

Evolutionary background for stress coping styles: Relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates

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Reactions to stress vary between individuals, and physiological and behavioral responses tend to be associated in distinct suites of correlated traits, often termed stress coping styles. A connection between physiology, behavior, and cognition was recently demonstrated in strains of rainbow trout (*Oncorhynchus mykiss*) selected for consistently high or low cortisol responses to stress. Compared to high-responsive (HR) fish the low-responsive (LR) strain display better retention of a conditioned response, and tend to show proactive behavior such as enhanced aggression, social dominance, and rapid resumption of feed intake in new environments. Marked differences between HR and LR trout in brain monoamine neurochemistry have also been reported. In line with these studies, experiments with the lizard *Anolis carolinensis* reveal connections between monoaminergic activity in limbic structures, proactive behavior in novel environments, and the establishment of social status via agonistic behavior. Together these observations suggest that within-species diversity of behavioral and cognitive correlates of stress responsiveness is maintained by natural selection over a wide range of animal groups. This diversity may underlie several seemingly different phenomena such as stress coping style, behavioral syndromes, and animal personalities.

1. Introduction

1. Individuality of the stress response - a widespread phenomenon

Few terms are as popular and frequently used in biology, medicine and psychology as "stress". The very definition of "stress" as a biological term, however, has a long history of controversies. Many of these controversies stem from the fact that what is stressful to one person, or animal, is not always stressful for other members of the same species. It is now recognized that stress coping is of fundamental importance to fitness and quality of life in a number of species (Cavigelli and McClintock, 2003; Janczak et al., 2003; Korte et al., 2005), including humans (e.g. Susman et al., 1999). In fact, understanding individual differences in coping ability has become a paramount task in biological psychiatry and stress research (see e.g. Bartolomucci et al., 2005; Korte et al., 2005).

Behavioral responses to stress have been described with a variety of terminologies (see e.g. Koolhaas et al., 1999; Wingfield, 2003). A distinction is often made between proactive (active coping, or 'fight-flight') and reactive (passive coping, or 'conservation-withdrawal') responses. During stress or during exposure to glucocorticoid hormones a transition from behavioral activation to inhibition is typically seen with increasing duration or severity of the challenge (Haller et al., 1998; Øverli et al., 2002a; Wingfield, 2003). The threshold at which the shift from an active to a passive behavioral response occurs is subject to great individual variation, and so is the period of recovery needed to restore normal behavior. It has also been firmly established that individual differences in behavior are associated with differences in the physiological stress response. Koolhaas et al. (1999) promoted the term stress coping style to describe this phenomenon. These authors defined coping style as "*a coherent set of behavioral and physiological stress responses, which is consistent over time and which is characteristic to a certain group of individuals*".

Koolhaas et al. (1999) also reviewed the evidence that two opposing stress coping styles (proactive and reactive) can be identified in mammals. A proactive stress coping style is characterized by

a high level of active avoidance, aggression, and other actions indicating active attempts to counteract the stressful stimulus. Reactive coping, on the other hand, involves immobility and low levels of aggression. Physiologically, the proactive strategy is associated with low hypothalamus-pituitary-adrenal (HPA) axis responsiveness, but high sympathetic reactivity, while the opposite is true for reactive coping. A genetic basis for the expression of behavioral and physiological components of individual coping styles has repeatedly been demonstrated (e.g. Driscoll et al., 1998; Lepage et al., 2000; Ellenbroek and Cools, 2002; de Boer et al., 2003; Veenema et al., 2003). Koolhaas et al. (1999), however, point out that genetic and epigenetic factors combine to generate a relatively stable trait characteristic that confers differential adaptation to environmental conditions such as population density, social stability and food availability. Persistence of a given set of characteristics could thus depend on fluctuations in environmental factors (Dingemanse et al., 2004; Höjesjö et al., 2004) as well as frequency dependent selection.

Compared to the literature in mammals, relatively little information is available on the occurrence of different individual stress-coping styles in other animal groups. Among teleost fish, consistent behavioral patterns that may reflect alternative coping styles have been observed in cichlids, sticklebacks, and salmonids (Huntingford, 1976; Francis, 1990; Van Raaij et al., 1996; Budaev et al., 1999; Bell and Stamps, 2004; Brelin et al., 2005; Schjolden et al., 2005a). Recent evidence suggests that divergent hypothalamus-pituitary-interrenal (HPI) axis reactivity is associated with consistent differences in behavior in rainbow trout (Pottinger and Carrick, 2001a; Øverli et al., 2002b, 2005). The HPI-axis is the teleost equivalent of the mammalian hypothalamus-pituitary-adrenal (HPA) axis (Mommsen et al., 1999), and in rainbow trout the magnitude of the cortisol response to stress is an individual trait which is stable over time, with a moderate to high degree of heritability (Pottinger and Carrick, 1999). Lines of rainbow trout selected for high (HR) and low (LR) post-stress cortisol levels have been established by T.G. Pottinger at the UK Natural Environment Research Council, Center for Ecology and Hydrology. LR fish show a strong tendency to become socially dominant (Pottinger and Carrick, 2001a), a more rapid

recovery of feeding after transfer to a novel environment (Øverli et al., 2002b), and a reduced locomotor response (interpreted as reduced anxiety, not reduced aggression, because no aggression was seen) in a territorial intrusion test (Øverli et al., 2002b). Furthermore, Moreira et al. (2004) recently demonstrated strain differences in the ability to form or recall memories of a stressful event: after a similar number of training trials, the consequent rate of extinction of a conditioned response was more rapid in the HR fish.

