

Temperament – a psychobiological approach to Harm Avoidance and Novelty Seeking

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Göteborg, Sweden 2006

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Printed in Sweden
ISSN 1101-718X
ISBN 91-628-6856-X
ISRN GU/PSYK/AVH--173--SE

*To my mother and father for always encouraging my curiosity and enthusiasm,
whichever shape it has taken.*

DOCTORAL DISSERTATION IN PSYCHOLOGY

ABSTRACT

Ray, J. (2005). Temperamental development in the rat.
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This thesis explores if rats can be said to have a temperament, and if that is the case, how it changes with age and how it is related to brain neurochemistry. Using the hole board and canopy tests (considered to measure exploration and anxiety respectively), behaviour was studied in Wistar rats. In **Study I** Principal Components Analysis (PCA) revealed two temperamental dimensions reflecting Harm Avoidance and Novelty Seeking. Sex differences were apparent, in that nonestrous females were more active than males and nose poked more in the hole board. In regard to the two temperamental dimensions, sex differences could also be observed, with males exhibiting high levels of Harm Avoidance, and more females exhibiting a high Novelty Seeking/low Harm Avoidant profile. In **Study II**, a longitudinal study, behaviour was observed from the age of 6 to 52 weeks. Correlation analyses showed substantial behavioural consistency over time, with subjects showing considerable rank order consistency in behavioural measures from their 11th week and on. PCA analysis revealed two temperamental dimensions in adult rats. However, only one dimension reflecting Harm Avoidance was present in the juvenile and older rats. Several behavioural variables showed age-bound mean level profiles.

In **Study III** connections between brain monoamines and temperamental dimensions were analysed using multivariate techniques. Harm avoidant subjects had low levels of striatal dopamine, and high levels of cortical noradrenaline and amygdaloid 5-hydroxyindoleacetic acid. High Novelty Seeking scores were linked to low levels of brainstem serotonin and dopamine, and to low levels of 5-hydroxyindoleacetic acid in amygdala and accumbens. Moreover, rats scoring high on Novelty Seeking had higher-than-average levels of noradrenaline in the thalamus and of serotonin in the amygdala. **Study IV** went on to explore potential similarities in behaviour between male sibling rats, finding small and non-significant correlations. In contrast to this, weight correlated highly between siblings both at the start and the end of the testing period.

Overall the findings in this thesis support the position that temperament is a temporally enduring dimension but that it also changes over the course of an organism's life cycle. Tentative connections between chemistry and temperamental dimensions are made, and findings on siblings in Harm Avoidance and Novelty Seeking point in the direction of little or no temperamental similarity.

Keywords: Harm Avoidance – Novelty Seeking – Continuity – Longitudinal–Monoamines - Siblings

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ACKNOWLEDGEMENTS

I would like to thank my supervisor, Professor Stefan Hansen, for all those hours of support in many and varying ways on this excursion into science. It has been a pleasure to be able to delve into my studies in such a relaxed and friendly environment and I have enjoyed our cooperation tremendously.

Thanks are also due to all my fellow PhD students at the department that have readily shared their time, knowledge, laughter and worries with me! I would especially like to single out Maarit Marmendal, PhD, for generous help at the laboratory explaining how to use the activity box.

Among the teaching staff here I would especially like to thank Professor Sven G. Carlsson for kindly worded food for thought and further reflection, and Reader Jan Johansson Hanse for a soothingly practical approach to the daunting task of climbing Mount Statistica.

Also, to Kjell Söderberg, Bengt Carlsson, Gunilla Palm, Marie-Louise Rydberg, Ann Backlund, Madelene Kröning, Paul Svensson and Ricardo Berrio for all your endless and cheerful help with countless practical details, I am extremely grateful.

I am also indebted to Mrs Birgit Linder for the laboratory assistance without which there would be precious little data or results!

Finally, to my wife Johanna, my friends Marcus and Hans, and all my family in all the far off corners of the world, thanks for all those discussions along the way that have helped me think more critically about what I am doing.

The Swedish Research Council financially supported the empirical work on which this thesis is based.

LIST OF PUBLICATIONS

This thesis is based on the following research papers, which will be referred to by their Roman numerals:

- I. Ray, J., & Hansen, S. (2004). Temperament in the rat: Sex differences and hormonal influences on harm avoidance and novelty seeking. *Behavioral Neuroscience, 118*, 488-497.
- II. Ray, J., & Hansen, S. (2005). Temperamental development in the rat: The first year. *Developmental Psychobiology, 47*, 136-144.
- III. Ray, J., Hansen, S., & Waters, N. (2006). Links between temperamental dimensions and brain monoamines in the rat. *Behavioral Neuroscience, 120*, 85-92.
- IV. Ray, J. & Hansen, S. (2006). Why are rats in the same family so different from one another? (Manuscript in preparation).

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INTRODUCTION

“Individual humans show consistent differences in their behavioral tendencies. Compared to others, some people are relatively assertive, or bold, or friendly, or deceptive. Analogous patterns of individual variation have been documented in several primates, domesticated animals, laboratory rodents, and a scattering of other animals (Gosling and John, 1999; Gosling 2001). In humans, these differences have been termed personality type (Pervin and John, 1999). In other taxa, they have been referred to as coping styles, temperaments, behavioural tendencies, strategies, syndromes, axes, or constructs (Gosling 2001).”

(Sih, Bell, Johnson & Ziemba, 2004)

The subject matter of the present thesis is temperament, and more specifically two dimensions of temperament in the rat. The potential usefulness of this particular endeavour to the field of psychology might not immediately be apparent so let us briefly consider this question before getting into definitions of terms and presentation of earlier work in the field. Laboratory rats form the basis of a huge amount of research in areas as diverse as stress research, the effects of maternal separation, the propensity to take drugs and develop an addiction, not to mention the effects and properties of psychopharmacological agents. All these fields have this one feature in common – they use animal models to explore the biology and psychology of one species, the rat, in the hope of discovering useful information for another species – ourselves. Now, it is possible that different personality types are more likely to become addicted (Bardo, Donahew, & Harrington, 1996) to recreational drugs. It is also possible that different personality types are more susceptible to psychological illness (Matthews, Deary, & Whiteman, 2003). It would follow from this that it might be relevant to pause and consider the effect temperament and personality might have on the effects of drugs, stress experiences and coping techniques, or the interaction between individual and psychopharmacological agent, not only in humans, but also in rats, the very animals the experimental groundwork is originally performed on. But not only that – on another, and far more fundamentally important level, it would also be of great interest to know whether temperament and personality are at all relevant in a larger evolutionary perspective or whether they are just random noise around an optimal adaptive mean. To do this, however, it must first be established if rats can really be said to have temperaments that could be studied in relation to the previously mentioned fields – if not we might truly be off on a wild rat chase. Thus the thrust of this research project has been to explore temperament in the rat – is it present, and does it develop and change over time?

Temperament can be conceived of as those predispositions for behaviour that form the substrate on which experience and life events work to form the traits that later build a personality. Temperament is discernible in individual differences that emerge in very young human infants and is presumed to be partly heritable. Temperament, traits and personality are concepts that are hard to distinguish from each other in the literature, with different authors using different approaches (Matthews et al., 2003). Many authors in the animal research literature reserve use of the word personality for people, using the word temperament for animals, but in child developmental work temperament is frequently used to describe humans. Although traits and personality are usually used to describe humans, a number of researchers use the term personality

to describe animals other than humans (e.g. Capitanio, 1999; Gosling, 2001), ranging from octopi to rhesus macaques. It would thus seem to this author that at the present time there is no clear and meaningful distinction in terminology.

Unfortunately, sorting out which label to use on this level in no way ends the confusion, because different researchers use different labels, seemingly with the same meaning, for temperamental (or personality) dimensions. Take anxiousness as an example. This very fundamental dimension has been described using, among others, the following terms: behavioural inhibition, fearfulness, emotionality, Neuroticism, shyness, timidity and Harm Avoidance. In the present thesis I have adopted the term Harm Avoidance to describe one dimension of the behaviours I have studied and will attempt to clarify my approach in the following text.

One of the premises of this thesis is the behavioural continuity of species. It follows from modern genetics and evolutionary reasoning that the methods and concepts suitable for studying one mammal can be usefully applied to other mammals. This is based on the fact that mammals share the same genetic ancestry to a very great degree, and that this genetic code has been adapted to build structures that deal with the same problems and opportunities in life – finding food, a mate, and shelter, avoiding predators and raising offspring. All the above require the ability to both initiate and inhibit actions and to strike a balance suited to the particular needs of the organism in question which will differ if that organism is predator or prey, nocturnal or diurnal, herbivore, carnivore or omnivore, etc. The laws of evolution specify that within any normal population there will be a variation of characteristics and that it is upon this variation that natural selection will operate (e.g. Freeman & Herron, 2003). Although it has not been proven it would seem plausible that the sum of tendencies to activate or inhibit action that we call temperament, and, in more cortically advanced species, traits and personality, would also exhibit this kind of normal variation in mammals. In fact perhaps this would be the case in any complex organism that has to move around to mate and find food.

In relation to variability a further question arises. Is the variability in behaviour, and at a deeper level, personality or temperament, to be considered as random noise or as some form of useful or functional descriptor? King (2003) nicely sums up the argument for adaptation by proposing two alternatives. In the one scenario personality would be totally random. If this were the case we would be continuously in the dark as regards the motives and behaviour of our conspecifics, never being able to predict their future behaviour on the basis of our observations of previous behaviour and our assumptions about their temperament or personality. This is clearly not the case. Another scenario would have us all homogenous, which is also clearly not the case – we daily see differences in, say, persistence or desire for novel stimuli in those around us.

Another line of thinking as regards variability is the person-situation debate (Fleeson, 2004). Essentially one can describe the situationist claim as maintaining that traits do not influence or predict individual behaviour in a given situation. Indeed there is a large variation within individuals as to how they will react to different situations. The other side point out that traits do indeed predict trends in behaviour over a long period of time. This approach can also point to evidence showing that despite the large variability, a given individual varies around a central point with a high degree of

consistency if measured over a period of time. In a sense this can be reduced to statistics – many phenomena can be described as an average tendency over time, but this still does not allow accurate prediction of behaviour on one particular occasion – life is too complex for that. It does, however, allow accurate prediction of behavioural trends over a great number of occasions. In other words, temperament, in the words of Grigsby and Stevens (2000), can be described as a *probabilistic* phenomenon, meaning that an individual with an anxious temperament will not invariably be anxious in all situations, but the probability of presenting anxious behaviour will be higher in this individual than in, say a low anxiety extravert. Funder (2006) has recently published a study that clearly spells out many of the misconceptions that keep this debate alive, a debate based largely on misunderstandings around the false dichotomy of persons and situations.

Although none of the above proves the adaptive value of temperament (such proof is sadly hard to obtain in almost any evolutionary account of antecedents), it points us in the scientifically constructive direction of wondering what the significance of variable behavioural strategies might be on the life-and-death playing field of Darwinian evolution. In the last decade of the 20th century and the first few years of the 21st century, a number of studies focussing on the adaptive value of personality/temperament from a biological fitness perspective have been published, and we will look at some of these below and return to these interesting ideas and findings in the Discussion section.

Temperament and personality

Humans

A great deal of work has been done on temperament and personality in humans over the past fifty years, ranging from basic research to applied psychology in psychiatric care settings, candidate evaluations in business and organisations, medical treatment and neuropsychological studies. Parallel to this temperament has been studied in the child developmental literature (Kohnstam, Bates, & Rothbart, 1989) with a slightly different focus – that of development, change and heritability. Research in temperament and personality can be divided into two major areas as defined by the directions mentioned above. One key area is the possibility to describe behaviour in terms of underlying factors or traits as measured by individual differences. The other is concerned with the long-term stability and change of these factors and traits. Of course the two fields largely merge in many cases.

