THE RELATIONS BETWEEN STRESS, SOCIAL RANK, PERSONALITY, AND COGNITIVE PERFORMANCE IN YUCATAN MINIPIGS (*SUS SCROFA*)

by

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ABSTRACT

Individuals differ consistently in their behavioural and hormonal responses to environmental challenges, and these differences can impact cognitive ability. In gregarious species, individual differences in personality, stress hormone concentrations, and cognitive performance can correlate with social rank, although studies have yielded conflicting results on the relations among these variables. Using 10 male and 10 female Yucatan minipigs (*Sus scrofa*), we aimed to: (1) explore the potential for inter-individual consistency in behavioural and hormonal traits; and (2) characterize the relations among personality, social rank, the stress response, and cognitive performance on an object location memory task. We found that pigs varied along two personality dimensions, labeled curiosity and timidity. Some stress hormone biomarkers were repeatable in males, but not females. The sexes also differed in activity of the two major stress systems, with females showing a greater asymmetry between HPA axis and sympathetic nervous system activity, symptomatic of chronic stress. Application of an acute stressor immediately before the object location memory task impaired subsequent performance, but only in the males. Finally, the effect of timidity on object location memory differed between the sexes, with performance tending to be better among less timid males and more timid females. The plethora of sex differences in our study suggests that results from one sex cannot be generalized to the other and underscores the necessity of considering both sexes in behavioural and physiological studies.

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INTRODUCTION AND OVERVIEW

Stress, which involves a stressor or threat to an organism's homeostasis, and a behavioural/physiological stress response, activates both the sympathetic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis (McEwen, 2010). These systems secrete catecholamines (epinephrine and norepinephrine) and glucocorticoids (cortisol in fish and most mammals), respectively. The glucocorticoids and catecholamines modulate activity in limbic brain structures including the hippocampus, amygdala, and prefrontal cortex. These three brain areas mediate declarative memory, defined as the conscious or voluntary recollection of previously learned information (Milner et al., 1998). Individual behavioural differences can influence the direction of stress effects on memory, as an individual's personality is often associated with a consistent physiological response pattern in reaction to a stressor (Koolhaas et al., 2010). In social species, an individual's social rank may be correlated with cognitive performance (Humphrey, 1976), as well as with personality (David et al., 2011) and the stress response (Creel, 2001). While inter-individual variability was traditionally dismissed as nonadaptive "noise" around an adaptive mean (Wilson, 1998), it is now recognized as having important ecological and evolutionary consequences (Williams, 2008). Elucidating the relations between the physiological stress response, cognitive performance, personality, and social rank can help us to better understand individual stress vulnerability, as well as the evolution of behavioural, cognitive, and hormonal traits.

Stress Effects on Declarative Memory

Stress effects in learning and memory have been investigated using a diverse selection of stressors that are imposed at different phases of a cognitive task. Cognitive tasks can measure different domains of learning and memory. It is widely accepted that memory is composed of multiple distinct systems supported by different brain pathways (Squire, 2004). Declarative memory has been the most investigated with respect to stress effects, as the related limbic structures are highly sensitive to stress hormones. There is evidence that stress influences hippocampus-independent forms of memory as well (Schwabe et al., 2012), but these memory systems have received much less attention (but see Guenzel et al., 2013; Quirarte et al., 2009).

Declarative memory is temporally defined in three phases: encoding (initial learning/acquisition of a task), consolidation (or storage), and retrieval (or recall). Encoding and consolidation depend on limbic structures, while retrieval engages chiefly the neocortex (Squire and Zola, 1996). The timing of the stressor relative to the different memory phases is critical in determining whether stress facilitates or impairs memory (Joels et al., 2006, Roozendaal, 2002).

Stressor severity is another important factor influencing the direction of the effect. The relation between the degree of stress and cognitive performance was first described by Yerkes and Dodson (1908) and has since become known as the Yerkes-Dodson Law. The law states that moderate levels of arousal should induce optimum levels of performance, while performance falls away at lower or higher levels (i.e., an inverted-U

relationship). As task difficulty increases, the optimal level of stress decreases. Inverted-U shape curves have been described for glucocorticoid (Lupien and McEwen, 1997) and catecholamine (McGaugh, 1989) effects on memory.

The influence of stress on subsequent memory for material unrelated to the stressor is studied using a variety of laboratory stressors, such as footshocks, restraint stress, noise, or psychosocial stressors. The material to be remembered after presentation of the stressor may be emotional or neutral in nature. The effects of stressful events on subsequent memory appear to depend on the nature of the to-be-remembered material (Lupien et al., 2007).

Glucocorticoid effects

Generally, glucocorticoid elevations in response to a stressor are more likely to affect subsequent memory for emotional than for neutral information (Abercrombie et al., 2006; Buchanan et al., 2006; Cahill et al., 2003; Kuhlmann et al., 2005; but see Andreano and Cahill, 2006; Maheu et al., 2005). These effects depend on the timing of the stressor, with stressor-induced increases in glucocorticoids enhancing encoding and/or consolidation, while impairing retrieval (Roozendaal, 2002). Many studies report an inverted-U dose-response curve for glucocorticoids and declarative memory (Lupien and McEwen, 1997). These dose-dependent effects can be explained by considering the specific role of the two glucocorticoid receptor types. The ratio of Type I/Type II occupation plays a critical role in mediating glucocorticoid actions on memory (de Kloet et al., 1999). Cognitive performance is optimal when Type I receptors are saturated and

Type II receptors are partially occupied. Since endogenous levels of glucocorticoids are higher in the morning than in the afternoon, a stressor will affect the ratio of Type I/Type II occupation differently depending on the time of day when it is applied. In support of these predictions, a meta-analysis by Het et al. (2005) showed that studies conducted in the morning tended to find impairing effects of glucocorticoids, while studies in the afternoon observed enhancing or no effects. Thus, the severity of the stressor and the time of day when it is administered substantially influence the direction of glucocorticoid effects on memory.

Catecholamine effects

Very little research has examined the effect of stressor-induced catecholamine elevations on subsequent memory for material unrelated to the stressor. An early study in rats demonstrated a dose-dependent relationship between norepinephrine released following a footshock and memory for material unrelated to the stressor (Gold and McGaugh, 1975). Recent human studies have reported contradictory findings. Blockade of β-adrenergic receptors prior to stressor application did not affect short or long-term recall of the material-to-be-remembered, suggesting that catecholamines do not modulate memory for material unrelated to the stressor (Maheu et al., 2005). However, a study by Segal et al. (2012) showed that norepinephrine increases following exposure to emotionally arousing stimuli enhanced memory in a subsequent non-arousing task.

Recent data suggest that direct noradrenergic activation alters memory for material that is not inherently emotional in nature. Post-training infusions of

norepinephrine into the basolateral amygdala dose-dependently enhanced 24-h memory of an object recognition task, which induces minimal levels of emotional arousal (Roozendaal et al., 2008). Similarly, stimulation of the locus coeruleus, the main source of norepinephrine in the brain, immediately preceding a learning episode selectively facilitated memory of object-place associations for that episode (Lemon et al., 2009). Both studies also showed that blockade of β-adrenergic receptors impaired memory, providing further evidence that noradrenergic activation enhances memory, even when the information to be remembered is not itself arousal inducing.