In summary, the HR-LR lines appear to demonstrate that a heritable association between increased cortisol production, anxiety-like behavior, and altered cognitive function is present in a comparative vertebrate model system. In this review we also recount studies indicating the existence of similar trait associations both in another poikilotherm species, the lizard *Anolis carolinensis* (Summers et al., 2005a,b; Korzan et al., 2006a,b), as well as in non-selected aquaculture populations of fish (Øverli et al., 2004a, 2006; Brelin et al., 2005). Comparisons with studies on mammals are also made, which together suggest that some trait associations are consistent throughout the vertebrate sub-phylum. Finally, we give a brief overview of some possible underlying physiological mechanisms.

2. The HR-LR trout model

2.1. Selection program

The HR and LR rainbow trout lines were initiated in 1996 by repeated stress testing (3h confinement in 50 L water in groups of 6-7 individuals once monthly) of passive integrated transponder (PIT) tagged 2-year-old rainbow trout (for details see Pottinger and Carrick, 1999). Mean post-stress plasma titers of the teleost corticosteroid hormone cortisol across five episodes of confinement were then calculated for each fish, and individuals were ranked accordingly. Confinement stress testing of the 1st generation offspring (F1), consisting of 15 HR and 14 LR families each resulting from a unique male-female crossing, were carried out on five different occasions between September 1997 and September 1998, and on five occasions in 1999. A highly significant regression of mid-parent cortisol response on progeny response was seen (estimated r^2 [h²] value = 0.41), and the six LR families with the lowest mean cortisol response

and the six HR families with the highest mean cortisol response were identified and used in further breeding (Pottinger and Carrick, 1999). The F2 generation consisted of 11 HR, 11 LR and 3 unselected families. At present, studies are carried out on the F4 and F5 generations of fish.

2.2. Behavior of HR and LR trout

Adult female F1 generation HR and LR fish was used in experiments investigating the effect of selection for stress responsiveness on behavior and brain monoaminergic activity (Øverli et al., 2001, 2002b). In this experiment, HR and LR trout were transferred from communal holding tanks to isolation in observation tanks. After 6 days in isolation, locomotor activity was quantified while observing each fish first in an undisturbed condition and thereafter during a territorial intruder test. In this test, both HR and LR rainbow trout increased their activity level when the intruder was present (intruders are smaller, previously unfamiliar individuals of the same species that are suddenly introduced in the holding unit of a territorial fish, c.f. Höglund et al., [2001]). HR trout displayed higher locomotor activity than LR trout in the presence of an intruder, but not while they were undisturbed. Furthermore, approximately 40 % of the LR fish were feeding during the one week experimental period experiment, while no HR fish ingested any food during this time. Hence, low cortisol production during stress coincided with both a low locomotor response to stress and more rapid reversal of stress-induced anorexia (Øverli et al., 2002b).

Behavioral differences between the HR-LR trout lines were also investigated by Pottinger and Carrick (2001a), who observed the outcome of fights for social dominance between juvenile fish from the F2 generation in a series of dyadic encounters. Juvenile rainbow trout, in common with other predominantly stream-resident salmonids (Keenleyside and Yamamoto, 1962; Taylor, 1990; Hutchison and Iwata, 1997), are intensely territorial animals. When introduced simultaneously into an arena they will engage in agonistic activity that results in the establishment distinct dominance hierarchies (Jönsson

et al., 1998; Winberg and Lepage, 1998; Øverli et al., 1999, 2004b). Using position of the fish within the tank, locomotor activity, agonistic behavior, feeding, and plasma cortisol as criteria of social dominance, Pottinger and Carrick (2001a) reported that LR fish became dominant in a majority (43 out of 46) of HR-LR pairings. The mechanistic basis for the apparent co-selection of competitive ability and stress responsiveness is yet undetermined. Clearly, both direct effects of cortisol (DiBattista et al., 2005) and the involvement of central signaling substances such as corticotrophin releasing hormone (CRH) and brain monoamine neurotransmitters (Winberg and Nilsson, 1992, 1993) require further investigation.

In summary, some of the features of the LR trout line suggest that they represent selection for a pro-active stress coping style, as defined by Koolhaas et al. (1999). It should, however, also be pointed out that the behavior of the HR-LR lines of fish is highly context dependent, and the fishes are influenced by factors such as novel environments and group size (Schjolden et al., 2005b, 2006). It can therefore not yet be ascertained to what degree the behavioral and endocrine profiles of HR and LR rainbow trout correspond to the trait associations identified in other animal groups (Koolhaas et al., 1999; Steimer and Driscoll, 2003; Bolhuis et al., 2004, 2005; Groothuis and Carere, 2005) and humans (Bohnen et al., 1991; Sgoifo et al., 2003; Salvador, 2005).

2.3. Conditioned responses and brain monoaminergic activity in HR and LR trout

Moreira et al. (2004) performed an experiment where groups of HR and LR fish were exposed to a paired conditioned stimulus (CS) and unconditioned stimulus (US). After exposure to 18 CS-US pairings, at least 70% of the individuals of both lines acquired a conditioned response upon presentation of the CS only. Post-conditioning, the fish were tested by presentation of the CS at weekly intervals with no further reinforcement, and the extinction of the conditioned response in the two lines was compared. The frequency of individuals who retained the conditioned response was greater among the LR than HR fish at 14, 21 and 28 days after the last CS-US pairing took place.

To our knowledge, the study by Moreira et al. (2004) is the first to indicate a genetic basis for an association between cognitive, neuroendocrine, and behavioral traits in teleost fish. In mammals, it has repeatedly been shown that signal systems conveying the effects of stress on physiological processes are also involved in behavior and cognitive ability. For example, catecholamines released in the first phase of the stress response are known to promote consolidation and/or storage of novel information (McGaugh and Roozendaal, 2002). The influence of HPA-axis activation on cognition in mammals has been extensively explored (for reviews see e.g. Roozendaal, 2002; Wolf, 2003). The brain monoamine neurotransmitters serotonin (5-hydroxytryptamine, 5-HT), dopamine (DA) and norepinephrine (NE) have also been shown to modulate learning and memory in a number of different training tasks and contexts (e.g. Meneses, 1998; Liang et al., 2001; Dreher et al., 2002). For instance, there appears to be a link between dopaminergic cortical innervation and the development of cognitive capacities (Nieoullon et al., 2003), and the magnitude of dopamine release predicts accuracy of memory on a delayed response task (Phillips et al., 2004).