Of the many models of human temperament and human traits that have been developed, those of H. J. Eysenck and J. A. Gray are perhaps among the most well known within the framework of biological psychology. Another well-known model in this framework is that of C. R. Cloninger, the 7-factor psychobiological model of temperament and character. Cloninger conceptualizes temperament as consisting of a number of largely heritable automatic perceptual biases he calls Harm Avoidance, Novelty Seeking, Reward Dependence and Persistence. Character dimensions, consisting of Self-directedness, Cooperativeness and Self-transcendence appear as the individual matures (Cloninger, Svrakic, & Przybeck, 1993; Cloninger, 1994). Novelty Seeking then, is viewed as a temperamental bias in initiation of behaviours such as frequent exploratory activity in response to novelty, impulsivity, disorderliness and talkativeness, and also with a quick loss of temper and active avoidance of frustration. Harm avoidant individuals are biased in the direction of cessation of behaviours,

worrying, fear of uncertainty and increased risk of anxiety. Reward dependence is characterised by warm social affiliations. Persistent individuals, finally, are ambitious and determined, overachieving and eager. One feature of Cloninger's model is to allow for the study of important developmental features at the extremes of the human life span, such as the development of persistence at an early age and self-transcendence in old age. Cloninger envisages temperament as being connected to the procedural learning systems of the brain, whereas character is connected to propositional learning. Thus character is more open to cognitive influence, whereas temperament reflects habit systems. The sum total of temperament and character make up the personality of humans, with the two being causally independent but functionally interactive.

Questionnaires trying to measure personality and temperament rely heavily on evaluations of emotional content in relation to certain events and behaviours, with words like worried, angry, shy and irritated present in many questions. It could be argued that it is nigh on impossible to understand personality and temperament without understanding a good deal about emotions, and I would be remiss if I did not at least mention the field here. Strangely, the study of emotions and the study of personality constitute two separate fields of research within psychology, and rarely do the two meet. Authors like LeDoux (1998) have done a great deal to put emotions on a respectable footing in the world of science, in his particular case, the biological underpinnings of fear and anxiety. A truly ambitious project integrating neuroscience and emotion research is that of Panksepp (2002). He proposes a model of basic emotions with four major components, present in all mammals from soon after birth, and calls them SEEKING, FEAR, PANIC and RAGE. In the terms of this thesis, the dimension of SEEKING, characterized by forward locomotion, sniffing and investigation and being stimulus-bound and appetitive in nature, would correspond closely to Novelty Seeking. FEAR, characterized by freezing, flight and escape behaviour, and also stimulus-bound, would be more related to Harm Avoidance than PANIC, which has more to do with the seeking of social contact and distress at isolation and loss of significant caretakers. The RAGE system and the FEAR system are intimately related, which of course makes good evolutionary sense.

Overall, though, P. T. Costa and R. R. McCrae's (McCrae & Costa, 1997) Big Five model is by far the most influential human model of personality. A vast array of studies have been able to replicate the finding that personality can be described using the following five broad dimensions. Openness to experience reflects curiosity and the desire to expose oneself to the new and unfamiliar, contra conventionality. Conscientiousness reflects need for achievement and discipline. Extraversion is a measure of how sociable and talkative an individual is. Agreeableness is a measure of whether a person is trusting and good natured or uncooperative and rude. Finally, Neuroticism is a measure of worry, anxiety and insecurity contra calm and even temperedness. These traits are also known under the acronym OCEAN, or as the FFM – Five Factor Model. These findings have recently been extended beyond the domains of human research in a number of papers on Big Five traits in animals.

Temperament in nonhuman organisms

In a review of the field (Sih, Bell, Johnson, & Ziemba, 2004) spanning ecology, human psychology and evolutionary analyses, the authors propose the term behavioural syndromes to cover suites of behavioural traits correlated across time. In

their perspective, behavioural syndromes can be seen as tradeoffs in the organism's time budget over its lifecycle. This would mean that individuals living in shifting circumstances would be prone to using the same suite of strategies over a variety of situations – sometimes leading to suboptimization if one behaviour in one context alone is studied, but on the whole leading to a useful life strategy when a number of situations and life-contingencies are taken into account. According to the authors, this means that we need to study multiple behaviours, preferably in multiple contexts, to really be able to link “personality” – i.e. behavioural syndrome to the ecological niche and environment the creature lives in, rather than atomizing it. This, however, is not usually how animals are studied. Let us take a look at a few landmark studies here to acquaint ourselves with the field, and save a few others for the Discussion section.

Primates

Animals other than humans have received much less attention although the picture has begun to change over the past decade. Primate research has revealed interesting temperamental differences and development in monkeys and apes. In a study on temperamental development within and between species (Heath-Lange, Ha, & Sackett, 1999) a pattern of relative stability or gradual change was noted in development from day 1 -200 in infant macaques and baboons (temperament was studied in 50 day age blocks). Temperament was measured using behavioural variables reflecting latency to contact familiar human caretakers, response to capture, clinging, attention to environment and a number of other behaviours. As subjects matured their rank scores became more stable, with correlations over time not in evidence at first (i.e., when comparing the first two 50 day age blocks), but appearing as the infants got older.

In a review of reactivity in primates (Higley & Suomi, 1989), strong temperamental continuity from infancy (9 months) to childhood (18 months) and early adolescence (30 months) was reported and has subsequently been replicated in different laboratories and with different species. Reactivity is a measure closely associated with the concept of Neuroticism, anxiety and Harm Avoidance.

Factor analytical approaches in personality research on chimpanzees indicate that the human five factor model (FFM) of personality may be applicable to chimpanzees (Weiss, King, & Figueredo, 2000), and also that the age related changes observed in chimpanzees partially mirror human changes such as decreases in competition and social volatility. These studies have yet to be replicated on other wild or semi-wild populations, but recent work by the same authors mainly confirms the factor structure the authors initially described (King & Farmer, 2005).

Canines

It is natural, perhaps, to focus on primates as an object of study if one is operating from the assumption that there is some meaningful biological continuity between species closely related in terms of DNA. Another animal close to us, not genetically but as a long standing invited guest of the human family, is the dog. Do they have temperaments? In a recent cross-species study (Gosling, Kwan, & Oliver, 2003) judgements of personality were recorded in dogs and humans and the authors found judgements to be accurate in both, looking for internal consistency (“...the degree to which judgements about personality are consistent across observations or items thought to reflect the same behavioural dimension”), correspondence (comparing

owner's judgement with the dog's behaviour as rated by independent observers in a local park) and consensus (between owner's and independent judge's description). The authors found that the judgements were accurate and as substantial in size as those of humans. Interestingly, the FFM framework as applied to dogs reveals no Conscientiousness dimension, in fact an equivalent of Conscientiousness has to this author's knowledge only been found in chimpanzees.

Another study of interest assessed consistency of personality across tests in dogs (Svartberg, Tapper, Radesäter, & Thorman, 2005). Analyzing 29 breeds of dogs on behavioural personality tests, the authors assessed behavioural continuity. The test-battery consisted of ten separate subtests, including responses to metallic noise, chase, the appearance of an unfamiliar person and play. The dogs were rated on five dimensions of personality, labelled Aggressiveness, Curiosity/Fearlessness, Playfulness, Chase-proneness and Sociability. Statistical analyses yielded correlations from 0.57-0.89 for all traits over tests. The authors concluded that personality in these dogs was indeed stable over the two months of testing.

Predator and prey

But what of mammals further from humans? Let us take a look at predator and prey animals, large land mammals that in all likelihood would exhibit temperamental differences were we to follow functional evolutionary reasoning. In predators, the hyena has been studied (Gosling 1998) using behavioural ratings and factor analysis. Gosling obtained a factor structure with five personality variables labelled Assertiveness, Excitability, Human-Directed Agreeableness, Sociability and Curiosity. Females were found to be more assertive than males, regardless of age, replicating a well established finding in biology (females' circulating testosterone is very high in the hyena). Unfortunately Gosling did not have the possibility to study behavioural change from infancy through puberty to maturity in this work.

In herbivores/prey animals temperament in the bighorn ewe has been the object of study (Réale, Gallant, Leblanc, & Festa-Bianchet, 2000). This study focuses, among other things, on the global/domain specific dimension (closely mirroring the old trait/state debate). The findings in this long-term study support the existence of two temperamental dimensions labelled boldness and docility in ewes. Temperament was not affected by reproductive status or body weight. The authors reached the conclusion that temperament, although consistent over time and age in ewes, is also domain specific – that is, boldness in one situation will not necessarily translate to boldness in other settings. Why this should be the case is, to my mind, a crucial question, and one we may be able to discern an answer to after further consideration of the studies at hand.

Marine organisms

Mammals, then, have been the focus of increasing attention in temperament research. What of other mobile organisms? A handful of interesting studies involving fish and octopi have tackled temperament/personality underwater. In a study on 29 guppies (Budaev, 1997) evidence for two broad personality dimensions, Approach and Fear Avoidance, were found using a factor analytic approach. The author also stresses the fact that unidimensional characterisation of the organism in question in personality studies is problematic. The same fish can exhibit both bold and shy behaviour patterns. Budaev also maintains that random behavioural components would be more

pronounced in non-threatening situations, possibly masking consistent individual differences. This, of course, applies especially to prey animals such as many guppy populations.

An aquatic invertebrate predator, the octopus, also showed large individual differences when studied using factor analysis of behavioural measures (Mather & Anderson, 1993). Forty-four octopi were studied in three situations characteristic of their everyday lives, alerting, threatening and feeding situations, and a subsequent factor analysis of behaviour isolated three orthogonal dimensions – Activity, Reactivity and Avoidance. Tests were conducted every other day for two weeks, thus giving a temporal dimension to the study, albeit slight. The authors suggest that octopi would have three adaptive reasons for having evolved individual differences in personality, these being genetic drift, the inherent “usefulness” of variability in heterogeneous environments, and shifting selection pressures as a result of cephalopods belonging to a fast evolving group of organisms in competition with bony fish. Also, on a proximate level, variability would come into play in the life span of an organism so dependent on learning as the octopus. Mather and Anderson see octopus personality as something more than situation-specific effects. Rather, referring to earlier longitudinal studies (Mather, 1991) to support this claim, they consider personality in octopi to consist of long lasting adaptive individual differences.

In an elegant study of pumpkinseed sunfish (Wilson, Coleman, Clarke, & Biederman, 1993), the authors found consistent differences between groups of fish that could be classified along a bold – shy continuum. Those fish that were quick to explore novel objects were more likely to approach human observers, more likely to swim alone, and acclimated more quickly to a novel (laboratory) environment after capture. They were also more likely to be infested with parasites of a species indicating that the fish in question were foraging in deeper and riskier waters – i.e. taking higher risks in the trade-off between foraging success and risk of predation. These differences in behaviour seemed to disappear after a period in the laboratory. The authors make some interesting remarks based on their observations. Firstly, perhaps these bold-shy differences, although stable and present before capture, were due to behaviourally flexible individuals responding differently to ecological pressures (food shortage) – after all, as is pointed out “...the assumption that phenotypic stability implies innate differences between individuals is questionable. Environmental factors can reinforce differences between flexible individuals as easily as erasing them.” Three major problems with psychologists’ approach to the shy-boldness continuum are also mentioned. First, that the ecological consequences of shyness and boldness have never been studied in a natural population of any species. Second, that an evolutionary perspective is lacking – that is, no one has tried to predict and test adaptive patterns of shyness and boldness that might result from natural selection, and third, that the taxonomic distribution of the shy – boldness continuum is largely unknown. These are indeed crucial questions that psychology has for a long time consistently failed to ask.

As indicated in the previous paragraphs, a number of species have been studied within the personality or temperament framework, although each individual species has so far not been subject to much research and a great number of species have simply not attracted research attention in this field. I have chosen to use a number of focal studies to give the reader an overview of the field and the recurring themes, but there are of

course more individual studies on a large number of species, from piglets, spiders and grasshoppers to trout, great tits and rhinoceri!