Chronic stress effects

While moderate levels of acute stress enhance declarative memory, chronic stress is frequently associated with declarative memory impairments. Prolonged exposure to stress produces numerous changes in hippocampal structure (Conrad, 2010; McEwen, 2000; McEwen and Sapolsky, 1995; Roozendaal, 2002). Severe, traumatic stress in humans has been shown to kill hippocampal neurons. Individuals with post-traumatic stress disorder, recurrent depression, or Cushing's syndrome have a significantly smaller hippocampal volume and impaired declarative memory (Conrad, 2010; McEwen, 2000). Individual differences in stress responsiveness may put some individuals at greater risk of developing stress-related disorders (e.g. Sweis et al., 2013).

Inter-individual Variation in the Stress Response

It is well-known that individuals differ from one another in their physiological responses to challenges in their environment. However, studies that examine the stress

response in free-living or captive animals typically neglect to analyze inter-individual variation (Williams, 2008); unless looking for differences associated with life-history factors, such as sex, age, body condition, or season. A standard practice for such studies is to take a sample at only one time point from each individual and assume it is representative of the individual's true state. Only a few studies have explicitly tested this assumption by determining the repeatability of glucocorticoid titers from multiple samples of the same individual. Statistical repeatability (also known as the intraclass correlation coefficient) is a measure that describes the proportion of variation that is due to differences among individuals. Repeatability, r, is given by the formula: $r = s^2_A/(s^2 + s^2_A)$, where s^2_A is the between-group variance and s^2 is the within-group variance (Hayes and Jenkins, 1997).

Repeatabilities for glucocorticoid responses have been calculated most often for birds (Cockrem, 2013), but a few recent studies have found repeatable individual differences in glucocorticoid profiles in fish (Cook et al., 2011, 2012), amphibians (Narayan et al., 2013) and mammals (Smith et al., 2012). These studies report individual consistency in baseline glucocorticoid levels (Angelier et al., 2010; Ouyang et al., 2011; Romero and Reed, 2008; Smith et al. 2012), stress-induced glucocorticoid levels (Angelier et al., 2009; Cockrem et al., 2009; Cook et al., 2011, 2012; Rensel and Schoech, 2011; Wada et al., 2008), or both (Cockrem and Silverin, 2002; Kralj-Fiser et al., 2007; Narayan et al., 2013). In addition, some researchers observed that repeatability is dependent on context (Romero and Reed, 2008), sex (Wada et al., 2008), or physical condition of the animal (Cook et al., 2012). These findings suggest that caution be

applied when interpreting patterns in individual differences found on only one sampling occasion.

Artificial selection studies demonstrate that variation in glucocorticoid profiles has a heritable component, at least in some species. Selective breeding in the laboratory has established lineages of high and low responding zebra finches (Evans et al., 2006), Japanese quail (Satterlee and Johnson, 1988), and rainbow trout (Pottinger and Carrick, 1999). To my knowledge, no studies have investigated the repeatability or heritability of individual variation in baseline or stress-induced levels of catecholamines.

The functional significance of consistent inter-individual variation in hormonal traits is poorly understood (Williams, 2008). One unresolved issue is whether it is baseline glucocorticoid levels or stress-induced glucocorticoid elevations that are functionally important. Basal and stress-induced glucocorticoid concentrations have disparate physiological and behavioural effects and, therefore, probably have distinct fitness consequences (Romero, 2004). Chronic elevations of basal glucocorticoids can provoke numerous pathologies, including immune suppression, reproductive suppression, gastric ulcers and muscle wasting (Sapolsky, 1992a), and are therefore assumed to signal an individual or population with reduced fitness. A recent review reported that basal glucocorticoid levels can predict fitness, but the nature of this relationship varies across species, populations, and life-history stages, and is not always present (Bonier et al., 2009b). Similarly, in a review of the evidence for an association of acute glucocorticoid reactivity with fitness, Breuner and colleagues (2008) concluded that environmental

context largely predicts the nature of the relationship. Thus, inter-individual variation in the stress response may be maintained if high and low glucocorticoid responders represent alternative strategies with adaptive values that change according to environmental conditions (Blas et al., 2007). In contrast to the glucocorticoids, nothing is known about the fitness consequences of catecholamines in wild animal populations.

Animal Personality

Personality exists across the animal kingdom, from primates to insects and molluscs (Gosling, 2001). Animal personality is defined as inter-individual behavioural and physiological differences that are stable over time and across contexts, irrespective of sex or age (Carere and Maestripieri, 2013). This definition is analogous to that used by human-personality psychologists, with the exception that they include affective and cognitive traits in addition to behavioural traits (Gosling, 2008). A personality trait subsumes various specific behaviours (i.e., "biting" and "growling" at conspecifics comprise the trait "aggressiveness"). Non-human personality traits are most often measured using behavioural tests, but subjective ratings by knowledgeable observers have also proven to be a viable method of assessment (Gosling, 2008). Individuals often possess a suite of correlated traits referred to as a behavioural syndrome, a well-documented example of which is the aggressive-bold syndrome (Sih et al., 2004).

Personality has significant ecological and evolutionary consequences (Sih et al., 2012; Wolf and Weissing, 2012). Individuals differ in their responses to environmental challenges, and these differences are related to survival and reproduction (Smith and

Blumstein, 2008). On a proximate level, personality differences may be shaped by a combination of genetic, parental and environmental effects, as well as an individual's experience over time (Sih et al., 2004). The evolutionary mechanism maintaining inter-individual behavioural variation is a matter of debate. Several hypotheses have been proposed to explain the existence of personality using social niche theory (Bergmuller and Taborsky, 2010), evolutionary game theory (Dall et al., 2004), life-history trade-offs (Biro and Stamps, 2008), and sexual selection (Schuett et al., 2010).

Coping Styles

Inter-individual variation in the stress response has been associated with personality differences in a wide range of species (Koolhaas et al., 1999, 2010). The term "coping style" is often used to refer to a behavioural and physiological response pattern in reaction to a stressor that is stable over time and across situations (Koolhaas et al., 1999). Two distinct response patterns are distinguished: proactive and reactive coping styles. Coping strategies have also been labeled along other axes, i.e., shy-bold, slow-fast, and passive-active. These labels are often used interchangeably in the literature, as they discriminate between similar behavioural traits. Typically, proactive copers are fast but superficial explorers, are aggressive, easily develop routines, and are novelty seekers. Conversely, reactive individuals are characterized by slow but thorough exploratory behaviour, low aggression, high flexibility and reactivity to environmental changes, and fear of novelty.

Generally, proactive coping is associated with high sympathetic reactivity to stressors whereas the reactive coping style tends to have a higher HPA axis reactivity (Koolhaas et al., 1999, 2010). Several studies support the prediction that shyer or more reactive personalities are linked to an increased release of glucocorticoids in fish (Raoult et al., 2012), rodents (Cavigelli and McClintock, 2003; Veenema et al., 2003), birds (Atwell et al., 2012; Carere et al., 2003; Korte et al., 1997; Kralj-Fiser et al., 2007; Lendvai et al., 2011), pigs (Hessing et al., 1994; Ruis et al., 2000) and non-human primates (Byrne and Suomi, 2002). However, it has been suggested that the relationship is in fact more complicated, varying with the season and social context (Koolhaas et al., 2010; Martins et al., 2007). While the hormones of the HPA axis receive the majority of attention, some studies have also shown a positive correlation between proactive coping and sympathetic reactivity (Fokkema et al., 1995; Korte et al., 1997; Sgoifo et al., 1996).