Øverli et al. (2001) described differences in brain monoaminergic function between HR and LR rainbow trout. In this study only a limited number of fish could be sacrificed for analysis of brain neurochemistry (and only post spawning females were available), hence the results may not give a complete picture of monoamine metabolism in these strains of fish. However, the pattern of stress induced elevation of brain stem and optic tectum concentrations of 5-HT, NE, DA, and their metabolites, suggested that both synthesis and metabolism of these transmitters were elevated after stress to a larger degree in HR than in LR trout. A divergent pattern was seen in the hypothalamus, where LR fish displayed elevated levels of 5-hydroxyindoleacetic acid (5-HIAA, a serotonin metabolite) and 3-methoxy-4-hydroxyphenylglycol (MHPG, an unsulphated norepinephrine metabolite in salmonids). In the telencephalon, LR fish had higher baseline 5-HIAA/5-HT ratios, while there were no significant differences between stressed HR and LR fish. Like in mammals, brain monoamine neurotransmitters are

centrally involved in the control of behavioral and endocrine stress responses in teleost fish (Winberg and Nilsson, 1993; Winberg et al., 1997, 2001; Øverli et al., 1998, 1999; Höglund et al., 2001, 2002a,b; Clements et al., 2003; Larson et al., 2003; Perreault et al., 2003; Lepage et al., 2005), but the possible role of these signal substances in memory function has not been extensively studied in fish.

3. Observations on hatchery strains of rainbow trout

3.1. Feeding behaviour as an indicator of stress coping style

In previous experiments with HR and LR trout (Pottinger and Carrick, 2001a; Øverli et al., 2002b), the most striking differences in behavior between the lines were seen in the locomotor response to territorial intrusion (higher in HR), the tendency to regain feed intake after stress (more rapid in LR), and the ability to gain social dominance in pairs (higher in LR). Later experiments addressed whether the same trait associations are present also in non-selected hatchery populations of rainbow trout (Øverli et al., 2004a, 2006). It was found that the duration of appetite inhibition after stress predicted social dominance with near 100% certainty, with rapid reversal of stress-induced anorexia being characteristic of individuals likely to become dominant in later contests (Øverli et al., 2004a). The tendency to become dominant did not depend on a size advantage in animals that rapidly resumed feeding. In fact, even if they took food on more occasions, these individuals displayed significantly lower growth rates during the experimental period (Øverli et al., 2004a). This observation is in line with previous reports that high metabolic rates predict social dominance in salmonid fishes (Metcalf et al., 1995; McCarthy, 2001).

The general approach in our studies with hatchery populations of rainbow trout has been to remove individuals from a large homogenous population, and then study the behavior and physiology of these fish in isolation (Øverli et al., 1999, 2004ab, 2006). The weeklong isolation period, and the fact that all fish come from the median size interval in a larger population, is intended to minimize the effect of previous social interactions. After the acclimation period, fish are subjected to various treatments such as

tests of stress responsiveness, or social interaction in pairs. This experimental approach is summarized in figure 1. A similar approach is used in studies with the lizard *Anolis carolinensis* (described in chapter 4), with the main difference being that these animals are caught in the wild before being transferred to the laboratory.

In some experiments (Øverli et al., 2006; Sørensen, 2006), feeding behavior was described accurately by assigning a score to each fish on each feeding occasion (once daily during one week). Feeding behavior was rated according to a 4-step scale following the criteria listed in table I, and all points obtained during one week were later summed for each individual. All fish would advance through the stages listed in table I with varying speed of progress. The resulting measure would depend on how rapidly fish resumed normal feeding behavior, with high points indicative of quick acclimation to the new environment. Total feeding score was found to predict several other behavioral and physiological measures. The relationship between feeding score and plasma cortisol levels in undisturbed socially isolated fish is graphed in figure 2 (Ø. Øverli, C. Sørensen, and G.E. Nilsson, unpublished results). Other clear patterns were that fish that obtained a high total feeding score showed lower brain stem 5-HIAA concentrations, high aggression, and less locomotion during acute confinement stress, and (table II, summary of unpublished data and data from Øverli et al., 2006).

3.2. Boldness, hunger, or learning?

Obviously, it is not straightforward to adjudge whether fish that start to eat quickly are bolder (i.e., the anxiogenic effect of novelty and transport wear off faster) or simply hungrier in the feeding test (i.e., the anorectic effect of stress wear off faster). Furthermore, boldness and motivation to feed are tightly linked through common neuroendocrine signaling systems (see e.g. Bernier and Peter, 2001; Carr, 2002). It should, however, be kept in mind that the rainbow trout used in our studies are poikilotherm animals kept in relatively cold water (5-15°C). These fish can live for long periods without food, if necessary. Hence,

in most situations where active feeding behavior would imply both benefits and potential costs, salmonid fish are more likely to decline feeding than would be expected from mammalian studies. In fact, anyone who has ever fished for salmonids using prey imitations as lures can subscribe to how easy it is to disturb these fish so that they refuse to open their mouth. In a study by Øverli et al (1998) it took one week to restore normal feeding behaviour in previously subordinate fish after they had been removed from their dominant partner. At this point the effect of social position on brain serotonin metabolism had also been reversed (Øverli et al., 1998). Hence, restoration of feeding after stress is likely to reflect downregulation of the physiological stress response, and feeding score would seem to be a very precise indicator of stress coping style in salmonid fish.