Rats

To round this review of previous research off, let us turn to the animal in focus in this thesis – the rat. In doing this we are suddenly confronted with an amazing paradox. The rat is probably *the* single most used and studied mammalian animal in the world of psychological research, and yet the number of studies dealing directly with the temperamental makeup of this creature is tiny! Nowhere is this clearer than in longitudinal research. A number of studies have been conducted where rats were observed for a few days or a week, but work extending over a few months or a year is hard to come by. What then do we know of temperament in rats? To try to answer this question let us look at a few studies that highlight various angles.

In a critical evaluation of behavioural methods currently used to study emotionality (a concept closely related to Harm Avoidance) and stress in the rat (Ramos & Mormède, 1998) several key issues relevant to this thesis are raised. As described in the opening paragraphs of this introduction, there is no strictly defined and structured terminology linking observable rat behaviour to higher-level categories. Ramos and Mormède approach the subject from the angle of stress research, but the crucial problems remain the same whether one is looking at temperament or stress in animal studies – those of knowing what we are actually studying! Rather than getting caught up in quibbles over wording Ramos and Mormède set out the evidence for the merits of a multidimensional factor analytically defined approach. Drawing on evidence from the literature from 1934 and onwards, the authors make the case for the proposition that behavioural measures such as ambulation and defecation originally thought to measure anxiety do not correlate in consistent ways and are highly dependent on experimental methods. It would seem that the original supposition that behavioural measures relate to unitary constructs in a simple manner is untenable, and that rodents exhibit a multidimensional structure of behavioural dimensions much as humans do in trait research. Emotionality, in other words, is comprised of a number of distinct forms that will be independently displayed in different conditions. In a study using a factor analytical approach (Boguszewski & Zagrodzka, 2002), two groups of rats aged 4 and 24 months respectively were tested on a variety of measures in the open field, elevated plus maze and social interactions test. The authors reported finding two factors in the open field reflecting activity and anxiety, whereas the analysis of the elevated plus maze yielded three factors reflecting anxiety, motor activity and decision-making. The authors reported a higher level of anxiety and lower levels of motor activity in old rats as compared to young. In a study designed to test the reliability of high and low anxiety-related behavioural measures (Salomé et al., 2002), rats were tested on the elevated plus-maze, the forced swim test, and the black-white box test (latency to leave initial “safe” point of entry). Using multivariate analysis the authors found that the factor of anxiety represented 43.4% of the total variance, with a second factor, “locomotion” and a third, “escape”, accounting for 20.4% and 11.7% respectively. These differences, present in High anxiety and Low anxiety behaviour rats were independent of laboratory where the tests were conducted. Using the elevated plus maze to study the effect of anxiogenic stimuli, Treit, Menard, and Royan (1993) showed that rats do not habituate to the maze – entering open arms showed no significant increase over 18 sessions, in fact there was a slight decrease in

propensity to enter open arms after the first session, which the authors suggest might reflect increased fear.

Another important area of research has been sex differences, where a number of studies have found that female rats show less anxiety related and harm avoidant behaviour than males (although this has been contested and it would seem that the type of fear provoking situation is critical for differential responses in the two sexes). In a factor analytic study of fearfulness in almost 800 rats from two genetically heterogeneous inbred strains (Augilar et al., 2003) using a battery of novel/threatening tests including the hole board, open field, plus maze and activity, and also classical fear conditioning, researchers obtained a three factor structure consisting of a learned fear factor, a fear of heights/open spaces factor, and an emotional reactivity factor. Although male rats, in agreement with much previous research, showed more fear than females, the factor structures were shared by both sexes. This conclusion contradicts the findings in another study (Fernandes, González, Wilson, & File, 1999) also investigating sex differences in behaviour using a factor analytic approach. This study involved behavioural assessment of 115 Wistar rats (59 females) in the hole board, elevated plus maze, and a test of sexual orientation. The authors reported different factor structures in male and female behaviour, with females primarily exhibiting activity and males primarily exhibiting sexual interest and anxiety. The question remains whether the temperaments of male and female rats actually do differ to the extent reported by Fernandes et al., or if they share a common basis and factor structure for their temperaments. I am tempted to put much of this discussion down to methodological differences, but obviously the question itself is still important.

On a species level a great number of strains and breeds¹ of rats can be distinguished, with different strains exhibiting varying levels of reactivity, emotionality and anxiety. As an example, Long-Evans strain mother rats show more licking towards pups than Fisher 344 mothers, and generally have a more ordered litter than both Wistar and Sprague-Dawley rats [see Whishaw & Kolb (2005) for a more thorough treatment of the concept of rat strains]. Molecular genetic research involving quantitative trait loci can now correlate gene polymorphisms with traits, thus greatly enhancing the power of our scientific toolbox (Mormède et al., 2002). For the purposes of the present study, however, I am not so much interested in differences between strains as those existent *within* a particular strain. The research community at large is well aware of between strain differences – indeed these have been intentionally bred for so as to be able to perform various experiments. Temperament within each strain is, however, not yet clearly understood and is indeed very much under-researched. Very few of the published articles deal directly with longitudinal stability of temperament in the rat – this is usually secondary data that can be gleaned from examining the reported behaviour on the various tests adopted – the hole board, the elevated plus maze and the open field. At present the research in this field indicates that individual differences in temperament within strains is in evidence, but that a good deal more work must be done before we can safely generalise findings across species boundaries. I would also

¹ Strains describe physiological differences such as resistance to cold or heat and may therefore be internal and invisible, whereas breed is a morphological concept relating to form and function such as body weight or length of tail, relating to visible external differences

like to clarify at this point that although only two dimensions of temperament were studied in the articles making up this thesis, this must not be taken to mean that I consider rats to only have two dimensions of temperament. Other dimensions are present in the rat to study, such as social behaviour and dominance if one has the time and resources.

Temperament and brain function

Part of the present research focuses on possible links between brain monoamines and temperament. An overview of the monoamines measured, and their metabolites follows. As these areas are hugely complex I cannot hope to cover this properly here – that would be outside the scope of this thesis.

Serotonin

Of the many transmitter substances in the brain, serotonin (5-HT) has been under intense investigation due to its apparent role in anxiety and the promise of discovering anxiolytic medications if this monoamine were to be fully mapped out and understood. As with the rest of our brain, things have turned out to be very complicated, and a full understanding of serotonin is still some way off. Serotonin is involved in a wide array of behaviours ranging from appetite and aggression (Ferrari, Palanza, Parmigiani, de Almeida, & Miczek, 2005), to sexual behaviour and circadian rhythm (Lucki, 1998), in fact, the range of behaviours somehow affected by 5-HT is so great as to render the experimental data available so far extremely confusing. Nevertheless, this substance is thought to be of relevance in most models of anxiety and behavioural inhibition, although authors debate over exactly how it is relevant.

The serotonergic pathways arise chiefly in the raphe nuclei and project to a large number of brain structures including the amygdala, hypothalamus, nucleus accumbens, striatum, hippocampus, thalamus and neocortex (Hensler, 2006). This abbreviated little list covers large parts of the brain, which might give the reader a hint as to the sheer complexity and number of processes 5-HT must be involved in. This has not stopped some authors from attempting to explain 5-HT on a more general level. Graeff (2002) envisages 5-HT as having the dual role of regulating defensive behaviour through enhancing learned responses to possible (or distal) threat through actions in the forebrain, while inhibiting unconditioned responses to immediate (proximate) threat by acting on the periaqueductal grey. In psychiatric terms the former would be related to generalized anxiety, and the latter to panic disorder. A number of other interesting more speculative theories for the role of serotonin have been proposed (Daw, Kakade, & Dayan, 2002; Doya, 2002), but we are still far from an overarching theory substantiated by research findings that can really explain the workings of serotonin at much more than a rather fragmentary level.

In the rat, Piazza and co-workers have reported that high-responding rats (exhibiting greater locomotor response to novelty) had lower overall 5-HT content in the frontal cortex, nucleus accumbens and striatum than low-responder rats (Piazza et al., 1991). Schwarting, Thiel, Müller, and Huston (1998) tested 24 male Wistar rats (age not specified) for 5 minutes on the elevated plus maze for two consecutive days. Based on measures in this test the rats were divided into high and low anxiety groups. The animals had also been observed in an open field test 11 days prior to the plus maze test, where various activity measures were taken. On the day after the last test the animals were anaesthetized and decapitated, and the medial frontal cortex, ventral

striatum, neostriatum, ventral hippocampus, and amygdala were dissected out and analysed for levels of 5-HT and other neurotransmitters. The authors found that rats spending a lot of time in the open compartment of a plus maze showed higher levels of 5-HT in the ventral striatum than those spending significantly less time in the open compartment. No differences were found between the high and low anxiety animals with respect to noradrenaline (NA) and dopamine (DA) levels.

In a study on rhesus macaques (Higley, Suomi, & Linnoila, 1996), correlations have been reported between decreased cerebrospinal fluid (CSF) 5-HIAA (a metabolite of 5-HT) and later excessive alcohol consumption – a finding which also has been found to be relevant to man. Interindividual differences in CSF 5-HIAA in the rhesus macaques were also reported to be stable over time. Significantly lower CSF 5-HIAA levels (which correlate positively with levels in the prefrontal cortex, involved in impulse control) have been found in a group of 43 impulsive alcoholic offenders with antisocial personality disorders when compared to healthy volunteers (Virkkunen et al., 1994). In another human study, Zald and Depue (2001) found significant inverse correlations in both positive and negative affect in the prolactin response to d,l-fenfluramine (an index of 5-HT functioning) as measured three times daily over two weeks in 31 healthy male subjects. Positive and negative affect are mood concepts that reflect levels of alertness and pleasurable engagement, contra fear, guilt and nervousness, and can be described as Extraversion and Neuroticism related traits (see Watson, Clark & Tellegen, 1988). The results indicate that 5-HT acts as a constraint on these traits. This finding makes sense in terms of the ability of 5-HT to reduce DA-facilitated incentive-motivational behaviour in animals.

Catecholamines: dopamine and noradrenaline

The dopamine systems, which constitute a much larger proportion of brain cells than both the serotonergic and noradrenergic brain systems, modulate a number of behaviours. They are involved in incentive motivation, such as reward and sex. In the rat, dihydroxyphenylacetic acid (DOPAC) is the most common brain metabolite of dopamine, with a short term accumulation of DOPAC in the striatum being an accurate reflection of activity in dopaminergic nigrostriatal neurons (Cooper, Bloom, & Roth, 2003). In humans, homovanillic acid (HVA) is the most common metabolite (Ibid.). Dopamine is involved in drug addiction and is perhaps best known in connection with Parkinson's disease, where dopamine deficiency plays a major role (reviewed by Carlsson, 2001). There are three major dopaminergic pathways. The nigrostriatal system originates in the the substantia nigra pars compacta and projects to the dorsal striatum. The mesolimbic system originates in the A10 area of the ventral tegmental area (VTA) and innervates the ventral striatum and portions of the limbic system. The mesocortical dopamine system projects from the VTA mainly to prefrontal areas of the neocortex (Kandel, Schwartz, & Jessell, 2000; Panksepp, 1998).

Another monoamine under investigation in Study III, NA, projects to large areas of the brain from the locus ceruleus, following five major tracts. The major brain metabolite of NA is MHPG (3-methoxy-4hydroxy phenethyleneglycol). One current hypothesis concerning NA is that the locus ceruleus and its projections determine the global orientation of the brain concerning the external world and also within the viscera – increased noradrenergic activity being associated with unexpected events in the external environment, and decreased activity with behaviours mediating restful states (Cooper et al., 2003). Locus ceruleus noradrenergic activity in both rodents and

primates has been associated with attention mechanisms (Aston-Jones, Rajkowski, & Cohen, 1999).