It is unclear exactly how neuroendocrine mechanisms relate to personality dimensions (reviewed in Carere et al., 2010, Coppens et al., 2010; Koolhaas et al., 2010). Koolhaas and colleagues (2010) argue that differential stress system activity is mainly a consequence rather than a cause of individual behavioural variation. Proactive and reactive individuals differ in physical activity and thus have dissimilar metabolic and cardiovascular requirements, which may be reflected by differential activity of the HPA axis and sympathetic nervous system. Conversely, hormones may influence the development of personality through organizational and activational effects on the brain and behaviour (Sih et al., 2004). Thus, the proximate mechanism maintaining coping styles likely involves ongoing feedback between behaviour and physiology.

Social Rank

Social rank is another biologically important axis of behavioural variation, as dominance hierarchies occur in a range of species, with the dominant individuals in a group having priority of access to preferred resources (Drews 1993). Thus, dominance relationships can have important fitness consequences, with dominant individuals tending to enjoy greater reproductive success (Ellis, 1995; Majolo et al., 2012). Social rank is commonly measured using competitive orders, and, to a lesser degree, using aggressive orders. Competitive orders rank an individual according to their priority of access in approach or avoidance situations, while aggressive orders determine rank by an animal's agonistic behaviours towards conspecifics (Syme, 1974).

Social rank and personality

Two hypotheses have been put forward to explain how a hierarchy is established: (1) the 'prior attributes' hypothesis, which states that an individual's prior attributes will determine that individual's position in the group's hierarchy, and (2) the 'social dynamics' hypothesis, which views rank order as resulting from the dynamics of social interaction (Chase et al., 2002). While evidence shows that the formation of dominance hierarchies is a complex phenomenon and likely involves both processes (Chase et al., 2002), many studies provide support for the 'prior attributes' hypothesis by demonstrating that individual attributes are correlated with rank. While age, sex, and physical size are among the most common attributes studied, neophobia is increasingly being linked to social rank. However, the direction of the relationship between neophobia

and dominance varies between studies and across species (Colléter and Brown 2011; David et al., 2011; Fox et al., 2009; Verbeek et al., 1999).

Social rank and the stress response

Social subordination has traditionally been associated with a chronically overactive stress response in most vertebrates. Within the classical picture of a social hierarchy, subordinate animals are more likely to be the subjects of resource deprivation, predator attacks, and harassment by dominant animals. They also have fewer means of coping and social support. However, it is also plausible to hypothesize that dominant animals engage in more agonistic interactions in order to maintain their rank, and thus are more socially stressed than subordinates. A large body of research on the relationship between social rank and glucocorticoid secretion indicates that the degree of social stress associated with a particular rank depends on how social status is acquired and maintained, rather than the rank per se (Abbott et al., 2003; Creel, 2001; Creel et al., 2013; Goymann and Wingfield, 2004; Sapolsky, 2005).

Different social ranks vary in energetic expenditure due to differences in associated activity patterns. Thus, Muller and Wrangham (2004) suggested that metabolic stress, rather than psychosocial stress, may commonly mediate the relationship between rank and glucocorticoid secretion. Of course, many factors other than the stress associated with social status can influence glucocorticoid profiles, including environmental conditions (Creel et al., 2013), group size or composition (Goymann et al., 2003), and personality (as discussed in Coping Styles).

Individual Differences in Learning and Memory

Although animal personality is arguably the fastest growing field in behavioural biology, individual variation in cognition has received surprisingly little attention (reviewed in: Carere and Locurto, 2011; Sih and Del Giudice, 2012; Thornton and Lukas, 2012). In humans, individual differences directly affect many aspects of cognition, including attention, perception, learning and memory. In contrast, animal cognition studies focus largely on species-level cognitive capacity, dismissing individual variation as noise around an adaptive mean. This 'cognitive capacity' perspective imposes limitations on the field of comparative cognition. Many studies use too few individuals to be able to conclude whether a cognitive trait is present or absent in a species. Furthermore, this implies a binary distribution of cognitive traits among species, with species either possessing them or not, when it is far more likely that cognitive traits fall along a continuum within and between species (Thornton and Lukas, 2012). Also, focus at the individual level may reveal that poor performance can be explained by reasons other than cognitive deficiency, such as lack of motivation or attending to an incorrect cue. Finally, the 'cognitive capacity' perspective overlooks the fascinating possibility that variation in learning is related to personality differences.

Personality and individual learning

Personality may account partially for the unexplained variation in cognitive ability. Sih and Del Giudice (2012) suggest that personality may affect three different stages of the learning process. First, the animal must encounter a new situation. Neophilic

individuals should have a higher encounter rate than their neophobic counterparts. Consequently, they should be faster at learning a cognitive task. Several studies corroborate these predictions in ravens (Range et al., 2006), black-capped chickadees (Guillette et al., 2009), guppies (Dugatkin and Alfieri, 2003), rainbow trout (Sneddon, 2003), and rhesus macaques (Coleman et al., 2005). In contrast, pigs that avoided novel objects learned a Go/No-go task faster than their neophilic counterparts (Lind and Moustgaard, 2005). Unlike the other studies, this study used both positive and negative reinforcement techniques. As discussed previously, different personalities are associated with differences in stress reactivity. Thus, differential stress reactivity to the negative reinforcer may have affected the learning rate in the pigs by influencing their motivation to perform the task.

Second, according to Sih and Del Giudice (2012), animals assess changes in their environment. Neophilic individuals are thought to be less sensitive to environmental changes. Thus, neophobic individuals should perform better on tasks that require them to pay attention to new information. In agreement with these expectations, slow-exploring, reactive individuals are more successful in reversal learning of a task than their fast-exploring, proactive counterparts (black-capped chickadees: Guillette et al., 2011; pigs: Bolhuis et al., 2004). Similarly, fast-exploring male great tits were more likely to continue returning to the place where they had learned to expect food after the food location had changed (Verbeek et al., 1994). These results are consistent with the idea that neophilic individuals tend to form behavioural routines, whereas neophobic individuals are more flexible to changing task demands.

Finally, in the last stage of learning animals must alter their behaviour in response to the updated assessment. The lower behavioural flexibility in neophilic individuals may result from failure to attend to environmental changes (2^{nd} stage) or from failure to alter behaviour in response to processed new information (3^{rd} stage). However, it is difficult to distinguish between these two stages empirically (Sih and Del Giudice, 2012).

The aforementioned studies suggest that individual learning propensity may result from different personalities. It should be noted, though, that differences in cognitive ability can impact the development of an individual's personality. It is likely that genetic make-up, individual experiences, as well as interactions between the two, determine an animal's personality (Sih et al., 2004). For example, Sundstrom et al. (2004) found that brown trout of sea-ranched origin were bolder than fish of wild origin. Rainbow trout modified their response to novelty in response to positive or negative prior experiences (Frost et al., 2007). Genetic and developmental influences on cognitive ability have also been shown (reviewed in Boogert et al., 2011). It is also possible that personality and learning do not influence each other, but are regulated by a third variable.

Social rank and individual learning

Social rank can dramatically influence everyday aspects of an animal's life. An individual's position in the hierarchy may either facilitate or inhibit behaviours, such as cognitive performance. Nevertheless, few studies exist on the relation between social rank and learning. Humphrey's (1976) "social intelligence hypothesis" proposed that an animal's social success depends on his or her cognitive ability. Individuals that are

superior at calculating the consequences of their own and others' behaviour are more likely to acquire a high rank. An individual's aptitude in the social intelligence domain may generalize to broader cognitive domains, such as learning and memory. In male starlings, for example, high-ranking individuals mastered a foraging task faster than low-ranking conspecifics (Boogert et al., 2006). Dominant chickadees also performed significantly better than subordinates on a spatial memory task (Pravosudov et al., 2003).