Several other factors, such as differences in metabolic rate, are of course likely to influence feeding behavior. A fish with a relatively high metabolic rate will consume energy resources more rapidly, and physiological hunger signals will take effect. Consider, for instance, the graph in figure 2: Fishes with higher levels of the catabolic hormone cortisol generally have low feeding scores, but scores appear to rise again at the high end of the cortisol range. Notably, very few individuals contributed to the high-cortisol part of the curve. Nevertheless, the curvilinear approach (second order polynomial with $y = 0.061x^2 - 2.1341x + 19.533$) yielded a much better fit ($r^2=0.78$) than a linear trend line ($r^2=0.41$), and a runs test for deviation from linearity came out marginally significant ($p=0.047$). Hence, with respect to the control of feed intake in rainbow trout, a likely conclusion is that cognitive and emotional aspects of HPI-axis activation can outweigh metabolic effects for extended periods of time. This notion may, however, only be valid up to a certain level of cortisol exposure.

As pointed out by Sneddon (2003), an apparent difference in boldness could also come from a difference in learning (i.e. some fish are faster to learn that feeding in the new environment was not associated with danger or other negative experience). Again, the association between stress

responsiveness and cognitive abilities recently demonstrated by Moreira et al. (2004) suggests that learning and/or memory retention is important for the expression of alternative stress coping styles.

4. The *Anolis carolinensis* model

4.1 General description of the *Anolis* behavior

Wild-caught male *Anolis carolinensis* lizards have been recruited for many studies investigating physiological responses associated with the establishment of social rank (Summers, 2002; Summers and Greenberg 1994; Summers et al., 1998, 2005a,b; Korzan et al. 2002, 2004, 2006a,b; Höglund et al., 2005). The study of stress associated with agonistic behavior in *A. carolinensis* is facilitated by their eyespots. Eyespots are specialized patches of skin located postorbitally to each eye (Hadley and Goldman, 1969; Vaughan and Greenberg, 1987), which darken when the animal is agitated or aggressive. This response has been reported to depend on both sympathetic activation and brain monoaminergic neurotransmission (Summers and Greenberg, 1994; Larsson and Summers, 2001; Höglund et al., 2005).

These visual signals are especially utilized when males defend territories against conspecifics (Korzan et al., 2000; 2002). Rival males perceive the presence or absence of this sign stimulus and as a result modify their behavioral output. For instance, Korzan et al. (2000) found that male *A. carolinensis* showed more aggressive displays towards their own mirror image if the eyespot was masked by green paint. Furthermore, males that had their eyespots permanently darkened by black paint became dominant in paired interactions (Korzan et al., 2002). Hence, manipulation of the eyespot signal can dictate social rank by influencing behavior of the individual viewing the signal. The effect of the sign stimulus is however, not as potent as memory of a recent interaction with a specific opponent (Korzan et al., 2004; Forster et al., 2005; Summers et al., 2005b; see 4.3 Opponent recognition).

Not surprisingly, males that express the eyespot signal first become dominant in paired contests when visual cues have not been experimentally manipulated (Summers and Greenberg, 1994; Larson and

Summers, 2001). Thus, it would appear that a rapid and large catecholamine response to social challenge is indicative of a pro-active stress coping style even in lizards. In the study of Larson and Summers (2001) latency to eyespot darkening was delayed by treatment with the serotonin reuptake inhibitor sertraline, which in turn lead to lower social status. Hence, it could be hypothesized that differences in brain serotonergic activity mediating individual differences in behavior and physiology exist prior to social interaction. Whether such differences predict social rank in *Anolis* was later tested by Summers et al. (2005a).

4.2 Predicting social dominance

Like the rainbow trout, *A. carolinensis* posses behavioral and physiological characteristics that make it possible to predict future social standing of an individual prior to agonistic interaction (Summers et al., 2005a, Korzan et al., 2006b). Korzan et al. (2006b) found that males responding to stimuli, such as the presentation of a receptive female or food, with a shorter latency than an opponent will become dominant in the majority of paired interactions (see table III for summary). The fact that the latency to respond to stimuli allows accurate prediction of future dominant status was later used by Summers et al. (2005a,c) to study serotonergic and corticosterone responses in males destined to achieve dominant or subordinate social status. These authors found that among future dominant individuals baseline serotonergic activity was reduced in the septum, nucleus accumbens, striatum, medial amygdala, anterior hypothalamus, raphe, and locus ceruleus, but not in the hippocampus, lateral amygdala, preoptic area, substantia nigra, or ventral tegmental area. In the lizard model, higher baseline levels of circulating corticosterone was found to predict social dominance (Summers et al., 2005c). Corticosterone and serotonergic activity also rises faster in animals that become dominant (Summers et al., 2005c). These authors suggested that even if the permissive effect of glucocorticoids on aggression does not suggest an active role for the hormone, these steroids may be necessary for full expression of aggressive behavior (Summers et al, 2005c).

In summary, similar to rainbow trout male *A. carolinensis* exhibit differences in stress coping strategies that predict future social status. The shorter the latency to respond to any stimulus reliably predicts behavioral output and future social standing. These behavioral predictors of dominance are underlain by more rapid neural changes in monoamines and plasma hormones during stressful social confrontation.

4.3 Opponent recognition

Among male *A. carolinensis* behavioral output is potently influenced by direct aggression, stereotyped displays of aggressive intent, and visual sign stimuli (Summers and Greenberg, 1994; Korzan et al., 2000; 2002). None of these social signals are, however, as potent as memory of previous opponents (Forster et al., 2005; Summers et al., 2005b). This results in relatively stable dominant-subordinate relationships, which may last up to a week (Larson and Summers 2001; Forster et al., 2005), and individual lizards remember specific opponents also when reintroduced after separation of socially interacting pairs. Learning opponent identity and, perhaps, appropriate behavior for specific social settings appears to be influenced by the hippocampus, where NMDA receptors (particularly those that include the NR_{2B} subunit) increase during social interaction (Meyer et al., 2004).