Previous studies on the topic of brain chemistry–behaviour have focused on finding monoaminergic correlates to individual behavioural items, such as locomotor activity or specific exploratory- or anxiety-related responses, rather than to overall traits or response styles. Thiel, Müller, Huston, and Schwarting (1999) found that the frequency of rearing in rats in a novel environment was associated with increased levels of DA and HVA in the ventral striatum, lower levels of 5-HT in the frontal cortex, and a greater HVA/DA ratio in the dorsal striatum. Bradberry, Gruen, Berridge, and Roth (1991) reported significant positive correlations between nose poking and drug-evoked ventral striatal DA release. Piazza et al. (1991) reported significant correlations between locomotor activity in a novel environment and various DA-related measures in the nucleus accumbens, striatum and prefrontal cortex. For example, there was a positive association between DOPAC in the nucleus accumbens and locomotion in a novel environment. Olson and Morgan (1982) found that rats moving little in an open-field arena had more whole-brain NA than non-emotional animals.

Considering the personality dimension of Extraversion, Depue and Collins (1999) make a case for approaching Extraversion (which is roughly equated with Novelty Seeking in their article) as a higher order expression of underlying neurobiological facilitation systems found in all animals. They point out that dopaminergic activity emanating from the VTA affects a wide range of locomotion, incentive motivation and appetitive behaviour. Results from neuroendocrine studies are also consistent with the view that individual differences in dopaminergic transmission are correlated to Novelty Seeking related traits (Gerra et al. 2000; Hansenne et al., 2002).

DA, then, seems to be involved in Extraversion related traits. However, a study by Tomer and Aharon-Peretz (2004) showed that the situation may be more complex than this. In a study of Parkinson's Disease, they found associations between Harm Avoidance, Novelty Seeking and hemisphere of original disease onset. Using Cloninger's Tridimensional Personality Questionnaire on a group of left onset, right onset and age matched healthy controls, patients with greater dopamine loss in the left hemisphere showed reduced Novelty Seeking, whereas patients with greater dopamine reduction in the right hemisphere reported higher Harm Avoidance than controls. The authors concluded that approach and avoidance are related to different patterns of dopaminergic activity where reduced Novelty Seeking reflects left hemisphere deficits in the mesolimbic ascending dopamine system, and increased Harm Avoidance is connected to greater loss of dopamine in the right striatum. Another indication that dopamine is not solely related to Novelty Seeking traits in a simple way, is the study by Farde and colleagues, who measured DA D2 receptor density in 24 normal volunteers. This revealed a strong association ($r = -0.68$) between the personality trait of social detachment as measured with the Karolinska Scales of Personality, and D2 receptor density (Farde, Gustavsson, & Jönsson, 1997).

Personality in relation to brain activity and structure

A number of studies have examined relationships between personality and brain in humans, using a variety of techniques. Pujol et al. (2002) found that hemispheric asymmetry in the cingulate gyrus was associated with variance in Harm Avoidance,

with a large anterior cingulate being related to high levels of fear and worrying. Another functional magnetic resonance imaging (fMRI) study in humans has shown that individual differences in the trait of Persistence may be linked to specific areas of the ventral striatum and the lateral orbital and medial prefrontal cortex (Gusnard et al., 2003). In another study, Kagan and colleagues (Schwartz, Wright, Shin, Kagan & Rauch, 2003) found that fifteen adults categorised as inhibited at two years of age showed greater amygdalar response to novel versus familiar faces compared with those previously categorized as uninhibited. A regional cerebral blood flow study (Stenberg, Risberg, Warkentin, & Rosén, 1990) on 37 healthy volunteers reported regional differences in blood flow distribution detectable as different patterns of activity in introverts and extraverts (dimensions in Eysenck's personality model roughly similar to Harm Avoidance and Novelty Seeking), with higher blood flow in the temporal lobes for introverts than for the extraverts.

Sibling differences and similarities in temperament

In the final article that makes up this thesis similarities in Novelty Seeking and Harm Avoidance between sibling rats are examined. Correlations between siblings and dizygotic twins on personality dimensions such as Extraversion and Neuroticism in humans are low, and rarely exceed 0.25 (reviewed by Bouchard & Loehlin, 2001, Bouchard & McGue, 2003, Plomin & Daniels, 1987; Plomin, DeFries, McClearn & Rutter, 1997). Plomin and Daniels (1987) account for a number of factors that might contribute to these differences apart from the obvious nonadditive genetic effects. Systematic differences might be related to sibling interaction (differential treatment), parental treatment – also differential treatment and birth-order (perhaps more relevant to humans than rats). Nonsystematic differences could be due to accidents, illnesses and trauma. There seems to be no work treating differences and similarities in personality/temperament in rodents, although one study (Galsworthy et al., 2005) has been done on general cognitive ability in mice, revealing low sibling correlations.

SUMMARY OF THE PRESENT STUDIES

The aim of the articles presented here was to study change and continuity in temperamental dimensions as assessed by multiple behavioural measures, to explore links between temperament and brain monoamines, and to examine sibling differences in temperament.

Study I aimed at establishing whether repeated measures of rat behaviour would yield some form of temperamental coherence and whether sex differences would be evident over the period of weeks that the study was conducted.

Study II followed up on the findings from the first study, and increased the scope to the entire first year of the rats' lives thus making it possible to study possible change and continuity in behavioural dimensions of temperament as the rats developed from infancy to sexual maturity.

Study III examined levels of brain monoamines in several areas of the brain and correlated these measures with temperament.

Study IV explored similarities and differences between rat siblings using the behavioural measures assessed in the first three studies and adding a novel test situation.

Method

Subjects

In Study I 64 rats (Wistar strain, 32 males) from Scanbur BK AB were allowed a minimum of two weeks to adapt to the laboratory environment prior to experiments. They were housed in unisex groups of four in clear plastic cages (52 x 30 x 18 cm) with ad lib access to food and water. The lights in the housing room were turned off from 0900 to 2100. All behavioural observations were conducted during the nocturnal phase of the rats' light/dark cycle. Subjects were 3-4 months old at the time of testing. Females were tested for behavioural estrus at the end of each test by the manual stimulation technique described by Blandau, Boling, and Young (1941).

In Study II thirty-two 25 day-old rats (16 males, 16 females) were purchased and were subject to identical conditions as above (with the exception that males were housed pair wise after week 37 due to their size).

In Study III 27 female rats were purchased and housed in groups of three to four, with feeding and circadian rhythm as in Study I above.

In Study IV fifteen pairs of sibling male rats were used, also purchased, fed and on a day/night cycle as in Study I. The rats were housed in sibling pairs. Subjects were around four months of age at the start of testing.

Procedure and apparatus

In this thesis I have studied rats' responses to two environments, with an additional environment added in the final study. To assess tendencies in exploratory/ambulatory and anxiety related behaviour in rats the elevated plus maze and open field test or variants thereof such as the hole board and canopy tests are commonly used. Their purpose is to elicit anxiety-like and exploratory behaviours. One problem with these tests is that they potentially elicit complex combinations of both anxiety-like and exploratory behaviour. To partially overcome this difficulty the canopy test, which primarily elicits anxiety, can be conducted in bright light conditions, which are aversive to prey animals such as rats, and the hole board test can be conducted in near darkness. The automated activity box, used in Study IV allows for very exact measurement of behaviour in a novel environment.

The hole board test

The hole board test apparatus consisted of a wooden, brown hole board (78 x 78 cm; walls 29 cm high). The floor was divided into 16 squares by white lines. Each square contained a hole, 4 cm in diameter and 2.5 cm deep. The apparatus was placed in a darkened room (9 lux) to alleviate the possible anxiety related effects of bright light on the rats. Behavioural measures in the hole board test were the number and cumulative duration of nose-pokes into the holes, the number of lines crossed and the number of rearings where the rat stood on its hind legs, either against a wall or unsupported.

The hole board test evokes the rat's tendency to use its snout to explore its physical environment. There is evidence that nose poking is a valid measure of exploration and

that it is governed by factors partly other than those regulating ambulation (Abel, 1995, File & Wardill, 1975).

The canopy test

The apparatus comprised a circular (104 cm diameter), deep green platform elevated to 73 cm above ground level (Grewal, Shepherd, Bill, Fletcher, & Dourish, 1997). A clear red Perspex circular canopy (70 cm diameter) was supported 10 cm directly above the platform by a central pillar. This divided the apparatus into a covered closed zone, and an outer open zone (referred to as the exposed zone). Eight white lines were drawn radially from the centre of the platform. The arena was illuminated by normal fluorescence room lighting, yielding a level of illumination of approximately 165 lux in the covered zone and 560 lux in the exposed zone. The 5-min test was started by placing the animal under the canopy.

Behavioural measures in the canopy test were the number of stretched attend postures, the number of lines crossed and the time spent in the outer exposed zone (defined as half of the rat's body or more extending beyond the canopy) of the arena. A stretched attend posture was defined as flexed hind limbs and a flattened lower back position with extended forelimbs; usually the response was accompanied by either a lack of movement or a very slow gait. Behaviours were recorded by two observers sitting on opposite sides of the platform. The canopy test can be considered to capture anxiety related behaviour, partly because it is sensitive to anxiolytic drugs (see Grewal et al., 1997).

The automated activity box

In Study IV rat behaviour was also measured in an activity box, a 70 x 70 x 35cm high plexiglass box constructed by Kungsbacka Mät och Reglerteknik AB, in which two sets of infrared photocell beams (with the higher set at 14 cm from the box floor and the low level at 4cm from the box floor, creating a grid of 9 x 9 cm squares) measured locomotion activity. The following variables were recorded at 5 minute intervals: horizontal activity (number of beam interruptions on the lower grid), peripheral activity (lower beam interruptions around box edge), rearing (number of high beam interruptions), peripheral rearing (edge high beam interruptions), corner time (seconds spent in corners), rearing time (cumulative measure of total high beams interruption in seconds) and locomotion (increasing by one count per beam interruption, differentiating it from horizontal activity, where the rat could theoretically be going back and forth between the same two squares the whole time).

Study I

Behaviour was measured in the hole board and canopy each week for three consecutive weeks. The hole board test lasted 10 minutes for each rat with the animal placed in the centre of the apparatus at the beginning of the test. The canopy test sessions lasted 5 minutes for each rat beginning with the animal being placed under the protective canopy. The effects of behavioural estrus were recorded using those females that happened to be in estrus at the time of testing.

A randomly selected group of 32 rats (16 of each sex) were gonadectomized (8 of each sex) or subjected to sham surgery (8 of each sex). 5 weeks after the operation these rats were tested once a week on separate days on the hole board and canopy tests. This was repeated for three consecutive weeks.

Study II

Behaviour was measured on the hole board and canopy apparatus. The rats were tested twice a week for two weeks in a row at 6 and 7, 11 and 12, 16 and 17, 21 and 22, 37 and 38, and 51 and 52 weeks of age. On each test week they were tested once with the hole board test and once with the canopy test. The two tests were conducted on different days and the average scores for these occasions were used in the computations. For convenience these ages are referred to as weeks 6, 11, 16, 21, 37 and 52. For ease of presentation we have adopted the convention of referring to 6-11 week olds as juveniles, 16-21 week olds as young adults, and 37-52 week olds as mature rats (the normal life expectancy of a laboratory rat is 2.5 to 3 years). As for females, only data from tests given during non-estrous stages of the cycle were used in the averages, owing to the fact that female behaviour differs strongly in estrous and non-estrous phases.

Study III

The rats were tested twice a week for three consecutive weeks. Each week they were tested once with the hole board test (10 min) and once with the canopy test (5 min). The two tests were conducted on different days. After testing was concluded the rats were sacrificed as follows.

Immediately before decapitation, each rat spent 15 min in a novel, diffusely illuminated black Plexiglas arena (46 x 33 x 35 cm). Their brains were rapidly taken out and put on an ice-chilled petri dish. Brains were dissected by free-hand into frontal cortex (medial prefrontal part), remaining cortex (i.e. the entire cortical mantle except the frontal cortex), thalamus, striatum, nucleus accumbens (including olfactory tubercle and the ventral pallidum), hippocampus, amygdala, hypothalamus, mesencephalon and brainstem.