While the social intelligence hypothesis suggests that learning propensity predicts social rank, social rank may affect learning. Barnard and Luo (2002) found that after pairing mice together, the dominant of the pair performed better than the subordinate on a radial maze task when tested in isolation, whereas no performance differences were found prior to establishing rank relationships. These results show that rank acquisition affects learning performance.

As is the case with many learning studies, it is uncertain exactly what a performance deficit reflects. Drea and Wallen (1999) propose three contrasting hypotheses to explain why subordinates lag behind their dominant counterparts in learning tasks. The "cognitive disadvantage hypothesis" is synonymous with the social intelligence hypothesis (i.e., subordinates are cognitively inferior to dominants). The "failure to learn hypothesis" proposes that performance is not related to cognitive ability, but rather the presence of dominant animals disrupts learning in subordinates. The "failure to perform hypothesis" posits that subordinates learn just as well as dominants, but inhibit their performance in the presence of dominants. They found that dominant

rhesus macaques performed well on a discrimination task when tested in a group and in isolation, but that the subordinates performed well only when tested in isolation, providing support for the "failure to perform hypothesis". Their results emphasize the importance of distinguishing between learning and performance as manipulation of different factors can lead to markedly different conclusions about an animal's cognitive ability.

Why Pigs?

Pigs (*Sus scrofa*) have been increasingly recognized as an ideal model for studying learning and memory (reviewed in: Gieling et al., 2011; Held et al., 2002). These gregarious, inquisitive animals demonstrate well-developed cognitive abilities (Broom et al., 2009; Kouwenberg et al., 2009), as well as sophisticated social behaviour (Held et al., 2010; McLeman et al., 2008). Moreover, pigs are an excellent species for personality studies, as they show high inter-individual variation in behavioural responses to a task (Forkman et al., 1995; Janczak et al., 2003; Spoolder et al., 1996).

From a comparative perspective, pigs share many physiological similarities with humans. In particular, the anatomy, growth and development of the pig brain resemble that of the human brain more closely than do the brains of rodents and other small laboratory animals (Lind et al., 2007). Such parallels between their species and ours make pigs a promising model for investigating learning and memory.

Pig cognition studies can also aid in improving animal welfare. In 2010 there were an estimated 966 million domestic pigs worldwide, more than 90 percent of which

are kept in unnaturally overcrowded conditions that prohibit them from fulfilling their behavioural needs (Singer and Mason, 2006). Since the welfare of the animal is thought to depend solely on whether his or her cognitive needs are being met (Duncan and Petherick, 1991), a developed knowledge of pig cognition is critical in determining ways to maximize welfare. Specifically, investigating how husbandry stressors impact learning and memory can aid in minimising stress-related cognitive disorders. Studies on individual variation in cognition are also necessary in addressing welfare concerns as individuals may require different cognitive and physical enrichment.

Natural behaviour

The behaviour of feral domestic pigs is quite similar to that of their ancestral species, the Eurasian wild boar (D'Eath and Turner, 2009). Wild boars or feral domestic pigs live in family groups known as "sounders". A sounder typically comprises a few mature sows and their offspring from that year. Female sub-adults may remain with their dam's group or join an adjacent group. Male sub-adults leave the group around 6-10 months of age and live alone until the mating season at which time they will compete with other males for access to a female group. Neither sounders nor solitary males are territorial and the size of the home range is largely determined by resource availability. Pigs are scavenging omnivores, and spend most of their time rooting, grazing and exploring substrates with their snout. A stable, linear dominance hierarchy is maintained in sounders and overt aggression is rare (D'Eath and Turner, 2009).

Sensory capacities

The visual capacities of pigs are somewhat limited in comparison to primates (Zonderland et al., 2008). The pig's hearing range exceeds that of humans, although sensitivity is slightly poorer. Vocalisations function significantly in communication and social recognition, and convey information about the sender's identity and their arousal state (Held et al., 2009). Olfaction is well-developed in pigs and plays an important role in the transmission of social information (Kristensen et al., 2001; Meese et al., 1975; Mendl et al., 2002). McLeman et al. (2008) showed that pigs are able to discriminate between group members based on the presence of only one or two of the three principal sensory modalities (vision, olfaction and audition). In a foraging task, pigs can learn to use both visual and olfactory cues to find the food reward (Croney et al., 2003). Tanida and Nagano (1998) found that miniature pigs relied more on visual and auditory cues than olfactory cues to discriminate between people.

Personality

Coping styles have been extensively studied in pigs. The backtest, in which a pig is held on its back and the number of escape attempts is counted, is a commonly used measure of coping, however it has been criticized for being arbitrary as the motivational and functional significance of the behaviours observed in the test are unclear (Jensen et al., 1995a). High-resisting and low-resisting pigs have been shown to differ in responses to social encounters (Hessing et al., 1993), basal cortisol concentrations (Geverink et al., 2002a; Hessing et al., 1994) and HPA reactivity in response to a stressor (Geverink et al., 2002b; Ruis et al., 2000). Other studies have found no evidence for the existence of two

discrete coping strategies (D'Eath and Burn, 2002; Forkman et al., 1995; Jensen et al., 1995b; Spake et al., 2012; Spoolder et al., 1996). Likewise, later attempts to determine whether an individual's backtest score predicts endocrine responses to acute stress failed to find a correlation (van Erp-van der Kooij et al., 2003; Velie et al., 2012), casting further doubt upon the value of the backtest. In light of these findings, further research on pig personality has benefited from moving away from the concept of distinct coping styles and focusing more on personality as a multidimensional phenomenon. Open field tests, human approach tests, and novel object tests are widely used to assess behavioural traits in pigs and may be more applicable to the study of pig personality than the increasingly obsolete backtest (Brown et al., 2009; Donald et al., 2011; Janczak et al., 2003; Magnani et al., 2012).

Cognitive abilities

Pigs have well-developed spatial memory abilities. For example, pigs can remember the previous location of food in a foraging arena, but stressors imposed during the retention interval disrupted this ability (Laughlin et al., 1999; Mendl et al., 1997). Recently, Elmore et al. (2012) showed that piglets are able to solve a spatial T-maze task using extra-maze visual cues. Pigs can also acquire a spatial holeboard discrimination task (Arts et al., 2009; Bolhuis et al., 2013; Gieling et al., 2012). Environmental enrichment has been shown to enhance acquisition of a spatial task in some studies (Bolhuis et al., 2013; Sneddon et al., 2000), while others have found no effects (Jansen et al., 2009; de Jong et al., 2000).

The ability of pigs to recognize objects has been assessed in several studies. Spontaneous object recognition tests have shown that Göttingen minipigs explore novel objects significantly more than the familiar object, indicating recognition of the familiar object (Kornum et al., 2007; Moustgaard et al., 2002; Sondergaard et al., 2012). Yucatan minipigs also display object recognition memory after a one week delay interval (Kouwenberg et al., 2009). Gifford et al. (2007) reported that pigs failed to show a novelty preference after 10 min exposure to the sample object, but did discriminate between familiar and novel objects when the exposure time was increased to two days.

A couple of studies have examined whether pigs can remember multiple aspects of a past event. Held et al. (2005) demonstrated that pigs are able to discriminate between two food sites of different relative value and remember their respective locations, indicating that they are able to remember the "what" and "where" of an event. Kouwenberg et al. (2009) extended this finding to show that Yucatan minipigs can simultaneously recall what object is encountered where, and in which context, providing the first evidence of episodic-like memory in this species.