This effect is not associated to learned or conditioned defeat, because defeat alone will not inhibit aggressive behavior. That is, males reintroduced to the same male whose domination created their subordinate status one, three or seven days earlier, respond with a dramatically reduced aggressive response, and the previous social order is maintained. However, males that lose a first interaction to one male significantly increase their aggressive output during a second interaction with a different opponent, but still dominant male (that is, this dominant male has recently won an aggressive interaction with a different male) three days later (Forster et al., 2005). Although social rank relationships remain relatively stable, this time period is limited. After ten days, aggressiveness increases between previously

paired lizards and approximately half of the social rank relationships are reversed.

These data suggest that proactive and reactive strategies are plastic and limited temporally in the lizards; perhaps because their adaptive value may change with time and the neuroendocrine condition of the potential opponent. Most likely, altered behavioral strategies are dependent on changes in neural mechanisms. For instances, elevation in dopaminergic activity may increase aggression (Kramarcy et al., 1984) or shorten latency to attack (Höglund et al., 2001) and increases the propensity for dominant status (Winberg and Nilsson, 1992). Chronically elevated serotonergic activity, on the other hand, inhibits aggression, and may reverse dominant status (Larson and Summers, 2001; Summers et al., 2005b). The plasticity of pro- and reactive strategies, while rare, may follow because social rank must occasionally be reassessed, and adopting a more proactive strategy for a second interaction may be adaptive.

5. Observations in other species, synthesis, and discussion

5.1. Stress and cognition

In both animals and man an increasing amount of evidence suggests that psychological processes associated to how an individual assesses a given situation might be equally important factors as the actual physical challenge in determining the severity of the stress response (Von Holst, 1998; Ursin and Eriksen, 2004; Eriksen et al., 2005). Expectancy of the outcome of stimuli is one example of a powerful cognitive modulator of the stress response. Furthermore, in several laboratory reared rodent lines it also appears that genetically determined differences in stress coping characteristics are associated with divergence in cognitive ability (e.g. Castellano et al., 1999, Nguyen et al., 2000, Balogh and Wehner, 2003, Brennan, 2004) and, vice versa, if one selects for variation in cognitive ability, concomitant differences in stress coping style arise (e.g. Giorgi et al., 2003; Steimer and Driscoll, 2003; Aguilar et al., 2004). In particular, rodents exhibiting behavioral and physiological traits typical of a reactive stress coping style perform poorly in the acquisition of a conditioned response in aversive trials (e.g. Willig et

al., 1991). Similarly, in an unselected population, individual differences in cognitive performance in rodents have been shown to predict stress induced corticosterone release and mesolimbic levels of brain monoamine neurotransmitters (Tomie et al., 2000).

It is well known that chronic stress and exposure to glucocorticoid hormones may impair memory and learning (see e.g. McEwen and Sapolsky, 1995; McEwen, 2000; Roozendaal, 2002; Wolf, 2003 for reviews). It is far from clear, however, whether the association between stress coping style and cognition reflect acute effects of circulating glucocorticoids (Oitzl et al., 1998; Beylin and Shors, 2003), organizational effects of chronic differences in hormone exposure during development (Catalani, 1997; Steimer et al., 1998; McEwen, 2000; Grootendorst et al., 2001; Meaney, 2001), or inherent differences in brain function. There is also a possible evolutionary implication of the relationship between glucocorticoids, cognition and behavior that is rarely discussed: It has been hypothesized that proactive behavioral responses are maladaptive under repeated, uncontrollable or unpredictable stress (Haller et al., 1998; Wingfield, 2003). Proactive responses should instead be attempted against known controllable stressors where the outcome of the behavior can be predicted. It follows that a reduced capacity to make use of memories of actual events may decrease controllability and predictability, which in turn should increase HPI/HPA-axis activation, and inhibit proactive behavior. The results of Moreira et al. (2004), suggesting a more rapid extinction of conditioned responses in the HR rainbow trout, are clearly in line with this argument.

5.2. Mechanisms behind variation in stress responsiveness

In the following we review some potential mechanisms that may be involved in heritable variation in the stress response. First, we have concentrated on those biochemical pathways where polymorphisms altering gene function already have been related to stress coping or other personality traits in mammalian species, as this provides evidence that mutations in these genes can survive in a population. Thereafter,

we the review the possible role of brain structural plasticity and the process of neurogenesis in the association between physiological, behavioral and cognitive traits.

5.2.1 Cortisol production and interrenal sensitivity to adrenocorticotrophic hormone

The behavioral differences between HR and LR trout are consistent with some reported effects of corticosteroid hormones in poikilotherms (decreased appetite: Gregory and Wood, 1999; increased locomotor activity: Cash and Holberton, 1999; Øverli et al., 2002a). In fish, like in mammals, these steroids have time-, context-, and dose-dependent effects on behavior (Øverli et al., 2002a). Cognitive effects of cortisol have to our knowledge not been reported in teleost fish, but the conserved nature of the central signal systems in the stress response suggest that such effects are present.

Pottinger and Carrick (2001b) reported that stress-induced adrenocorticotrophic hormone (ACTH) levels are similar in HR and LR fish, but exogenous ACTH elevated plasma cortisol levels to a significantly greater extent in HR than LR fish. Hence, interrenal sensitivity to ACTH is different in HR and LR fish. Corticosteroids are synthesized from cholesterol by side chain cleavage mediated by the enzymatic activity of the P450_{SCC} complex located in the inner mitochondrial membrane. Pregnenolone, the product of this cleavage, then undergoes a series of isomerizations and hydroxylations to produce cortisol. In mammals, it has been shown that this synthetic activity depends on the availability of cholesterol in the inner mitochondrial membrane. One of the factors that regulates this availability is the steroidogenic acute regulatory protein (StAR), a sterol transport protein, which allows rapid transport of cholesterol from the outer to the inner mitochondrial membrane following an acute stimulus, thereby making the precursor available to P450_{SCC}.

