them from experiencing stress, it might be better to house them individually. Females on the other hand show no aggression while being housed socially (Arndt *et al.* 2009). Arndt *et al.* (2009) tested socially and individually housed inbred mice in a modified holeboard to see the effects of and the stress response on the way of housing. It was found that males and females show no effect of individual housing if compared with social housed animals in the holeboard test. Arndt *et al.* (2009) even showed that there is another factor which affects the behaviour of the animals, the order of testing. Animals housed in a group might communicate stress after being tested. The social reactions in the group due to the removal of one group member could also be of influence on the behaviour of the other individuals (Arndt *et al.* 2009).

There are other factors, apart from the social context in which animals are housed, that might influence test results. Therefore these factors like light, feeding schedules, enrichments etcetera are kept the same for all animals in the test. However, some of these factors are specific for a laboratory and cannot be standardized. These are factors like the season, experimenter and humidity. (Lewejohann *et al.* 2011). Repetition of an experiment in another laboratory might therefore result in different conclusions. This has been shown by Crabbe *et al.* (1999). In this experiment, genetically identical mice were tested in three different laboratories. Although experiments were kept as synchronized as possible, every laboratory yielded different results. Differences were especially found in measures concerning anxiety and exploratory activity. Wahlsten *et al.* (2003) performed a follow-up study where the results from the experiment of 1999 were analysed more extensively. This showed that it is probably impossible to yield exact same results from tests done in three different laboratories. Even if an experiment is repeated using the same set up, different conclusions may be found. Apart from the laboratory specific factors which affect this different behaviour of the animals, there are the factors that form an individual during its ontogeny which might have an influence as well (Freund *et al.* 2013, Lewejohann *et al.* 2011). These origins of behavioural differences will be explained later in this review.

Tests on the influence of the environment on individual behaviour of animals have for example been done by Nomakuchi *et al.* (2009). They performed a test on the influence of the environment on joining and exploration behaviour of two groups of stickle backs (*Gasterosteus aculeatus*). One group, the benthic group, feeds alone or in a small shoal on benthos and one group, the limnetic group, feeds in shoals in water columns on plankton. This difference in feeding behaviour reflects in their social joining behaviour. Fish from the limnetic group follow each other significantly more often than fish from the benthic group (Nomakuchi *et al.* 2009). The amount of exploration behaviour showed (alone and with a demonstrator) however, was not correlated to their feeding behaviour. The individual

variation found in this trait might be under influence of the risk sensitivity of the individual fish. Some fish only show joining behaviour if there are more than three fish to follow, not just one. This study showed that fish which displayed a higher level of exploration behaviour ("bold" fish) in the single exploration test, followed the demonstrator in the following test (Nomakuchi *et al.* 2009). This study is an example of the fact that individual behaviour of animals is dependent on the environment they live in (Uher 2011). An animal will react to the environment in a way which is functional for that environment. Some animals react faster to stimuli in their environment than others. Other animals might even manipulate their environment to suit their personality or go to a specific environment which fits with their personality.

On a social level, age and rank in the hierarchy can have an influence on the personality of animals (Uher 2011). The relation between rank in the hierarchy and personality has been looked into by different researchers. Shimozuru *et al.* (2006) for example tested the influence of social hierarchy on scent-marking behaviour in Mongolian gerbils (*Meriones unguiculatus*). Scent-marking is typical behaviour of dominant males. They perform this behaviour more often compared to subordinate males. This study suggests that scent-marking behaviour is correlated with the rank of the animals in the social hierarchy (Shimozuru *et al.* 2006).

Nathaniel *et al.* (2007), Suarez-Jimenez *et al.* (2013) and Dingemanse and Goede (2004) also focussed on the relation of dominance and personality. Nathaniel *et al.* (2007) tested mice (*Mus musculus*) and naked mole rats (*Cryptomys foxi*) individually and in a social group. Their territorial aggressive behaviour and non-territorial aggressive behaviour were tested. The first was tested by placing a group of small, ranked by age intruders in another adult animal's territory. For the non-territorial aggressive behaviour, several animals were grouped in a cage which was not the territory of any of the animals. The results showed that, in the intruder test, the resident animal showed most aggression and highest dominance. In both tests, the age hierarchy had no role during the aggressive encounters. These results show that it is not the age hierarchy but the individual behaviour of the animals that defines their reaction to the environment (Nathaniel *et al.* 2007).