The tissue samples were weighed and immediately frozen when placed on aluminium-foil resting on dry ice. The samples were stored at -80°C until neurochemical analysis, at which time they were homogenized with perchloric acid (0.1M), ethylene-diamine-tetraacetic acid (EDTA), (10%), glutathione (5%) and alpha-methyl-dopa (2.3637µM), and centrifuged. Analysis of tissue concentrations (ng/g tissue) of DA, NA and 5-HT, and their metabolites DOPAC, HVA, 3-methoxytyramine (3-MT) and 5-HIAA, was done by High Pressure Liquid Chromatography HPLC separations and electrochemical detection. The HPLC separation was performed on a reverse phase column (4.6 x 150 mm) packed with Nucleosil ODS, 5µm, 150*4.6mm i.d.) with an aqueous mobile phase (1ml/min) containing 40mM citric acid, 12mM K₂HPO₄, 1,7mM H₃PO₄, 0.35mM sodium octylsulfat, 6% (v/v) of methanol and 0.05mM EDTA.

Study IV

In the fourth study, the hole box and canopy apparatus were used again, with the rats being tested twice a week for three consecutive weeks. Each week they were tested once with the hole board test and once with the canopy test. One week after these tests the rats were exposed to another novel environment. This consisted of a 30 minute test in an activity box, as described above.

Statistical analyses

For all four studies, the statistical procedure was to check the distributions of the data and test violations against normality using Fisher's test. Skewed variables were

rescaled using the ladder of powers procedure (Velleman 1988) to approximate normality and then evaluated with parametrical methods using SPSS 11.5.

In both studies I and II we used the SIMCA-P software package (version 9.0, Umetrics AB, Umeå, Sweden) to perform principal components analyses (PCA) on the average values over time. SIMCA-P uses unit variance scaling and mean-centering to pre-process data. The significance of each principal component is determined by cross validating a model based on part of the database and testing its validity in relation to the remaining data. One of the major advantages of the SIMCA-P software is that it can handle data sets with many variables and few observations “short and fat data tables”, to quote Eriksson, Johansson and Kettaneh-Wold, (2001). As an example, in one study using SIMCA the authors tried to distinguish between brains with and without cancer (Jellum, Bjørnson, Nesbakken, Johansson, & Wold, 1981) using only 16 brains and single gas chromatographic measurements from each brain recording a large number of variables (105). They were able to clearly distinguish between brains with and without cancer. (It should be noted that the algorithm used in this program is not the one used in standard statistics packages like SPSS). The PCA allowed us to extract components that reflect separate temperamental dimensions.

In Study III the data was summarized as medians for the three hole board and canopy tests. Data from females in estrus were not used, as behaviour in the hole board and canopy is changed during this period. After following the skewness and re-expression procedure described above, PLS (Partial Least Squares), a SIMCA-P software application was used to look into relationships between brain monoamines and temperament. The temperamental model used was the same as described below in Study IV.

In Study IV, following the skewness detection and reexpression procedure delineated above, intraclass correlations were calculated on sibling behaviours. The raw results (medians across tests) on the three hole board- and canopy tests were fed into a multivariate model for extracting Novelty Seeking and Harm Avoidance. This model had been created on the basis of data from more than 60 rats receiving three tests in the hole board- and canopy situations under identical conditions (Ray & Hansen, 2004). For the purposes of the present study, this model served as a standardized ‘personality test’, ‘trained’ on an independent large sample to extract individual differences in Harm Avoidance and Novelty Seeking on the basis of data from the hole board- and canopy tests. All of the 30 subjects used in the present study fitted into this model with none falling outside the Hotelling's T^2 95% tolerance area (see Eriksson et al., 2001).

Results

Study I

Study I explored the stability of individual differences in rats over a 3-week period. There were significant and stable individual differences in behaviours expressed in the hole board and canopy test (Table 1).

There were also sex differences in that non-estrous females showed more locomotion than males in the hole board test, and were more variable in their behaviour than males as expressed in time spent outside the canopy in the canopy test. When the

females were in behavioural estrus and sexually receptive they seemed less anxious as evidenced by their spending more time in the exposed zone and being more active. We found no sex differences in anxiety related behaviour in the canopy test when only comparing non-estrous females and males.

Table 1. Test-retest Spearman correlations for behaviour recorded in the hole board and canopy tests.

	Test 1-2	Test 1-3	Test 2-3
Hole board test			
Nose pokes (frequency)	+0.50**	+0.61**	+0.49**
Nose poke duration (sec)	+0.29*	+0.37**	+0.45**
Rear (frequency)	+0.65**	+0.69**	+0.81**
Activity (frequency)	+0.74**	+0.74**	+0.67**
Canopy test			
Stretched attend postures (frequency)	+0.55**	+0.57**	+0.52**
Activity (frequency)	+0.68**	+0.47**	+0.52**
Exposed zone (sec)	+0.69**	+0.69**	+0.60**

* $p < 0.05$, ** $p < 0.01$.

Gonadectomy reduced hole board locomotor activity in both sexes and also reduced hole board nose poking in the males.

Finally, following the multivariate analysis two behavioural dimensions were obtained that contain behaviour from both tests situations, with one reflecting Harm Avoidance and the other Novelty Seeking (Fig. 1). More males than females had high levels of Harm Avoidance and fewer males than females had a low Harm Avoidance and high Novelty Seeking profile (Fig. 2).

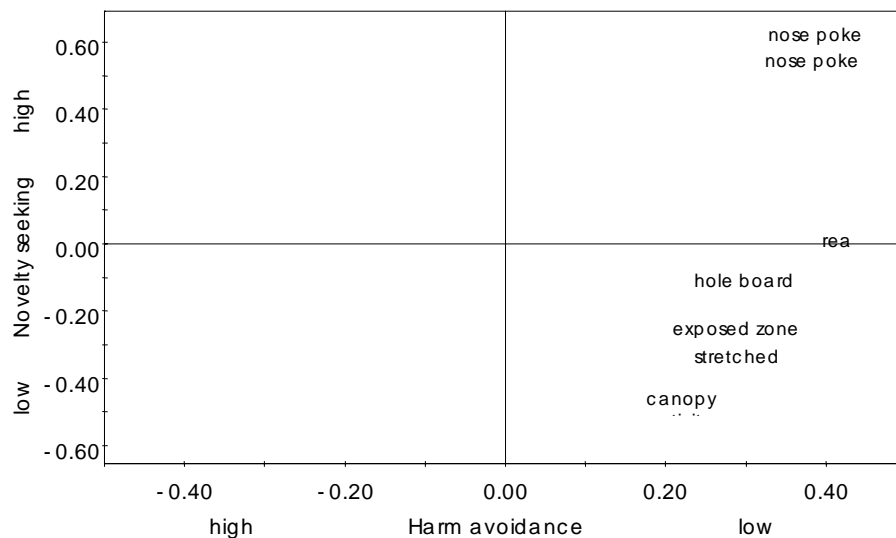


Figure 1. Plot of loadings on first (horizontal axis) and second (vertical axis) principal components.

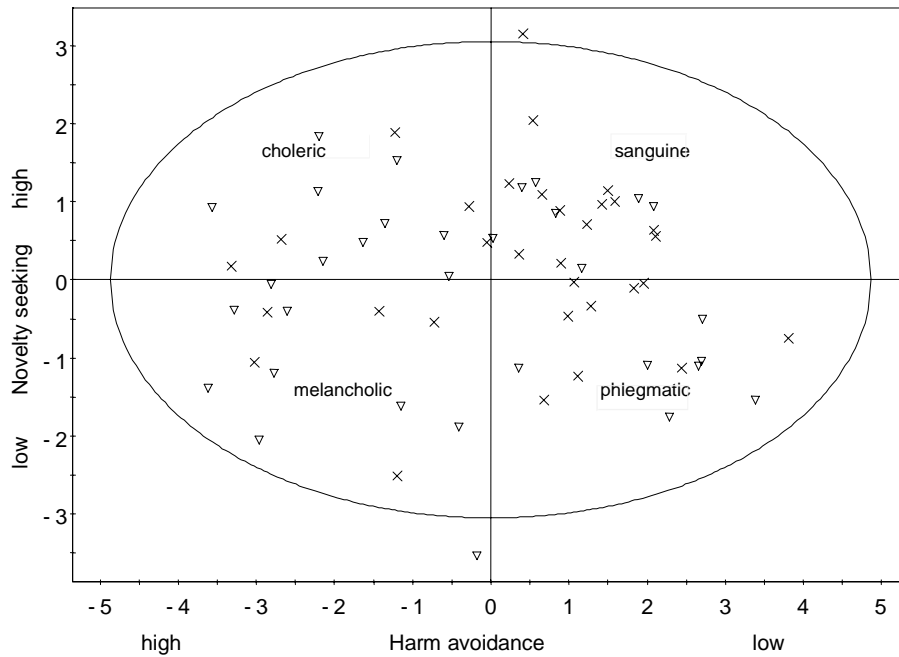


Figure 2. Plot showing the distribution of scores, i.e. weighted averages on the two scales for Harm Avoidance (horizontal axis) and Novelty Seeking (vertical axis). Its format makes it superimposable on Figure 1. Each symbol represents one individual. Crosses: female rats; triangles: male rats. The oval represents Hotellings T^2 95% confidence ellipse (Eriksson et al., 2001).

Study II

In the 52-week longitudinal study 16 week old rats formed our reference category with which younger and older rats were compared. The findings can be conveniently grouped into four areas, firstly behaviour in 6-11 week-old rats (referred to as juveniles), including sex differences and comparisons to 16 week olds (16-21 week olds are referred to as young adults). Secondly, behaviour in 37-52 week-old rats (referred to as mature) including sex differences and comparisons to 16 week olds, third behavioural consistency over time and finally the results of principal components analyses.

The typical pattern for juvenile rats was for them to be more active (locomotion) in the canopy test and to nose poke more. The juvenile rats also very seldom spent any length of time outside the protective canopy on the canopy test, whereas a larger proportion of the adults spent time in the exposed zone. Also, a sex difference apparent in adults was not in evidence in juveniles: adult females rear more and show more hole board locomotion than males, but this is not the case in juveniles. Juvenile females did however spend more time outside the canopy and showed more stretched attend postures, considered to be a form of risk assessment, than juvenile males.

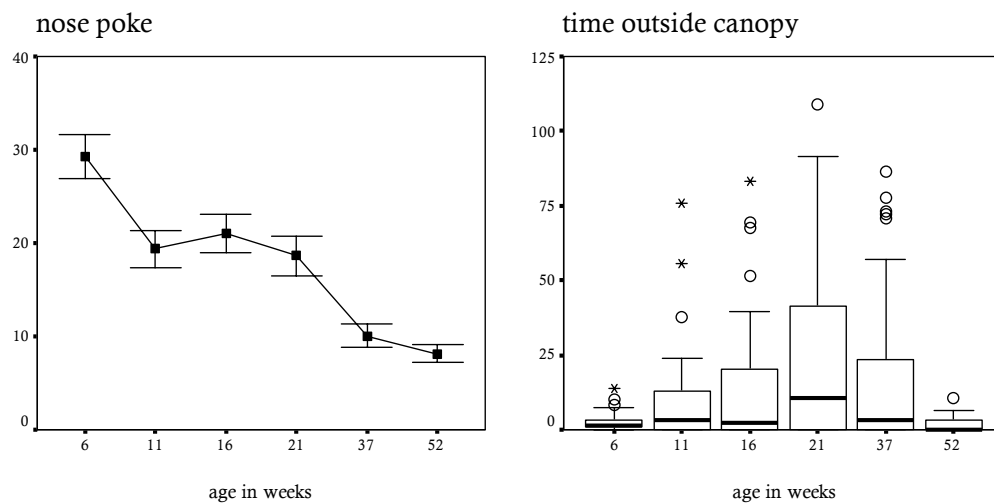
Mature rats, on the other hand, showed decreased nose poking and rearing as compared to a 16 week old. Mature rats also moved less in the canopy test and were highly unlikely to leave the protective canopy and move around in the exposed zone

as compared to 16 week olds. Mature females reared more and showed more hole board locomotion than mature males, and also showed more stretch attend posture.