A multitude of genetic polymorphisms are known in this pathway in humans (see e.g. Wedell and Luthman, 1993; Stratakis and Rennert, 1999), which are all connected with debilitations in untreated patients. Polymorphisms in corresponding genes could be present, but have less severe effects, in the

tetraploid trout genome. Furthermore, an ACTH receptor promoter polymorphism that results in a lower promoter activity in vitro and lower cortisol secretion in response to ACTH stimulation in vivo was recently reported by Slawik et al. (2004). Hence, the possibility should be explored that differences in stress induced cortisol concentrations might arise from differences in interrenal function and sensitivity, rather than in central HPI/HPA-axis control. On the other hand, variability in adrenal function does not have to be of genetic origin. For instance, seasonal variations in stress-induced plasma corticosteroid levels have been correlated to alterations in adrenocortical steroidogenic capacity in lizards (*Sceloporus undulatus*) (Carsia and John-Alder, 2003).

5.2.2 Brain neurotransmitter systems

In humans and other mammals it has long been recognized that genetically determined alterations in the brain serotonin system is associated with personality and temperamental traits, as well as the propensity to develop mood disorders. The most well known examples are polymorphisms in monoamine oxidase and the serotonin transporter (5-HTT) gene or promoter region (Lesch et al., 1996; Shih et al., 1999; Fernandez et al., 2003). Glucocorticoid hormones regulate the expression of 5-HTT, but the response to these hormones is attenuated in the 5-HTT 'short' type (Glatz et al., 2003). Thus, it could be proposed that the functional link between 5-HTT polymorphisms and personality traits is, at least in part, dependent on interaction with glucocorticoid hormones.

In the HR-LR trout model, it seems likely that other monoamine neurotransmitters, and neuropeptides such as corticotrophin releasing hormone (CRH, or corticotrophin releasing factor, CRF) are also involved in determining behavioral profile. In juvenile chinook salmon (*Oncorhynchus tshawytscha*), intracerebroventricular injections of CRH induced hyperactivity, an effect that was shown to depend on concurrent 5-HT activation (Clements et al., 2003). Interestingly, CRH administration also increases dopamine concentrations in dorsal medial hypothalamus of newts (*Taricha granulosa*) (Lowry

et al., 2001), and one of the main neurochemical differences between HR and LR rainbow trout is that HR fish respond to stress by increased DA concentrations and turnover in several brain areas, while LR fish do not (Øverli et al., 2001).

Increased DA synthesis and release may, however, also be an effect of acute elevations in glucocorticoid concentrations (Barrot et al., 2001). Furthermore, genetically determined differences in DA systems have been reported in several mammalian models (Rots et al., 1996a,b,c; Lecca et al., 2004). Thus, at present it is not known whether differences in DA systems between HR and LR trout are a cause or a consequence of hormone dynamics, but there are interesting parallels to mammalian models which suggest the presence of evolutionary conserved trait correlations. For instance, a negative relationship between DA reactivity and social competitive ability has been demonstrated in cynomolgus monkeys (*Macaca fascicularis*) (Morgan et al., 2000).

The physiological and behavioral role of brain dopaminergic systems has also been studied in the *Anolis* model (e.g. Waters et al., 2005; Korzan et al., 2006a). Elevated motivation requires dopaminergic action (Phillips et al., 2003), and aggressive social interaction stimulates dopaminergic as well as serotonergic activity in limbic brain regions (Miczek et al., 2002; Winberg and Nilsson, 1992). Dopaminergic activity in different brain regions appear to influence aggression (Kramarcy et al., 1984; Höglund et al., 2005), social status (Winberg and Nilsson, 1992), motor activity (Waters et al., 2005), learning, and perhaps most important, motivation and reward (Korzan et al., 2006a). All of these factors influence social interaction, and are integrated as a part of neural regulation of social stress. During social interactions between male *A. carolinensis* increased hypothalamic DA, and increased DA in substantia nigra and ventral tegmental areas (SN/VTA) were associated with increased aggressive behavior and status (Korzan et al. 2006a). Elevated dopaminergic activity is usually associated with greater aggressiveness and elevated social status, but very high physiological levels may even limit aggressive interaction (Höglund et al., 2005).

Like in most vertebrates, locomotor activity stimulates dopaminergic activity in striatum of *A. carolinensis* (Waters et al., 2005). However, during aggressive displays and attacks, changes observed in dopaminergic activity in nuclei associated with motor activity like striatum seem to be coupled with expression of specific stereotyped movements associated with social communication more than general motor activity (Korzan et al 2006a). The striatum and nucleus accumbens are closely linked, as are level of motivation and stereotyped social behavior. Increases in DA and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the nucleus accumbens transpire when males effectively attain dominant status and may be characteristic of impetus and reward (Korzan et al., 2006a). Effects of social interaction on dopaminergic activity in hippocampal and amygdalar nuclei appear consistent with current literature with neurochemical changes associated with memory formation (Korzan et al., 2006a). Together these data suggest that the combination of social signal perception, social rank, and behavioral manifestation, but not any solitary factor, may be the impetus for the changes in dopaminergic activity associated with formation of dominant and subordinate status. Herein, the DA systems seem to link behavioral responses to stimuli, and control of stimuli influences not only the behavioral response but also the neural machinery involved with these response strategies.