Objectives

In collaboration with Dr. C.J. Walsh and Dr. G.M. Martin, I conducted a series of experiments, presented below as two manuscripts formatted for submission to Hormones and Behaviour. The purpose of the first manuscript was to evaluate whether pigs show consistent individual differences in behavioural and hormonal traits and, subsequently, to characterize the relations among personality, the hormonal stress response, and social

rank. We also assessed the influence of litter origin and sex in shaping individual behavioural and hormonal profiles. In the second manuscript, we investigated the effects of an acute stressor on object location memory, a form of declarative memory. The effects of personality, social rank, and sex on performance in the memory task were also examined in order to better understand individual variation in cognition.

CO-AUTHORSHIP STATEMENT

Manuscripts 1 and 2 are co-authored with Dr. Carolyn Walsh and Dr. Gerard Martin at Memorial University of Newfoundland. As the first author, I formulated specific research questions, conducted a literature review, performed data collection and analysis, interpreted data, and wrote the manuscripts. Dr. Walsh and Dr. Martin assisted in the identification and design of the research project, supervised development of work, covered the costs of the animals and saliva sampling, and helped to evaluate and edit the manuscripts.

MANUSCRIPT 1

Individual differences in Yucatan miniature pigs (*Sus scrofa*): Personality, social rank, and the stress response

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Abstract

Behavioural traits representing personality exist across the animal kingdom. Few studies have examined inter-individual variation in endocrine traits, namely glucocorticoid and catecholamine levels, the stress hormones secreted by the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system, respectively. Personality and stress hormone levels are often linked, and, in social species, these variables can correlate with social rank. Using 20 Yucatan minipigs (*Sus scrofa*) we: (1) investigated the existence of consistent inter-individual variation in behavioural and hormonal responses; (2) described the relations between personality, social rank, and reactivity of the stress systems; and (3) examined whether litter origin and sex influence individual behavioural and hormonal variation. Principal components analysis on a set of behavioural variables revealed two personality traits, labeled curiosity and timidity. We observed sex differences in: (1) repeatability of salivary stress markers (cortisol, alpha-amylase, chromogranin A); (2) litter origin influences on social rank; and (3) reactivity of the stress systems. We also found social rank differences in sympathetic activity that were dependent on condition (stressed vs. non-stressed). The prevalence of sex effects in our study highlights the importance of including both sexes in research addressing behavioural and hormonal variation.

Keywords: domestic pig (*Sus scrofa*), inter-individual variation, stress, social rank, personality, sex, salivary alpha-amylase, cortisol, chromogranin A.

1. Introduction

Consistent inter-individual variation in behaviour and physiology is a widespread and ecologically relevant phenomenon in both human and non-human animals (Koolhaas et al., 2010). Behavioural traits representing personality have been described across a wide range of species and have significant ecological and evolutionary consequences (Sih et al., 2012; Wolf and Weissing, 2012). Less well-studied are consistent differences in physiological traits among individuals. In particular, there is marked, but poorly understood, variability in how individuals of the same species respond hormonally to stressors in their environment. Perception of a stressor activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system, which respond by stimulating the release of adrenal hormones, the glucocorticoids and catecholamines, respectively (Romero and Butler, 2007).

The analysis of the magnitude, patterns, and functional significance of inter-individual differences in the hormonal stress response provides exciting opportunities to integrate endocrine studies with other fields of biology such as behaviour, ecology, and evolution (Williams, 2008). However, studies that examine the stress response in free-living or captive animals typically neglect to analyze inter-individual variation (Williams, 2008), unless looking for differences associated with life-history factors, such as sex, age, or body condition. A standard practice for such studies is to take a sample at only one time point from each individual and assume it is representative of the individual's true state. Only a few studies have explicitly tested this assumption by determining the

repeatability of glucocorticoid titers from multiple samples of the same individual. Statistical repeatability is a measure that describes the proportion of variation that is due to differences among individuals. Repeatability, *r*, is given by the formula: $r = s^2_A/(s^2 + s^2_A)$, where s^2_A is the between-group variance and s^2 is the within-group variance (Hayes and Jenkins, 1997).

Repeatabilities for glucocorticoid responses have been calculated most often for birds (Cockrem, 2013), but a few recent studies have found repeatable individual differences in glucocorticoid profiles in fish (Cook et al., 2011, 2012), amphibians (Narayan et al., 2013) and mammals (Smith et al., 2012). These studies report individual consistency in baseline glucocorticoid levels (Angelier et al., 2010; Ouyang et al., 2011; Romero and Reed, 2008; Smith et al. 2012), stress-induced glucocorticoid levels (Angelier et al., 2009; Cockrem et al., 2009; Cook et al., 2011, 2012; Rensel and Schoech, 2011; Wada et al., 2008), or both (Cockrem and Silverin, 2002; Kralj-Fiser et al., 2007; Narayan et al., 2013). In addition, some researchers observed that repeatability is dependent on context (Romero and Reed, 2008), sex (Wada et al., 2008), or physical condition of the animal (Cook et al., 2012). To our knowledge, no studies have investigated the repeatability of individual variation in baseline or stress-induced levels of catecholamines.

There is accumulating evidence that individual variation in the physiological stress response is associated with behavioural differences among individuals (Koolhaas et al., 2010). Generally, shyer or more reactive personality types demonstrate high HPA

axis reactivity to stressors, whereas bolder or more proactive types tend to have a higher sympathetic reactivity. However, it has been suggested that the relationship is in fact more complicated, varying with the season and social context (Koolhaas et al., 2010; Martins et al., 2007). The proximate mechanisms leading to such correlations are poorly characterized, but involve ongoing feedback between behaviour and physiology (Trillmich and Hudson, 2011).

In socially living species, personality and stress reactivity have been shown to correlate with social rank. Social rank is a biologically important axis of behavioural variation, as it may strongly influence fitness, with dominant individuals tending to enjoy greater reproductive success (Ellis, 1995; Majolo et al., 2012).

Several studies have hypothesized that subordinates should be less neophobic than dominant individuals, as subordinates may be restricted to forage in riskier novel environments. This hypothesis has found support in studies of barnacle geese (Stahl et al., 2001), rats (Robertson, 1982), jackdaws (Katzir, 1983) and black-capped chickadees (An et al., 2011). However, when tested in isolation, no relation between neophobia and rank was found in great tits (Boogert et al., 2006), suggesting that the response to novelty may not be a trait inherent to low-ranking individuals, but is instead evoked by the social context. Several studies have also shown that the behavioural response to novelty predicts the subsequent establishment of dominance relationships in birds and fish. However, the direction of the relationship varies between studies and across species. In zebra finches (David et al., 2011) and brown trout (Sundstrom et al., 2004), more exploratory

individuals are more likely to become dominant, whereas the opposite was observed in mountain chickadees (Fox et al., 2009) and great tits (Verbeek et al., 1999).

Physiological correlates of a particular rank are generally thought to emerge only after the rank is attained (Sapolsky, 2004). A large body of research on the relationship between social rank and glucocorticoid secretion indicates that the degree of social stress associated with a particular rank depends on how social status is acquired and maintained, rather than the rank per se (Abbott et al., 2003; Creel, 2001; Creel et al., 2013; Goymann and Wingfield, 2004; Sapolsky, 2005). In a comparative study of primates, glucocorticoid levels were elevated in subordinate animals if they experienced high rates of stressors and had little social support (Abbott et al., 2003). Hierarchy stability can also modify the rank-related pattern in glucocorticoid levels, with subordinates experiencing the most social stress in stable hierarchies, while the pattern is reversed during periods of instability (Sapolsky, 1992b). Little is known about the rank-catecholamine relationship, as the speed with which catecholamines are secreted precludes measuring basal plasma concentrations, and urinary and faecal metabolites do not preserve well (Sapolsky, 2005).