Two key concepts have been used to illuminate behavioural continuity – those of rank order and mean level consistency (Roberts & DelVecchio, 2000). Mean level consistency in this study shows whether there are reliable age-related changes in behaviour over time. Rank order consistency measures the extent to which individuals maintain their position in relation to other individuals in regard to measured variables, calculated using correlational analysis. There were clear age related changes in mean level consistency, some examples of which are illustrated below (Fig. 3).

Figure 3. Temporal profiles of nose poking behaviour and time spent outside the protective canopy. Results expressed as mean \pm SEM for nose poke and median \pm interquartile range for time outside canopy.

$^{\circ}$ outlier, * extreme outlier. Time outside canopy expressed in seconds.



The results pertaining to rank order consistency can be summed up in the following table (Table 2), which shows correlations over time for the various behavioural variables measured. As can be seen, the largest correlations are from week 11 and onward.

Table 2. *Pearson correlation coefficient matrix for canopy and hole board behaviours.*

Week	6-16	6-52	11-16	11-52	16-52	21-52	37-52
Hole board test							
Nose poke	+0.46**	+0.39*	+0.67**	+48**	+0.38*	+0.51**	+0.54**
Nose poke duration	+0.34	+0.48**	+0.54**	+0.50**	+0.67**	+0.68**	+0.66**
Locomotion	+0.43*	+0.43*	+0.81**	+0.75**	+0.75**	+0.84**	+0.84**
Rear	+0.53**	+0.32	+0.80**	+0.70**	+0.77**	+0.70**	+0.70**
Canopy test							
Stretched attend	+0.17	+0.35	+0.60**	+0.62**	+0.34	+0.43*	+0.22
Locomotion	+0.43*	+0.42*	+0.65**	+0.61**	+0.62**	+0.65**	+0.74**
Exposed zone	+0.15	+0.25	+0.55**	+0.61**	+0.78**	+0.58**	+0.58**
Body weight	+0.33	+0.33	+0.96**	+0.94**	+0.98**	+0.98**	+0.99**

* $p < .05$, two tailed. ** $p < .01$, two tailed.

Note: Time spent in exposed zone calculated with Spearman Correlation Coefficient.

The PCA indicated a difference in temperamental structure between adult rats and both juvenile and mature ones. The analyses showed that, as in Study I, adult rat behaviour as measured in these tests could be characterized by two dimensions, one reflecting Harm Avoidance and the other reflecting Novelty Seeking. In juvenile and mature rats however only one dimension, reflecting Harm Avoidance was extracted from the data.

Study III

Examination of the coefficients of variation (Table 3) revealed that the highest were all related to dopaminergic neurotransmission. The highest variability was in hypothalamic HVA levels. Frontal cortex DA, DOPAC and HVA also exhibited large individual differences; the same was true for DA and DOPAC in the amygdala.

Table 3. *Brain monoamines and their relationship to temperamental dimensions*

	Average level	Coefficient of variation	Harm Avoidance		Novelty Seeking	
			VIP	Direction of relationship	VIP	Direction of relationship
DA						
cortex	50 ± 11	0.22			1.23	↓
striatum	8274 ± 653	0.08	1.49	↓		
brainstem	55 ± 10	0.18			1.36	↓
DOPAC						
amygdala	94 ± 34	0.36	1.19	↑	1.24	↓
HVA						
frontal cortex	23 ± 7	0.30	1.34	↓		
NA						
cortex	317 ± 39	0.12	0.99	↑		
thalamus	518 ± 38	0.07			1.03	↑
5-HT						
thalamus	708 ± 91	0.13	1.10	↓		
striatum	586 ± 51	0.09	1.02	↓		
amygdala	1515 ± 216	0.14	1.17	↓	1.23	↑
brainstem	637 ± 80	0.13			1.76	↓
5-HIAA						
accumbens	571 ± 51	0.09			1.38	↓
amygdala	819 ± 144	0.18	1.46	↑	1.47	↓

Note. The direction of relationship column shows the relationship between the temperamental dimension and the neurotransmitter, with ↑ signifying a positive relationship and ↓ a negative relationship.

Table 3 shows the regression coefficients and VIPs for the most influential neurochemical predictors when related to individual differences in Harm Avoidance. Very influential predictors (i.e. with VIPs ≥ 1 , shown in bold in this simplified version of the full table in Study III) were striatal DA, frontal cortex HVA, and serotonin levels in the amygdala, thalamus and striatum. These predictors had negative regression coefficients meaning that increasing levels of Harm Avoidance was associated with decreasing brain levels of these compounds. Positive regression coefficients were assigned to 5-HIAA and DOPAC levels in the amygdala, as well as cortical levels of NA.

As to Novelty Seeking, brainstem 5-HT and DA, amygdaloid 5-HIAA and DOPAC, together with accumbens 5-HIAA and cortical DA received negative regression coefficients with high VIPs. By contrast, amygdaloid 5-HT and thalamic NA were assigned positive coefficients.

Approximately 20% of the variation in the neurochemical variables was related to Harm Avoidance and Novelty Seeking respectively, and the predictors explained around 60% of the variation in both dimensions.

Study IV

Intraclass correlations between rat siblings (Table 4) in the hole board, canopy test and activity box showed that only weight was significantly correlated between siblings (hole board rearing and activity box peripheral rearing reached significance, but the confidence interval overlapped zero).

Table 4 Means and standard deviations for rat siblings in hole board test, canopy test and activity box. Intraclass correlation coefficients for rat siblings on behavioural measures in hole-board, canopy test and activity box.

Variable	Mean ± S.D.	Intraclass correlation coefficients
<i>Hole board test</i>		
Nose poke	12 ± 6	0.21
Nose poke duration	11 ± 7	0.15
Hole board activity	61 ± 37	0.36
Rear	14 ± 8	0.48*
<i>Canopy test</i>		
Stretched attend posture	3 ± 2	0.04
Canopy locomotion	31 ± 15	0.06
Exposed zone ^a	0 ± 0	-
Harm Avoidance	2.36 ± 1.43	0.23
Novelty Seeking	-0.78 ± 1.10	0.07
<i>Activity box</i>		
Horizontal activity	1119 ± 334	0.37
Peripheral activity	595 ± 203	0.22
Rearing	16 ± 79	0.32
Peripheral rearing	104 ± 43	0.44*
Corner time	442 ± 275	-0.11
Locomotion	377 ± 143	0.40
Rearing time	101 ± 47	0,22
Weight (ca 2 months)	438 ± 45	0.76*
Weight (ca 4 months)	495 ± 58	0.70*

^a Time in exposed zone represented with median ± inter-quartile range.

* $p \leq 0.05$

DISCUSSION

Summarising the results for Studies I-IV before discussing them in more general terms, we see the following.

Study I

In Study I clear individual differences in the behaviours elicited in both test situations were in evidence. There was a sizable variation in behaviour between individual rats, and these individual differences were also stable over time, with significant positive correlations of behavioural measures between tests remaining high during the three test weeks. Rats, then, like humans, show large and consistent individual differences in tests designed to evoke exploration and anxiety.

There were also sex differences which partially support previous research findings indicating that females show more exploratory behaviour and less anxiety related behaviour than males (Gray, 1971). However, these differences were not apparent in the canopy test when using only nonestrous females. Nonestrous females also varied significantly more than males in the time spent in the unprotected zone of the canopy test, with lower intertest correlations and a three times greater range than males. Using data from estrous females alone, females can clearly be considered less anxious than males.

As for gonadectomy, the rats subjected to this procedure showed no behavioural changes in the canopy test. Comparing estrous and nonestrous females, ovariectomized females were about as anxious as nonestrous females, that is to say more anxious than estrous females. It would thus seem that the excess hormonal stimulation occurring in estrus has the effect of lessening anxiety related behaviour, whereas the lack of hormonal effects (as in ovariectomy) leaves the female at a base rate similar to that found in the nonestrous female. Gonadectomy did however effect behaviour in the hole board, with both sexes exhibiting less locomotion, and males decreasing their nose poking activity, suggesting a role for male sex hormones in exploratory behaviour. Overall, these results are in line with previous studies (e.g. Ferreira, Hansen, Nielsen, Archer & Minor, 1989; Hård & Hansen, 1985) suggesting that gonadal hormones regulate anxiety related behaviour in the rat.

All the original variables studied were represented by a PCA as two latent temperamental dimensions, reflecting Harm Avoidance and Novelty Seeking. The rationale behind using these labels is discussed below. Another finding with regards to the PCA is related to sex differences. Univariate analysis revealed just one significant sex difference in behaviour, whereas the PCA could detect an underlying pattern using all the variables, with more male rats showing a harm avoidant profile than females.

Study II

Generally speaking, this study showed statistically significant trends in mean level consistency in rats – that is to say, they showed reliable changes in anxiety and exploratory behaviours related to their age. Examples of this are the inverted U-curve in time spent outside the protective canopy, and the downward trend in nose poking. They also showed very high levels of rank order consistency – i.e. individual rats maintained their relative position over time in relation to other individuals.

In this study juvenile rats were found to exhibit more nose poking and locomotor activity but less rearing than 16 week olds, which at first might seem odd, as both are considered to be measures of exploratory behaviour. This will be discussed at greater length below. Juveniles were also much less likely to spend long periods of time outside the covered canopy than adults, and finally, juveniles did not exhibit a sex difference apparent in adult rats – i.e. that adult females show more hole board locomotion and rearing than males.

In comparing mature rats (i.e. 37-52 weeks old) to the 16 week-old adults, there were three principal differences, all reflecting decreases in behaviour. Mature rats nose poked and reared less in the hole board test, they moved less in the canopy test, and they were less likely to spend time in the exposed zone in the canopy test. All these behavioural indices point in the direction of a decline in exploratory activity and an increase in anxiety with increasing age. Humans are similar to rats in the first instance, but in humans Neuroticism (an anxiety related characteristic) actually decreases with age (McCrae et al., 2000), especially in women (Srivastava, John, Gosling, & Potter, 2003).

As regards latent temperamental structure, the juvenile and mature rats are characterised chiefly by the Harm Avoidance dimension, with two dimensions being in evidence in 16 week-old adults. As discussed further below, this might reflect a core dimension of the life of a rat with further dimensions coming into play along the developmental trajectory of its life.

Study III

Multivariate methods allowed us to discover relationships between brain monoamines and temperament. Harm avoidant rats had low levels of DA in the striatum and high levels of cortical NA and amygdaloid 5-HIAA. Low levels of 5-HIAA in the amygdala and accumbens were found in subjects high in Novelty Seeking, as were low levels of DA and 5-HT in the brain stem. The rats scoring high on Novelty Seeking also had NA levels exceeding the average in the thalamus, as well as higher than average 5-HT levels in the amygdala. More research must be done to try and replicate these findings and understand their possible implications.

Human studies have shown lower levels of DA and NA activity in anxious and depressed Parkinson's disease patients than in non-depressed patients in the left ventral striatum, left locus ceruleus, and in the amygdala and medial thalamus (Remy, Doder, Lees, Turjanski & Brooks, 2005), suggesting a connection between depression and anxiety and loss of dopaminergic and noradrenergic function in the limbic system in this DA related disease. It would seem valuable to continue exploring connections between anxiety related pathology, temperamental traits and neurochemistry across species to further our understanding of these systems.