Other transmitter systems that could well be involved in integrating physiological and behavioral aspects of stress coping style are gamma-aminobutyric acid (GABA) and endorphins. Modulation of the stress response includes glucocorticoid feedback at the hippocampal and hypothalamic levels (De Kloet et al, 1998) and regulation by neuronal pathways, including the GABAergic inhibitory system (Jessop, 1999). The neurotransmitter GABA, which is the main inhibitory neurotransmitter in the vertebrate central nervous system, inhibits the HPA-axis through its actions on GABA receptors expressed by CRH neurons within the paraventricular nucleus (PVN) of the hypothalamus. Furthermore, GABA inhibits the locus ceruleus norepinephrine system (Tsigos and Chrousos, 2002). Uhart et al. (2004) recently established a link between gene polymorphism in the GABA_A6 receptor subunit and stress

responsiveness in humans. Brain CRF neurons also express μ opioid receptors and are modulated by β -endorphin neurons originating in the arcuate nucleus. In humans a polymorphism in the extracellular N-terminal domain of μ opioid receptor has been identified (Bergen et al. 1997; Wendel and Hoehe, 1998), and a link to altered HPA-axis function was demonstrated by Wand et al. (2000).

5.2.3 Neurogenesis and neural plasticity

Structural processes in the brain may be highly important for behavior, mood, and cognition in both animals and human beings, and are affected by a range of environmental, endocrine, and pharmacological factors (Nilsson et al., 1999; Gould et al., 2000; Jacobs et al., 2000; Fuchs and Flügge, 2001; Garcia-Verdugo et al., 2002; Nottebohm, 2002; Song et al, 2002; Shors, 2004). Several reports indicate that new neurons are added to continually growing structures and replace old and dying ones in adults of all major vertebrate taxa, a process known as neurogenesis (although see Rakic [2004] for a counter argument). In mammals, chronic stress, glucocorticoids, aging, and repeated exposure to opiate drugs decrease adult hippocampal neurogenesis (Kuhn et al., 1996; Kempermann et al., 1998; Gould et al., 1997; Eisch et al., 2000), while voluntary exercise, enriched environments, and hippocampal-dependent learning increase neurogenesis (Gould et al., 1999; van Praag et al., 1999; Ambrogini et al., 2000). Increased adult neurogenesis also correlates with improved performance in hippocampal-dependent learning tasks (Nilsson et al., 1999; van Praag et al., 1999). These results have received a great deal of attention due to their implicit conclusion that more hippocampal neurons equal better memory function. Whether adult neurogenesis truly generates new nerve cells remains debated, although, in their review, Kempermann et al. (2004) submit ample evidence for function and contribution within cognitive processes. Reduced brain cell proliferation has also repeatedly been linked to depression and depression-like behavioral patterns (see e.g. Duman et al., 1999; Jacobs et al., 2000; Kempermann and Kronenberg, 2003).

Compared to mammals, teleost fish and other non-mammalian vertebrates have a much higher degree of adult neurogenesis (Zupanc, 2001). The size of a teleost brain increases with age, body weight and body length throughout life, and adult proliferation has been observed within all major teleost brain structures (Birse et al., 1980; Leyhausen et al., 1987; Brandstatter and Kotrschal, 1990; Zupanc and Horschke, 1995). Adult proliferation zones have been mapped in detail in the brains of the adult stickleback (*Gasterosteus aculeatus*) (Ekström et al., 2001), zebrafish (Zupanc et al., 2005), and gilthead sea bream (*Sparus aurata*) (Zikopoulos et al., 2000).

Compared to the number of anatomical and developmental studies, surprisingly little is known about the functional significance of neurogenesis in teleost fish. Ekström et al. (2001) noted a certain degree of interindividual variation in the labeling of proliferating cells in sticklebacks. They did not, however, investigate this further in their study, and called for a systematic exploration of this issue (Ekström et al., 2001). Sørensen (2006) recently showed that brain cell proliferation was reduced in socially subordinate rainbow trout, as compared to socially isolated and undisturbed controls. Similar observations have been made in mammals (Gould et al., 1997). It is not known, however, whether reduced brain cell proliferation is an effect of a general inhibition in growth rate, an effect of stress, or a persistent feature predisposing certain individuals for a subordinate social position and reactive behavior.

The above results warrant investigating whether rates of brain cell proliferation and neurogenesis differ between HR and LR trout lines, or predict social status in non-selected populations. The proteins needed for cell proliferation and cell fate determination are often highly conserved through evolution, and one would not expect to find functional polymorphisms in their genes that can be linked to altered stress responsiveness. Several systems with a regulatory role in cell proliferation, including the brain serotonergic system, are, on the other hand, likely to show such polymorphism. Notably, Veenema et al. (2004) recently demonstrated that there are several differences in HPA-axis responsiveness, 5-HT

responsiveness, and hippocampal cell proliferation in mouse lines selected for divergence in aggressive behavior.

6. Conclusions

Both genetic and environmental factors (e.g. social interactions and previous exposure to stress) contribute to extensive inter-individual variation in how stress affects behavior and physiology (Winberg and Nilsson, 1993; Koolhaas et al., 1999; Blanchard et al., 2001; Summers, 2002; Bartels et al., 2003, Sapolsky, 2003; Sgoifo et al., 2003; Entringer et al., 2004; Wust et al., 2004a,b,c; Korte et al., 2005). However, the existence of similar trait associations in the HR-LR strains and non-selected aquaculture populations of rainbow trout (Øverli et al., 2004b, 2005), and the apparent parallel between stress coping styles in mammals, the HR-LR trout model, and the *Anolis* lizard model suggests that that suites of correlated physiological and behavioral traits has been conserved by evolution. In other species variation in the physiological stress response is associated with a number of major axes of variation in behavior, including activity, shyness-boldness, and aggression (Korte et al., 1992, 2005; Koolhaas et al., 1999; van der Vegt et al., 2001; Cavigelli and McClintock, 2003; Popova et al., 2005). Such behavioral measures have all been identified as major personality traits in animals across a wide range of taxa (see Gosling and John, 1999; Gosling, 2001).