Behavioural and neuroendocrine characteristics may be shaped by a combination of genetic, parental, and environmental effects, as well as an individual's experience over time (Sih et al., 2004). In particular, sex and litter origin may mould personality development and its associated physiological traits. Numerous studies in both human and non-human animals have shown that males and females differ consistently in the mean

level of their expression of a behavioural trait (reviewed in Schuett et al., 2010). Moreover, physiological stress response patterns differ markedly between the sexes (Kudielka and Kirschbaum, 2005; Verma et al., 2011). An individual's litter of origin can also contribute to shaping individual differences in behaviour and physiology through genetic factors (Van Oers and Sinn, 2013), as well as maternal (Maestripieri and Mateo, 2009) and sibling (Hudson et al., 2011) influences on early development.

The pig is an ideal model in which to study the relations between personality, the hormonal stress response, and social rank. Previous studies have shown that pigs have personalities (Forkman et al.,1995; Janczak et al., 2003; Spoolder et al., 1996), and that they form semi-linear hierarchies in captivity (Fels et al., 2012; Puppe et al., 2008). In addition, several studies have shown that the stress response of the sympathetic nervous system and HPA axis can be reliably and accurately measured in pigs using salivary biomarkers. Salivary cortisol has been used extensively as a HPA axis marker in pigs (Escribano et al., 2012; Geverink et al., 2002; Merlot et al., 2011) and, recently, pigs have been shown to produce measurable increases in the sympathetic markers, salivary alpha-amylase (sAA; Fuentes et al., 2011) and salivary chromogranin A (CgA; Escribano et al., 2013), in response to a stressor.

The aims of the current study were to: (1) investigate the existence of consistent inter-individual variation in behavioural and hormonal responses in pigs; (2) describe the relations among personality, social rank, and reactivity of the two major stress systems;

and (3) examine whether litter origin and sex influence individual behavioural and hormonal variation.

2. Methods

2.1. Subjects

The subjects were twenty Yucatan miniature pigs (10 males, 10 females) born from six litters between September 23 and October 7, 2012 at the Memorial University of Newfoundland pig breeding facility (see Appendix A for birth records of subjects). Within the first two weeks after birth, the pigs were injected with iron and had their pin teeth clipped. Around two months of age, the pigs were ear-tagged, injected with *Erysipelothrix rhusiopathia* vaccine, and dewormed. Males in this study were not castrated. All procedures and daily husbandry practices were carried out according to guidelines set out by the Canadian Council of Animal Care. Male pigs were 28 weeks of age, and females 32-34 weeks of age, at the start of our experiment.

2.2. Housing

Prior to the experiments, the subjects were housed in an indoor room (5.8 x 6.7 m) divided into four pens separated by chain-link fencing. They shared the room with approximately fifteen other pigs who were not used in experiments. Males and females were kept in separate pens. For our study, the subject pigs were transferred to an adjacent room (4.5 x 6.7 m) where they stayed for the duration of the experimental period. Since the animals had reached sexual maturity, males and females were tested as separate

groups between April 23 and May 12, 2013, and May 20 and June 8, 2013, respectively. The test room consisted of a main pen with three side pens along one side (Appendix B). When tests were not being carried out the pigs had access to all pens. The floor consisted of red tiles covered partially with black rubber mats. The room was maintained under a 14:10 h light:dark cycle, with lights on at 6 a.m. Temperature was maintained between 17 and 21 ºC. Pigs were fed Co-op Pig Grower around 9:30 am and 4:00 pm every day throughout the experimental period. All animals had continual access to water. The floor of the pen was washed twice daily with a hose. Heavy rubber balls and hanging chains were provided as environmental enrichment objects for the pigs.

2.3. Social isolation and confinement

The subjects in the current study were part of a simultaneous study investigating the effect of an acute stressor on performance in an object-location memory task. The pigs underwent an 8-day habituation period followed by a trial every other day for 8 days, for a total of four trials per pig. A trial lasted 25 min. A stressor was applied immediately before the object-location memory task in two out of the four trials for each individual. We randomized the order of the stress and non-stress trials across individuals. The stressor involved social isolation in a crate for 10 min. The crate with the pig inside was moved just outside the test room to augment the experience of social isolation as well as to prevent the other animals from observing their crated pen-mate. The crate was kept outside the room when not in use. Animals in the non-stressed condition were let out of the side pen and allowed to wander around the main pen for 10 min before

commencement of the memory task. We restricted testing to between 12:00 pm and 4:00 pm, in order to control for the diurnal rhythm of the salivary analytes.

2.4. Saliva sampling

Three saliva samples were taken for each trial: 0 min (baseline), 10 min (post-stress), and 35 min (post-test) after stressor onset. In the non-stressed condition, saliva samples were collected with the same time-interval without the stressor present. A total of twelve samples were collected per subject. We used Salimetrics Children's Swabs (Salimetrics, LLC, Pennsylvania, USA) to obtain saliva. The pig was enticed to chew on the swab by placing it in front of his or her snout. If the pig was not interested, then the swab was gently inserted into the back of the animal's mouth to stimulate chewing. The pig was allowed to chew on the swab until it had become saturated (20-30 s). Saliva samples were immediately placed in storage tubes and frozen at -20ºC. We shipped samples on dry ice to Salimetrics LLC for analysis via enzyme immunoassay for sAA, cortisol, CgA, and testosterone. Saliva flow rate was not measured, as valid measurements of our analytes of interest can be obtained without the need for assessing flow rate (sAA: Rohleder et al., 2006; cortisol: Vining and McGinley, 1987; CgA: Escribano et al., 2013).

2.5. Personality tests

Three personality tests adapted from Janczak et al. (2003) and Spake et al. (2012) were performed 1-2 days before and 1-2 days after the object-location memory task: a solitary human approach test, a solitary novel object test, and a group novel object test. In

the solitary human approach test and novel object test, each pig was guided individually into a side pen in the test room. Depending on the test, a human or an object was positioned against the wall opposite the pen entrance prior to the pig's arrival. The amount of time the pig spent exploring the human or object during a 5 min period as well as the latency to approach the object were recorded. Individuals who did not make contact with the human or object were given an exploration time of 0 sec. In the human approach test, the test person wore rubber boots similar to those worn by animal care staff, but was unfamiliar to the pigs. The pen was hosed down following each test. The group novel object test took place in the main pen. An object was attached to a pole using a bungee cord and the pigs were allowed 20 min to explore the object. A knobby ball and a football were used as the novel objects in the solitary novel object tests, and a red truck and green tractor were used as the novel objects in the group novel object test (Appendix C). All tests were recorded using a Sanyo VPC-HD1010 (Sanyo Electric Co. Ltd., Osaka, Japan) handheld video camera at 60 fps.