The study of Suarez-Jimenez *et al.* (2013) showed that the rank of the mother in the hierarchy has an influence of the behaviour of her infants in rhesus monkeys (*Macaca mulatta*). Several tests were performed for this study; a Brazelton test, a human intruder test and a human intruder-startle test. Further, cortisol levels in reaction to stress were measured and interactions between the mother, her infants and other individuals were observed. The results of these tests and observations suggested that the behaviour of the infants is in accordance with the dominance rank of the mother. Apart from the role of the mother's dominance, early environment and other factors may also affect the behaviour of the infants (Suarez-Jimenez *et al.* 2013).

Dingemanse and Goede (2004) looked at individual differences in exploring new environments in relation with the dominance of the individual. To test this, great tits (*Parus major*) were used. Results showed that fast exploring, territorial birds had a higher dominance rank compared to slow exploring territorial birds. The contrary was true for nonterritorial birds. In these birds, slow explorers were more dominant over fast explorers. This suggests that, in great tits, dominance is not a good measure of personality because the relation between dominance and personality is dependent on the context (territorial/non territorial). Even if the context would be taken into account, it is suggested that dominance is not a good measure of personality. There are too many other factors which should be

taken into account like the environment, the characteristics and the way of coping with social defeat of the individual (Dingemanse and Goede 2004).

An animal can cope with a stressful situation, like social defeat, in two different ways, proactive and reactive. These two different coping styles are dependent on the personality of the animal. An animal which has a proactive coping style will act actively in a stressful situation by trying to control the situation. On the other hand, an animal which has a reactive coping style will act passively and show for example freezing behaviour (Daisley *et al.* 2004, Ijichi *et al.* 2013).

Origin(s) of behavioural differences (in inbred mice)

Apart from the environment, it was thought that by using genetically identical mice, the used animals would be 'standardized'. Since their genotypes are all the same, one might think their behaviour is the same as well. As said before, this is not the case, they still show individual differences in their behaviour (Lewejohann *et al.* 2011).

Lathe (2004) gives several explanations for this occurrence of personality in mice. There are factors which play a role early in the ontogeny of mice (Lewejohann *et al.* 2011) and factors which influence personality after birth. The first kind involves genetic changes like minisatellite variation and epigenetic factors like imprinting errors (Daisley *et al.* 2004, Lathe 2004, Lewejohann *et al.* 2011). As explained before, epigenetic factors like imprinting errors result in phenotypic variation and it is suggested that they might also lead to behavioural differences. An example of this effect of epigenetic factors on the behaviour of animals is shown by research of Weaver *et al.* (2004). Their research showed that the expression of the glucocorticoid receptor (GR) in rats is mediated by the behaviour of the mother. The expression of the GR depends on methylation of the GR promoter. More methylation of this promoter means a lower expression of GR. If the mother grooms and nurses her young, the methylation of the GR promoter will be lower. As an effect, GR will be expressed more and the pups will be less likely to show anxious behaviour (Weaver *et al.* 2004).

Next to this the position of the foetus in the uterus has an effect. For example a female mouse in between two male mice will be influenced by the male hormones and vice versa. The nutrition which they receive while in the uterus and twin effects (interactions between siblings in the uterus) also play a role. Stress and infection from the mother mice also affects behavioural differences in the offspring. This is of influence on the offspring before and after the mother gives birth to them (Lathe 2004).

After birth, personality will be affected by nutrition, the social interactions between siblings and the mother and, in adult animals, social status and hormones. The social status can influence personality in such a way that a difference in dominance can change behaviour in tests for anxiety, activity and aggression. Hormones play a role in females and males. In females the oestrus cycle and in males' testosterone levels contribute to personality. Females for example show increased aggressiveness during their pregnancy (Lathe 2004).

Daisley *et al.* (2004) performed a study on the effect of testosterone on the coping style of Japanese quail chicks (*Coturnix japonica*). Testosterone was injected in fertilized eggs. After hatching until the chicks were 3 weeks old, several behavioural tests were performed with them. Compared to the control chicks, the chicks in the test group showed a behavioural phenotype towards a proactive coping style. They approached and explored novel objects sooner than control chicks. This study showed that a female can have an

influence on the epigenetics of chicks by varying the amount of steroids in the yolk of an egg (Daisley *et al.* 2004).

Statistics

Considering statistics, first random variation was considered as a result of unstandardized factors in the test set-up. However, now it is known that this "random" variation might be a result of behavioural differences (Nomakuchi *et al.* 2009, Réale *et al.* 2007, Uher 2011).