Considering the tight links between neuroendocrine signal systems controlling physiological, emotional, and behavioral responses, and taking into account that few behaviors can be performed or emotions experienced without simultaneous physiological activation, one must conclude that behavioral syndromes observed in behavioral ecology (Sih et al., 2004), stress coping style (Koolhaas et al., 1999), and psychological and behavioral components of personality (Gosling, 2001) may all be reflections of the same phenomenon - individuality in the stress response. The fact that stress coping characteristics depend on genes as well as experience (and can even be transferred between parents and offspring by a

multitude of non-genetic mechanisms) is therefore relevant to the interpretation of results from all biological research on live animals, and may provide a common conceptual framework uniting subjects as seemingly different as speciation and personality psychology.

References

Figure legends

Figure 1. Generalized summary of the experimental approach used to describe stress coping styles in rainbow trout (c.f. Øverli et al., 2004a, 2006). Feeding behavior is observed during one week after transfer from group rearing to social isolation, followed by analysis of other trait characteristics such as the physiological and behavioral response to acute confinement stress, or aggressive behavior in pairs.

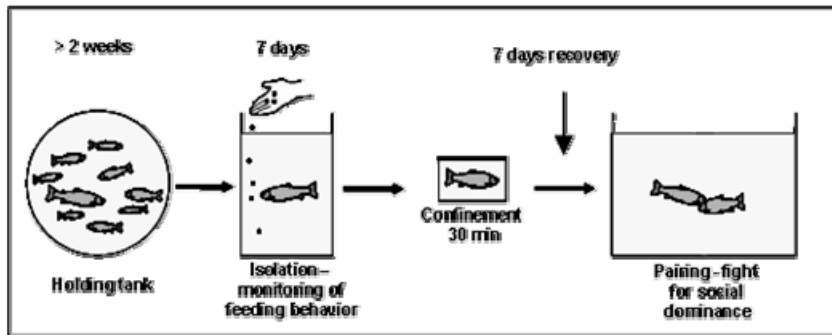
Figure 2. Regression between feeding points and resting plasma cortisol in isolated and undisturbed fish

Table I. Grading of behavior of individual fish held in isolation, and corresponding point scores in the feeding test (reprinted from Physiology and Behavior)

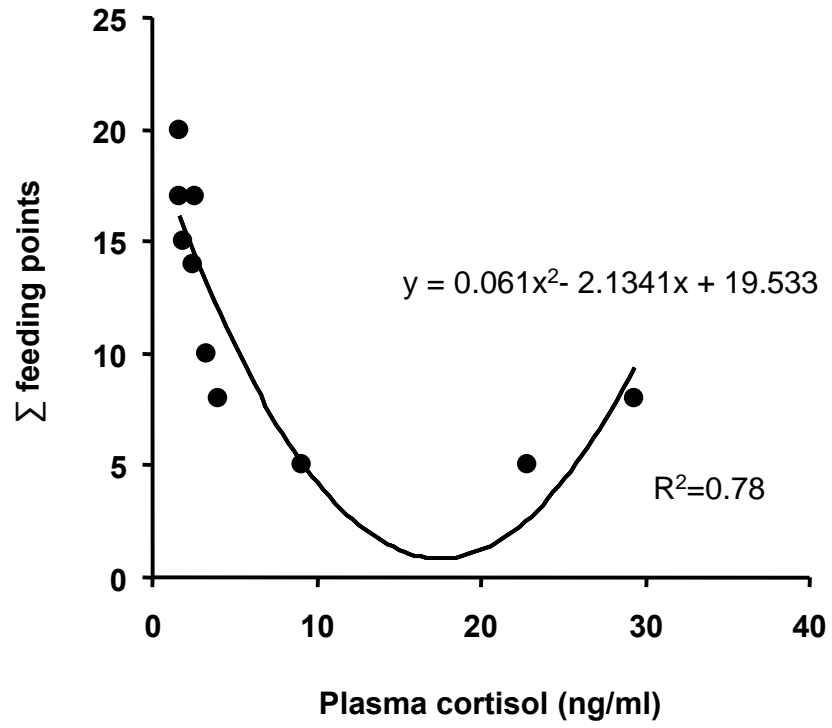
Table II. Correlations between feeding score and other behavioral and physiological parameters

Table III. The number of *Anolis carolinensis* pairs where the future dominant male showed the quickest (or highest) response prior to social interaction (reprinted from Acta Ethologica)

Øverli et al., figure 1



Øverli et al., figure 2



Øverli et al., table I

Points	Behavior
0	Fish does not respond to food
1	Fish eats only pellets that falls directly in front, and does not move to take food
2	Fish moves more than one body length to take food, but returns to original position in aquarium between each food item
3	Fish moves continuously between food items and consumes all food presented

Øverli et al., table II.

Correlation tested	R ²	p	Direction of regression
Feeding points vs brain stem [5-HIAA] (socially isolated fish)	0.77	0.002	Negative. Fish with a high feeding score show less baseline activation of brain stem 5-HT neurons after 1 week in isolation. (Ø. Øverli, C. Sørensen, G.E. Nilsson, unpublished data)
Feeding points vs locomotor response (socially isolated fish)	0.24	0.04	Negative. Fish with a high feeding score show a moderate locomotor response to acute confinement after 1 week in isolation (Published in Øverli et al., 2006)
Feeding points vs aggressive acts performed (dominant fish)	0.65	0.005	Positive. Fish with a high feeding score perform more aggressive acts when placed in a dominant social position after 1 week in isolation (Ø. Øverli, C. Sørensen, G.E. Nilsson, unpublished data)

Øverli et al., table III.

Behavior	Winners response first/highest/longest	Winners response last/lowest/shortest	Chi Square probability (expected 1:1 ratio)
Feed intake	11	3	0.03
Courtship	11	3	0.03
Aggressive displays, latency	14	1	0.008
Aggressive displays, frequency	15	4	0.01
Eyespot, latency	12	0	>0.001
Eyespot, duration	12	0	>0.001