2.6. Food competition test

After a hierarchy is established, pigs drastically reduce agonistic behavior, making it difficult to calculate a general 'aggressive order' (Langbein and Puppe, 2004). In stable groups in which overt agonistic interactions are rare, a common approach is to force agonistic encounters by restricting resource availability (e.g., food competition test), resulting in a 'competitive order'. Outcomes of social ranking fights during hierarchy formation and later food competition tests are highly correlated in pigs

(Hessing et al., 1994). Since both the male and female groups in our study had established stable hierarchies prior to the experiment, we determined the social rank of each individual by means of a food competition test, conducted 1-2 days before and 1-2 days after the object location memory task. Food was provided in a kennel bowl (21 cm L x 17 cm W x 6 cm H) attached to the chain-link of the middle side pen. The size of the bowl allowed only one animal to feed at a time. All pigs were familiarized with eating from the bowl prior to the tests. Each test lasted 70 min and was video recorded. Feed was replaced as needed. To ensure that the bowl was not monopolized by the dominant animals, different combinations of individuals were allowed to feed at a time, with the aim of maximizing encounters between all individuals. However, agonistic interactions were rare for less aggressive individuals or dyads with large discrepancies in rank, and so some unknown dyads were inevitable. We recorded the occurrence and outcome of all agonistic interactions in a dyadic interaction matrix with the rows labelled as wins and the columns as defeats (Appendix D). An agonistic interaction was defined as a fight or displacement initiated by one individual and followed by submissive behavior (i.e., turning away from an attack, fleeing, or displacement from the food bowl) displayed by the opponent (Langbein and Puppe, 2004).

3. Statistical analyses and results

All analyses were conducted using the statistical package R, version 3.0.2 (R Core Team 2013). High inter-observer reliability for all test measures was observed (see Appendix E for intraclass correlation coefficients).

3.1. Personality

3.1.1. Statistical analysis

Since we were interested in a general personality measure, we used principal components analysis (PCA) to reduce the number of variables measured in the personality tests (exploration duration in the first and second human approach test, novel object test, and group novel object test, and latency in the group novel object test). Since the variables measured have different ranges, we used the correlation matrix to calculate the principal components rather than the covariance matrix. Varimax rotation was performed on the components with eigenvalues greater than 1 in order to clarify the structure of the loadings matrix.

We examined the effects of litter origin and sex on the first two principal component scores using a two-way analysis of variance.

3.1.2. Results

PCA yielded three components with eigenvalues larger than 1 that explained a total of 71% of the overall behavioural variation (Table 1.1). The first component, PC1, which accounted for 36% of the variation explained, showed positive loadings exceeding 0.4 on four out of the six exploration duration measures. Therefore, increasing values of PC1 indicate more extensive exploratory behaviour. We subsequently refer to PC1 as a curiosity measure. The second principal component explained 21% of the variation and showed negative loadings exceeding 0.6 for duration spent exploring in the first novel

object test and the first group novel object test. Animals scoring high on PC2 can therefore be said to be more neophobic than animals with low scores. Thus, we chose to label PC2 as a measure of timidity. The third principal component was less readily interpretable, as it showed positive and negative loadings for latency to explore in the first and second group novel object tests, respectively. Thus, pigs who were slower to explore in the first group novel object test were quicker to approach the novel object in the second test. PC1 and PC2 were retained for subsequent analysis because they have clear behavioural meaning and together represent 57% of the variation.

Analyses of variance revealed a trend for males to score higher on curiosity than females ($F_{1,11} = 3.65$, $p = 0.08$; females (least squares mean (CI)): -0.22 (-1.21, 0.78); males (least squares mean (CI)): 0.28 (-0.76, 1.32)). Litter origin also marginally influenced curiosity ($F_{5,11} = 2.83$, $p = 0.07$). Neither sex nor litter origin affected timidity.

3.2. Social rank

3.2.1. Statistical analysis

We calculated sociometric indices with the R package *DyaDA* (Leiva et al., 2010). As males and females were not housed together, separate indices were computed for both sexes. Landau's improved linearity index *h'* (de Vries, 1995) measures the degree of linearity in a social hierarchy containing unknown or tied relationships. In order to assign each pig an individual rank position, linearity must be statistically significant. Since near-linear hierarchies for both sexes were found, we applied the I & SI method described in de Vries (1998) to rank individuals in an order that is most consistent with a

linear hierarchy. Given the high repeatability of social rank between the food competition tests (males: $r = 0.770$, $p = 0.003$; females: $r = 0.915$, $p < 0.0001$), data from both tests were combined and overall indices for the males and females were subsequently calculated.

One-way analyses of variance were used to examine the effect of litter origin on social rank separately for males and females.

3.2.2. Results

Table 1.2 presents the sociometric measures calculated for the male and female groups. A total of 149 agonistic interactions were observed over the two food competition tests for females. We observed fewer interactions for the males ($n = 62$). The improved Landau's index h' for males and females was 0.752 ($p = 0.01$) and 0.952 ($p = 0.0004$), indicating that males and females established semi-linear and near-linear hierarchies, respectively. Individuals were subsequently assigned ranks from 1 (most dominant) to 10 (most subordinate).

Litter origin significantly affected social rank in females ($F_{3,6} = 11.20$, $p = 0.007$), but not males ($F_{4,5} = 1.20$, $p = 0.42$).

3.3. Relationship between personality and social rank

3.3.1. Statistical analysis

We used Spearman's partial rank correlations to investigate the relationship between social rank with curiosity and timidity, controlling for sex.

3.3.2. Results

There was a marginal correlation between rank and curiosity (Spearman partial correlation, r = -0.39, p = 0.085). We found no correlation between rank and timidity.

3.4. Repeatability of stress hormones

3.4.1. Statistical analysis

Total hormonal output for sAA, CgA, and cortisol was expressed as the areas under the curve with respect to ground (AUC_G) according to Pruessner et al.'s (2003) formula. We divided the AUC_G of sAA by the AUC_G of cortisol to obtain an overall ratio variable of amylase over cortisol, named AOC_G, as a marker of stress system dysregulation (Ali and Preussner, 2012). 'Area under the curve with respect to increase (AUC_I)' was also calculated for sAA, cortisol, and CgA as it emphasizes the changes of the measurements over time (Preussner et al., 2003). Therefore, AUC_I can be interpreted as a measure of sensitivity of the system. AUC is a frequently used method to simplify statistical analyses by condensing repeated measurements over time into one variable. sAA AUC_G, CgA AUC_G, and cortisol AUC_G were positively skewed and so were log-transformed prior to analysis. We removed one outlier from the cortisol data due to its improbably high value (>4.0 ug/dL).

In order to determine if consistent inter-individual differences in stress reactivity exist we calculated repeatabilities for the individual log-transformed measurements of sAA, CgA, and cortisol, as well as for AUC_G and AUC_I of sAA, AUC_G and AUC_I of

cortisol, AUC_G and AUC_I of CgA, and AOC_G. Repeatabilities were analyzed with linear mixed models, using the restricted maximum-likelihood method and pig identity fitted as the random factor. We controlled for stressor as a confounding factor by including it as a fixed effect in each model (Nakagawa and Schielzeth, 2010). Repeatabilities for the individual samples were adjusted for stressor and sampling time (0, 10, and 35 min after stressor onset). Repeatability of female log-transformed testosterone levels taken at the third sampling time was also assessed, controlling for stressor. Male testosterone levels were not retained for analysis as the values were greater than 5x the highest standard (600pg/mL) even after dilution. 95% confidence intervals for the repeatabilities were estimated by parametric bootstrapping with 1000 permutations using the function rpt.remlLMM.adj of the *rptR* package (Nakagawa and Schielzeth, 2010).

3.4.2. Results

Repeatabilities for hormonal variables for males and females are presented in Table 1.3. Males had consistently higher repeatability estimates than the females. Individual CgA measurements were highly repeatable in males. Males also showed consistency in individual cortisol measurements. All other repeatabilities were not considered significant as their 95% confidence intervals included 0.