Réale *et al.* (2007) explain two ways of analysing personality. The first one uses an average value per individual over several observations. Uher (2011) also states that it is important to use aggregated measures for one individual to assess personality. Another possibility is to use repeated-measure ANOVAs with a focus on the between subject component (Réale *et al.* 2007).

To conclude personality, as said before, the behaviour has to be consistent over different and the same situations. One way of measuring this consistency, is by analysing the repeatability of behaviour. To check this repeatability, an ANOVA can be used with the different individuals as a fixed factor and a minimum of two measures per individual. The higher the correlation between these two measures, the better the repeatability (Réale *et al.* 2007, Uher 2011). The time in between the two measures and the environment in which the behaviour is tested can influence the results. For example, behaviour of animals changes with age. Therefore, measures that are collected a few years apart, may not be highly correlated. Concerning the environment, for tests performed in a natural situation, more than two measures are necessary because the unstandardized, changing environment has a greater effect on the behaviour than the relatively stable environment in a laboratory (Uher 2011). Another possibility is to use a mixed model with the individual as a random effect. Both for the ANOVA and the mixed model, the environment has to be constant (Réale *et al.* 2007).

Measures have to be looked at in relation to measures of other individuals from the same group. This is important because a personality is unique for an individual, therefore it has to be seen in relation to personalities of other individuals. Before looking at this between-individual variation, it is important to standardize the measures (Uher 2011).

Conclusion

In my opinion, there are several factors which could be improved concerning behavioural research of animals. First of all, it is important to use identical terms. At this moment, there are a lot of jingle/jangle fallacies. This means that, in case of a jingle fallacy, one term is used for different concepts and, in case of a jangle fallacy, different terms are used for one concept (Carter *et al.* 2013, Uher 2011). As explained before, Uher (2011) made an overview of which term means what. This could be a good guide to follow.

Next, methods which are used for measuring personality should be standardised. It would be best if the test measures one trait (Carter *et al.* 2013). As explained before, the traits boldness and exploration for example can be correlated. Therefore it is important to know which trait is measured. Concerning testing, there is also the environment which plays a role. It could be valuable to consider how the animal perceives its environment. In research conducted until know, the environment of testing is standardised according to factors which

we, humans perceive. These are factors like light, enrichment and temperature. However, it is for example known that rats can hear very high frequencies and Wahlsten *et al.* (2003) suggest that mice can perceive smells which humans cannot. This could influence test results.

Then there are the animals which are being used in the test. As showed by research from Lathe (2004) and Daisley *et al.* (2004), hormone concentrations have an influence on the individual behaviour of animals. One factor which is therefore important to know when using animals in tests, is their hormone profile. Another aspect which could be improved is the fact that some researchers do not use all animals which they first selected to be used in their experiment. For example, in case of a test where measuring personality involves the animals learning something, slow-learners are often discarded from the experiment. This means that not the whole sample population is tested (Réale *et al.* 2007). It would be better to integrate all personalities in the test.

One way of integrating animal personalities in research is by dividing animals in groups based on their personality. This has for example been done by Duclot *et al.* (2011). Rats were divided in two types of responders, high and low responders. This division in high and low responders was based on high or low activity in a novel environment test. Then they were exposed to social defeat to trigger a state which resembles a psychosocial stress-related mood disorder in humans. Concerning anxiety, body weight, sucrose preference and social avoidance, only high activity responders were affected. Both high and low activity responders were affected by the social defeat exposure, however only high responders showed a stronger reaction to a single social defeat. This research shows it is important to involve individual differences in research (Duclot *et al.* 2011).

Spoelder *et al.* (2013) divided their animals in groups as well. Rats were tested on how vulnerable they were for alcoholism and the relation of alcoholism with decision making. To assess their vulnerability for alcoholism, all rats received a water bottle and a bottle containing alcohol. Based on their consumption of the alcohol, rats were divided in high and low alcohol consumers (Spoelder *et al.* 2013).

To conclude, based on this review I think it is important to take individual differences into account while conducting a behavioural test. This is in agreement with Réale *et al.* (2007). This is not only important to receive more reliable results but for the welfare of the animals. It is for example important to know what coping style an animal has. Proactive animals will show an active reaction to a stressful situation. This makes it clear that they experience stress. Reactive animals on the other hand show freezing behaviour which might seem as if they do not experience stress. However, they do experience stress (Ijichi *et al.* 2013). As Ohl and van der Staay (2011) state in their article, every animal reacts in its unique way to the environment. This means that, to asses welfare of the animals, their personalities should be taken into account.

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