Study IV

The correlations in this study show few significant correlations between sibling pairs' behaviour, which is certainly an interesting finding, especially when contrasted with the high correlations in body weight. This study was designed to minimize influences due to unique environmental contributions. The rats were separated at weaning, housed together in sibling pairs and received exactly the same treatment in all variables we had any control over. Still, we cannot be sure that these rats did not

experience differential treatment within their litter prior to weaning, or that incidents such as being dropped or somehow severely frightened have not occurred to them during transport or during their stay at the research facility. Using Plomin and Daniels' (1987) framework, we would consider these rats as equivalent to dizygotic twins. They are born after each other, but within minutes, and should share the same proportion of DNA as human dizygotic twins. In humans, the estimate of personality heritability is around 0.43, with shared environment contributing only 0.06 and unshared environment accounting for 0.51 of the total variation in this trait (Plomin, DeFries, Craig & McGuffin, 2002). We must, of course, be careful here, replicating this research on larger groups before we can draw any conclusions, but it is nonetheless noteworthy that these rats that surely must be considered to have shared a huge part of their environment and had very little opportunity for unique environmental input are so dissimilar in behavioural measures.

General discussion

As with all attempts to move from behavioural measures to underlying temperamental dimensions, we are confronted with the difficult transition from correlations of behaviours over time and the clustering of these behaviours into some form of latent variables in the PCA to the interpretation of these clusters. Clearly the behaviours measured in the presented studies show correlations over time, and their mean levels vary in a consistent manner over time. From this it would be fair to assume that we are indeed dealing with some form of stable underlying trait or temperamental dimension and not just random variations in behaviour. But what exactly is it that we are seeing? Let us take the fact that juvenile rats show higher levels of nose poking than young adults, but lower levels of rearing, yet both rearing and nose poking are considered to be measures of exploration. How can we explain these seemingly paradoxical results? One line of reasoning, as developed in Study II, is that the juvenile rat is inclined to explore locally, but that the more distal exploratory action of rising up on the hind legs develops later in the life cycle (Rowe, Spreekmeester, Meaney, Quiron, & Rochford, 1998). It would seem that the overriding imperative for these juvenile animals would be to stay safe and stay local, that anxiety related behaviours would have the greatest survival value at this stage in development where the rats are still vulnerable and barely developed, and the incentives for bolder ventures further a field are small. As the rats evolve into sexual maturity the need to find a mate and forage for food would increase the incentive to override anxiety and replace it with the possible rewards of Novelty Seeking behaviour. From an evolutionary perspective this makes perfect sense, although, as with most evolutionary models it is difficult to conceive of a way of moving from persuasive argument to experiments that could make the theory stronger.

As noted in Study II, the rat data at hand only result in one dimension for the very young and very old, whereas the young adults show the two dimensions named Harm Avoidance and Novelty Seeking in these studies. The data in the PCA show that all behaviours (except time in the exposed zone) load into the single variable called Harm Avoidance and it could fairly be asked why one should label this Harm Avoidance and not Novelty Seeking or something else. The rationale behind this is that the same behavioural measure can conceivably express different temperamental constructs. A rat running into cover, for example, could be considered to be running from a perceived threat (anxiety/Harm Avoidance) or to a perceived reward – the comfort of a protective burrow [see Hughes (1997) for an excellent overview of methodological

issues and problems]. So, is this rat's behaviour motivated by punishment/anxiety/fear or by reward? We cannot know, but it is likely that we are dealing with a mixture of the two. Again, from an evolutionary perspective it would make sense to attribute primacy to anxiety in a prey animal such as the rat. Later in development such behaviours as stretched attend posture, typically conceived of as a measure of risk assessment (Blanchard & Blanchard, 1994), correlate negatively with our second PCA dimension (Novelty Seeking) whereas number and duration of nose poking correlate positively. The basic premise for this line of reasoning is that the juvenile individual is undifferentiated in its early days but as the potential benefits of exploring the world increase the temperament develops greater complexity. Interestingly enough, a recent study of humans (Abe, 2004) following preschool children from 3.5 years of age for 9 years found that the youngest children had an additional two personality dimensions beyond the Big Five, namely Irritability and Activity. As the children matured these dimensions were subsumed into Neuroticism and Extraversion respectively. Ratings of child personality at the early age of 3.5 were predictive of personality and behaviour in adolescence. It would thus seem that a tendency toward both change in levels of complexity (albeit not necessarily in the same direction) and stability over time are apparent in both rat and man.

In old age we see the trend reversed. Yet again the incentives of mating decrease and the dangers of exposure to the slow and older rat makes a drop in exploratory behaviour occur. This might reflect a general dissolution of functions in the rat. In humans, natural selection might well be operating even at a post-reproductive age, as older members of our species are valuable repositories of knowledge and serve important alloparental roles for our young (Diamond, 1998), but it is hard to see this as being the case in the rat. In humans the field of temperament and age is definitely under-researched and much remains to be done here (Matthews et al., 2003). Using the FFM, researchers can however conclude that the general pan-cultural trend is toward a decrease with age in Neuroticism, Extraversion and Openness to experience after adolescence, and an increase in Agreeableness and Conscientiousness (McCrae et al., 2004).

Conclusions from Study III and IV can only be more tentative, and perhaps more methodological in nature. The relationships between monoamines and temperament must be confirmed with more experiments before we can safely draw any conclusions, and work must be done trying to compare these results to those from other taxa as they are done. As to Study IV, the question remains essentially unanswered – but it does strike me as worth following up that these 30 siblings are so dissimilar – are we missing something here?

Limitations

Had more variables been included in the studies and a greater variety of situations been assessed, further temperamental dimensions would most likely have been in evidence. One important dimension to study in a group-living and socially flexible organism such as the rat would have been some form of sociability, perhaps observing rough and tumble play in the young rats and dominance aspects in the more mature rats.

A number of other questions remain in the present work, significantly the effects of repeated testing in relation to Novelty Seeking. It is a concern that the actual novelty

of the hole board can be called into doubt as the rat is repeatedly exposed to the opportunity to explore this environment, which in turn would conceivably effect the results. Comparisons were made between 6 – week olds and 3 – 4 month olds tested for the first time to control for this, and one could conceivably perform a number of cross-sectional measurements on rats of varying ages (corresponding to those in the longitudinal study) not previously exposed to the tests and compare these with the present results. Unfortunately this has not been feasible due to time constraints. Another interesting methodological issue relates to the choice of testing paradigm. Here again, other tests could have been added, and perhaps the use of the elevated plus maze would have allowed easier comparisons between these results and those of others using this far more widespread method.

In Study III, I feel the lack of precision in the brain regions analyzed is a major shortcoming. In future research I hope to have much more input as to the exact areas analyzed and also will endeavour to get exact images of the areas under investigation from the laboratory performing the dissections. As it stands now, the article points out interesting areas of research, but I feel it lacks greatly in precision. Also, we would need to replicate this study so as to be sure the model created is actually reliable. Although the results certainly seem to indicate that there is a connection between the temperamental dimensions and monoamines in question, a number of the confidence intervals for our regression coefficient values actually overlap with zero, which will remain a worrying source of uncertainty until we (or other researchers) have been able to validate the model on a new group of animals.

In Study IV it would have been desirable to have information about the actual genomes of the rats involved so as to add information using advances in molecular genetics technology to the study. It would also have been very interesting to actually study (by direct observation and with cameras) the rats' environment during those first days before weaning, to try and find possible sources of unique environmental influence.

The studies in this thesis support earlier findings in sex differences with females being less harm avoidant and more active than males (Gray, 1971), which in itself raises an interesting question. The pattern is the reverse in humans as far as we know, with females consistently being higher on anxiety measures [although notably not social anxiety – see Feingold, (1994) for a review], so why is this pattern different in humans and rats? In relation to many species the answer to why we observe different behaviour in the two sexes can be found in the structure of their social grouping, as might be said to be the case for the hyena, a matriarchal species where the female is clearly more assertive than the male. Biologically this can be connected to levels of testosterone, as mentioned earlier. In humans it would seem likely that with an increasing division of labour centred around our lengthy child rearing constraints, certain traits might conceivably have been more adaptively useful to the one sex as a whole (statistically speaking, of course) than the other. How this relates to rats, however, is not at all clear at present. I would find it intriguing if some researchers in the field dared speculate about this after presenting their conclusions. I wonder if this has a bearing on the validity of rat anxiety measures, or if there really is an adaptively sound evolutionary explanation for these documented differences in behaviour? In relation to the present thesis it is, of course, highly relevant to consider the implications of this reversed situation in the two species as this has consequences for

the applicability of findings across species. On a neurobiological level, further research may well help to reveal the bases for these differences, but without an additional understanding at higher levels of analysis it will be difficult to move from facts to theories. Despite the sex differences from humans, I would still maintain that results pertaining to individual differences in the rat are valuable in developing knowledge of the psychology of humans, as long as one keeps the different demands of different species in mind when trying to transfer findings across the species boundaries. Thus findings concerning the character and number of factors used to describe young and older animals are still valid in discussing the development of temperament as a factor of life span development and maturity.

As I suppose is often the case with these things, the position I am in as I approach the end of this thesis is a long way from where I began. Some of the most important work I have come across during this project had not even been written 4 years ago, and even if it had, I am not sure I would have appreciated its significance at that time. Perhaps one of the most striking findings along the path of studying temperament and personality in both rats and humans was the paucity of attempts to link the findings to biological thought as a whole. Open any textbook on biology, zoology, the cell, ecology – in fact any natural science subject touching on life and living organisms on this earth and you most likely will be met by a few introductory chapters on evolution, DNA and adaptation. These set the universally accepted framework in the natural/life sciences on which all else rests.

In personality psychology, this has not been the case. In the field of psychology, very few theorists outside of neuropsychology have tried to connect their findings to such basic ideas as adaptation and fitness, selection and ecological niche. In the life sciences as a whole, concepts such as these are the only ones used to try to understand form and function of systems and behaviour in all other life forms on earth – all others than man! Why is this so, one might wonder? Of the many reasons one might come up with, perhaps the highly tenacious historical concept in Western culture that man is fundamentally different from other life forms is one of the major stumbling blocks for progress even today, more than a century after Darwin formulated the first coherent versions of what was to become the theory of evolution.

It would seem, however, that all this is about to change. The methods and concepts of evolutionary theory – the only coherent scientific framework able to explain the complexity of function and form in life to date in the history of mankind, have recently started to be applied to psychology. Biologists and ethologists along with anthropologists, neuroscientists, developmental- and evolutionary psychologists are beginning to use the concepts of adaptation, selection, inheritance and fitness to explain diverse fields of human functioning, ranging from parent offspring conflict and altruism to temperament and personality. Since the discussion section allows for more wide-ranging thought so as to embed the experiments making up the thesis in a larger context, let us turn to these ideas to round this section off.

Personality was the theme in a recent issue of the journal *Behaviour* (volume 142, 2005), in which biologists set forth research on personality in a number of animal species ranging from fish to birds. In these papers, inquiry focussed on possible fitness benefits of various personality types. Instead of quibbling about how many traits animals might have, focus was on *how* the now well established fact that within

any species there will be a spread of boldness/shyness affects fitness. That is – do animals higher on bold (Novelty Seeking) behaviours stand a greater chance of survival, do they have a greater chance of mating, are they better adapted to changes in the amount of food available? Are there specific advantages in being more harm avoidant or shy, such as a better chance of surviving to have offspring if the amount of predators in an area suddenly increases? This line of inquiry suddenly connects observable behaviours (and therefore traits) to predicted outcome and actually allows for controlled experiments and observations in real wild populations which can be connected to current evolutionary thinking and fact.

In their introductory article, Dingenmanse and Réale (2005) distinguish between mechanistic and functional explanations of behavioural variation. Mechanistic approaches seek to evaluate how genes and environment combine to create phenotypes, whereas the functional approach looks at how the interaction between phenotype and environment affects fitness. The authors point out that this latter approach has not yet received much research attention even though it is only by combining these two approaches that we will be able to evaluate how behavioural traits might co-evolve under different environmental conditions. Individual differences in suites of correlated traits have been observed in many species and found to be moderately heritable over the life time of the phenotype, as pointed out in the introduction to this thesis. In a previous study (Dingenmanse, Both, Drent, & Tinbergen, 2004) the authors had studied fitness consequences of personality in a wild population of great tits. These birds had previously been captured and briefly tested exploratory behaviour in a laboratory, after which they were re-released. They were then studied over a period of two years. The authors could show clear advantages to male fast explorers and female slow explorers (in terms of highest survival) during years of relaxed food competition, whereas the trend was reversed in winters with little food. The authors went on to explain this in terms of intensified intra-sexual competition among females and territorial competition in males. This kind of study makes it possible to focus on immediate consequences for individuals, but also for long term effects over several generations, which will be a necessary perspective as a minimum if evolutionary processes are going to be evoked as causal agents in shaping personality/ temperament. An important feature of this kind of approach is to avoid a priori assumptions of fitness benefits from some particular trait or suite of traits (the “just so story” problem that plagues much of evolutionary psychology), but instead focus on actual observations in real environments over time.

So how does this relate to the rats I have studied, and, since we are dealing with psychology and not ethology (a totally defunct and obsolete distinction, in my opinion, but nevertheless still in place), to humans? Although we have tried to conduct analyses using multivariate methods, I strongly feel that using laboratory situations, while being eminently practical, will not allow us to fully link the types of behaviour we are seeing to their underlying evolutionary functions and fitness benefits. Longitudinal studies are a step in the right direction, and it would seem that a viable way forward for researchers in this field would be to move out of the laboratory and into wild environments studying species that are still subject to selection pressures. In the most general terms, laboratory researchers would benefit greatly from following the developments outside the laboratory, and many of those doing research in the field of personality and temperament would benefit from pondering the

possible place of their findings in an evolutionary framework to achieve greater clarity in what is actually being studied and discovered.

It is my firm conviction that this kind of work can be done on humans too. Be it via questionnaires solidly based on evolutionary themes, actual behavioural observations or life history interviews, we should be able to connect human temperament to fitness benefits in modern society, be they socioeconomic status, access to partners, offspring mortality or cooperation and support networks. In a much cited report (Eaves, Martin, Heath, & Hewitt, 1990) a survey on reproductive success in 1101 postmenopausal woman was analysed along with the women's scores on the Eysenck Personality Questionnaire, finding that lifetime reproductive success (a fitness measure) was related in a U-shape to Neuroticism and Extraversion, with the highest fitness for low extravert/high neurotic and high extravert/low neurotic combinations. Lowest fitness scores were correlated to high-high and low-low females. In another study (Nettle, 2005) using an evolutionary approach, 545 British adults were assessed using a personality inventory that correlates highly with NEO-PI-R (the FFM inventory). Extraversion was found to be a strong predictor of lifetime number of sexual partners. Male extraverts were more likely to have extra-pair sex and female extraverts were more likely to leave existing relationships for new ones. On the cost side, increased Extraversion increased risk for hospitalization or illness (although with small effect size), and higher Extraversion in females increased the risk of exposing their children to step parenting.

An interesting possibility opened up by an adaptationist perspective would be the chance to re-frame the trait state debate. As mentioned in the introduction, Sih et al. (2004) consider animal personality (which they call behavioural syndromes) in terms of correlated traits and reasons about whether they are adaptively flexible and plastic or not, and if so, why. In humans too, the trait/state debate might be meaningfully expressed in terms of whether fitness benefits accrue to particular correlated behavioural suites being more or less plastic, and research questions might deal with the nature of these correlated groups and the environments/contexts in which it would be better to be flexible or rigid in terms of possible different behaviour. Another author interested in the flexibility dimension (Dall, 2004, Dall, Houston, & McNamara, 2004), points out that the ability to be flexible will, in itself, incur costs to develop and maintain, and might thus be selected for in some particular niches and circumstances, but not others. In Dall's own words – “From an adaptive perspective, it makes sense for individuals to adjust their behaviour according to current conditions (including their internal state), and this can result in individual differences in behaviour if there is between individual variance in local conditions. More problematic, from this perspective, is how to make sense of individual differences in behaviour that are consistent over time (within or across generations) and/or different contexts”. Using the game theoretical framework of Maynard Smith (as cited in Dall et al., 2004), it is clear that different stable behavioural types can coexist in a population as a function of the frequency that different individuals use different behavioural strategies in relation to the fitness payoff of the behaviour – this is known as frequency dependent selection. If fixed proportions play each strategy consistently, personality differences could evolve. This works if individual organisms can also adapt their behaviour to feedback on other's behaviour. In the larger context of personality psychology in humans, this is obviously a chief feature of folk psychological concepts of personality – the possibility of predicting other people's

behaviour and assessing how reliable those people are. In fact, this is one major criticism of human personality tests I have heard from academics not in the field of psychology. Personality tests rely on generalized statements about behaviour at all times, and of course, most of us experience that the behaviour of others around us interacting with our state and our traits will determine individual reactions at any given time. Following the reasoning of Dall and others, individual people or animals can be locked into different regions of state space just by small stochastic differences in energy reserves, bacterial infections or interactions with other con-specifics within a dominance hierarchy (which could lead to a “reputation” for behaving in a certain way), which can go on to form stable differences in personality over time, even in genetically identical individuals. In a paper on this theme (Wilson, 1998), it is also pointed out that the reverse from the usual assumption can also hold – that is that the extreme phenotypes in a population may be better adapted than the norm! So, again, this leaves us with a much more tractable approach to personality - both traits and states – they must be understood as a function of evolutionary pressures: mating competition, sexuality, aggression as a result of territorial and resource competition, dominance hierarchies, boldness/Novelty Seeking/Extraversion/openness to experience associated with opportunities to exploit new resources, to food availability and dispersal, Neuroticism/Harm Avoidance/shyness related to predator pressure, bacterial threats, abiotic threats, dominance situations and threats from rival humans. The fact that no personality test has been constructed using this basis for the question items is truly astounding!

In this context, Budaev’s comment (1997) that behaviour observed in laboratory animals (guppies in his study) is more likely to be random and mask real individual differences is very pertinent, because modern Western humans share (at least) one major feature with laboratory rats, and that is the removal of selection pressures. This removal of selection pressures may be a clue as to why the rats in the studies presented here had such high correlations on body weight measures. All connections between differing strategies in obtaining food stemming from differences in Novelty Seeking and Harm Avoidance had been removed in the laboratory environment – access to more than enough food was easily available to all animals, regardless of how bold or timid they might have been, thus masking any potential benefits that might have accrued to particular profiles outside the laboratory. As for us humans, we live in an environment where most of our needs are taken care of and actual threats to our survival are very few. Thus we might find it difficult to know anything about the relationship of personality to survival and fitness – few of us see people getting killed due to personality traits. True, we may get ahead in terms of money and career due to a very outgoing personality, or end up in prison due to extreme aggressiveness, but will this ultimately really work as a selection pressure affecting our offspring’s number and survival, or is it just random noise? Despite thousands of studies on personality traits, we still don’t know, although the studies mentioned above are promising first steps.

Finally, a word on the applicability of Cloninger’s terminology to non-humans may be in order here. Of course the use of any system devised to describe humans is inherently problematic to use on beings whose ecological niche (and therefore brain and perhaps also temperament) is radically different from ours (an extravert rat seems implausible even to the most inveterate anthropomorphist!). The rat is a nocturnal animal that has had a long and intense selection pressure to avoid predators, especially

perhaps avian predators. It has well developed olfactory senses with which it finds its food and conspecifics. This can hardly be said of humans. All the same, our basic need to avoid potentially threatening situations and explore potentially beneficial ones is one we hold in common with all creatures that move around to find energy sources to metabolise, be they rats, octopi, mice or men. From this point of view it makes sense to explore similarities between animals and humans but not plants and humans – that is, temperament is considered to be an adaptive consequence of a highly mobile lifestyle. Although we cannot assume that character dimensions can be applied to rats, I argue that temperamental dimensions such as Harm Avoidance, Reward Dependence and Novelty Seeking are applicable in their basic senses to the rat. In this it would seem I am in good company as Cloninger himself refers to animals using these terms, describing animals high in Novelty Seeking as “...characterized by frequent exploratory activity, approach to novel stimuli and active avoidance or skilled escape from aversive stimuli.” (Cloninger, 1994), and describing how Harm Avoidance is measured in animal testing on rodents (Ibid.). The choice of Cloninger's terminology above other possible systems such as the Big Five partly reflects this author's hopes of identifying neurochemical correlates of temperament in future research, an area in which Cloninger has been very active. On a less scientifically well-founded level I also find the labels themselves to be convenient in that they lend an intuitive understanding of the concepts I have been trying to understand as applied to both rats and humans in a sense that terms like Neuroticism simply do not.

Future Directions

In future research a number of avenues of approach are open. Gosling (2003) suggests the use of multiple tests with multiple items, multiple accuracy criteria, comparison between two or more species, and multiple situations to best capture various dimensions, which would be an interesting approach. Budaev (1997) suggests the use of potentially threatening situations to minimize random behavioural noise, definitely an approach worth considering in the rat, which is a prey animal, perhaps using odours to evoke fear of predation. Effects of genetic relatedness contra rearing conditions would also be well worth looking into. On a more practical level further research on juveniles might benefit from a scale model of the test arenas being made to correspond to the smaller body size of juveniles which might be a confound when comparing with adults.

As for the relevance of this kind of research, it seems clear to me that any model of temperamental dimensions involving anxiety and curiosity, whether intended for pharmacological purposes or neuroscientific research would benefit from a more nuanced approach. If we treat experimental animals as all being essentially equivalent while, for example testing anxiolytics, when they in fact are highly and consistently variable individually and as a result of their life span and thus likely to respond very differently, we may draw erroneous and oversimplified conclusions as to the effects and mechanisms of medication. It is, in other words, not enough to focus research on between strain differences – we must also study within strain differences in the rat.

On a basic research level it would also seem to me that there is great merit in further deepening our understanding of such concepts as temperament, traits and personality. I am not alone in considering personality, temperament and traits to be extremely complex and dynamic. As explained briefly in the introduction, even the terminology of what, exactly we are studying, is very confused, both when it comes to conceptual

terminology (i.e. temperament, traits and personality) and in reference to lower level classifications (i.e. the various labels or names for factors and dimensions). I agree with Gordon Allport that the idea that there is no such thing as personality is indeed a siren song that leads to shipwreck (Funder, 1991), but simultaneously I doubt the validity of the concept both of personality and temperament as they are conceived today – I was highly sceptical of the field of trait psychology when I embarked upon this thesis, and am even more so now – but for entirely different and altogether more positive reasons. In my view, we need more specific research comparing species, developmental trajectories and where possible attempts to predict behaviour as based on temperament to move further beyond folk psychological characterisations and simplifications. In the introduction a study of pumpkinseed fish was mentioned (Wilson, Coleman, Clark, & Biederman, 1993), where a number of questions were asked of psychology from a behavioural ecologist's point of view regarding the lack of an evolutionary approach, the lack of knowledge of a taxonomic distribution of shyness-boldness, and the lack of studies in wild populations. Reading the questions again, we can see that some progress has indeed been made in the intervening decade or so. Recent studies cited (e.g. Dingenmanse et al., 2004; Dingenmanse & Réale 2005) have begun to deal with both the taxonomic distribution and ecological consequences of temperamental dimensions or behavioural syndromes in various species, and psychology is awakening to evolution, as evidenced by the spate of popular science books on evolutionary psychology written in the 1990's and on. But still, there is much to be done. I too am guilty of restricting my work to laboratory animals, to not trying to predict behaviour from observed temperament and to not having tried to tie in the behaviour of my laboratory animals to fitness and adaptive success. Evolutionary perspectives were in no way foreign to me when I began this project, although how on earth I might marry an interest in evolution with an interest in temperament and personality traits was a mystery to me. Now I feel confident that this can be done, and look forward to being one of those who tries to produce the facts and knowledge through experiment and further critical thought in this field.

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