3.5. Relations between social rank, personality, and stress reactivity

3.5.1. Statistical analysis

The effects of individual characteristics on hormonal profiles were assessed with eight linear mixed models. The response variables were sAA AUC_G and AUC_I, CgA AUC_G and AUC_I, cortisol AUC_G and AUC_I, AOC_G and testosterone. We included litter, sex, rank, curiosity, timidity, and stressor as fixed effects. The following two-way interactions were also included: rank and stressor, curiosity and stressor, timidity and stressor, sex and stressor, rank and sex, curiosity and sex, and timidity and sex. Sex was excluded from the testosterone model, as only female samples were analyzed. Pig identity was fitted as a random effect in all the models. The input variables were standardized following Gelman's (2008) approach prior to analysis to allow comparison of the parameter estimates (Grueber et al., 2011). sAA AUC_G, CgA AUC_G, cortisol AUC_G, AOC_G and testosterone were positively skewed and so were log-transformed prior to analysis. Inspection of residual vs. fits plots confirmed that all models had homogeneous variances. We used an information-theoretical approach based on Akaike's information criterion corrected for small sample size (AIC_C) to rank all possible subsets of the full model and weigh the relative support for each one (Burnham and Anderson, 2002).

Due to high model uncertainty in all the models, we used model averaging to obtain reliable parameter estimates. Model averaged parameter estimates were calculated from the 95% confidence set of models that included all models whose cumulative Akaike weights summed to 0.95 (Burnham and Anderson, 2002). Since each parameter does not appear an equal number of times in the model, the model set was averaged using the natural average method (Burnham and Anderson, 2002).

Linear mixed models were fitted using the R package *nlme* (Pinheiro et al., 2013). Information-theoretic model selection was carried out with the R package *MuMIn* (Barton, 2013).

3.5.2. Results

The linear mixed models revealed several factors that were important predictors of five of the eight hormonal measures. These effects are described below. All other factors did not predict hormonal profiles.

The ratio of amylase over cortisol (AOC_G) differed between the sexes, with females scoring higher than males (Sex: std. est. (CI) = -0.37 (-0.57, -0.17)).

Sex predicted cortisol AUC_G, with males scoring higher than females (Sex: std. est. (CI) = 0.24 (0.14, 0.34)). The interaction between timidity and sex was also important (Timidity*Sex: std. est. (CI) = -0.21 (-0.42, -0.008)). Bolder males tended to have a higher total cortisol output than shyer males, while the opposite was true for females (Fig. 1.1).

The interaction between sex and stressor was an important predictor of cortisol AUC_I (Sex* Stressor: std. est. (CI) = 5.29 (0.63, 9.95)). Males experienced an increase in cortisol AUC_I in the stressed condition relative to the non-stressed condition, while females showed no change across conditions (Fig. 1.2).

The interaction between sex and stressor also predicted CgA AUC_I (Sex*Stressor: std. est. (CI) = -249.9, (-493.8, -5.96)). However, the pattern was opposite that which was

found for cortisol AUC_I. Females had higher AUC_I values in the stressed condition than when in the non-stressed condition, whereas males showed no change in relation to condition (Fig. 1.3).

In order to explore the sex differences in cortisol AUC_I and CgA AUC_I further, we conducted a post-hoc correlation analysis on the two variables for males and females separately. Cortisol AUC_I and CgA AUC_I were strongly correlated in the males (Spearman's rho = 0.60, p = 0.005), whereas there was a marginal correlation in the females (Spearman's rho = 0.42, p = 0.07).

We found a significant rank by stressor interaction for CgA AUC_I (Rank*Stressor: std. est. (CI) = 334.4 (84.0, 584.8)). CgA tended to decline more with respect to baseline with decreasing social status in the non-stressed condition, while the opposite was true in the stressed condition, with high ranking individuals displaying lower AUC_I than more subordinate individuals (Fig. 1.4).

Rank predicted sAA AUC_G (standardized estimate (lower CI, upper CI) = 0.28 (0.01, 0.53)). Higher ranking animals had lower sAA ouput than more subordinate animals.

4. Discussion

4.1. Consistent inter-individual variation in behavioural traits

The PCA suggests two personality traits explain 57% of the variation seen in the behavioural tests. The pigs showed consistent performance across four of the tests: the

first and second human approach tests, and the second novel object test and group novel object test. In other words, an individual who explored a lot in one test performed similarly in a different test. We interpreted this trait as a measure of an individual's curiosity. Interestingly, the pig's performance in these tests did not predict how much he or she explored in the first novel object test and group novel object test. Rather, the amount explored in the first novel object test and group novel object test reflected a second trait, which we tentatively labelled timidity, as exploration decreased with increasing scores. While we had predicted similar within-test responses over time, the novelty of the situation may have been greatly reduced during the second round of testing, effectively altering the trait that the second tests were measuring (see Biro, 2012, Biro, 2013, and Edwards et al., 2013 for a debate on this issue). Prior to the first personality tests, the pigs experienced very little novelty in their environment. Following the first personality tests, they participated in cognitive testing, which exposed them to several novel objects, after which they underwent the second round of personality testing. At this point, they may have habituated to novelty in their environment, and could express their curiosity that was previously inhibited by their emotional state in the first test. In support of this interpretation, a study by Wemelsfelder and colleagues (2000) showed that pigs housed in impoverished conditions were more fearful of approaching a novel object than pigs in enriched conditions. Thus, the cognitive tests may have acted as a form of enrichment, reducing timidity and stimulating curiosity. This effect was not seen in the human approach tests, possibly because the pigs experienced daily human interaction and so the situation was not unfamiliar to them. The same finding was

reported by Brown et al. (2009), with pigs performing consistently in the human approach test over time, but not in the novel object test.

Several studies have examined the existence of personalities in pigs. A PCA conducted by Forkman et al. (1995) on several behavioural measures revealed three personality factors: aggression, sociability, and exploration. While we did not consider aggressive or social variables in our PCA, the curiosity trait that emerged is consistent with the Forkman et al. (1995) exploration trait. Studies which investigated within-test repeatability in the human approach test and novel object test have variously reported consistency in both tests (Janczak et al., 2003; Spoolder et al., 1996), only the human approach test (Brown et al., 2009), or only the novel object test (Van Erp-van der Kooij et al., 2002). Recently, Spake et al. (2012) found that the latency to explore an object and the time spent exploring an object were repeatable between novel object tests. Such discrepancies between studies are likely due to variations in test protocol. However, taken together, the findings confirm the existence of a personality trait reflecting exploratory drive in pigs.

Males tended to be more curious than females, and siblings tended to be more similar to each other in curiosity scores than to non-siblings. Curious individuals also tended to occupy higher ranks in the hierarchy than those who explored less. A similar pattern has been found in zebra finches (David et al., 2011) and fish (Colléter and Brown, 2011; Sundstrom et al., 2004). To date, studies that have examined personality and social rank in pigs have typically reported no relationship (Bolhuis et al., 2005; Brown et al.,

2009; Forkman et al., 1995; Ruis et al., 2002). However, personality traits equivalent to curiosity are positively related to food intake and growth rate in a diverse array of species (reviewed in Biro and Stamps, 2008), and, as a consequence, curious individuals are more likely to be successful in social disputes. In accordance with this interpretation, dominant pigs in our study were visibly larger in body size than subordinate animals, although weights were not obtained.

Timidity was not affected by sex or litter origin, nor did we find a correlation between timidity and rank. There was a trend for females to show an increase in timidity with increasing cortisol output, whereas the opposite pattern was observed in the males. While intriguing, this result should be considered exploratory in nature and further tests are required to replicate these findings.

4.2. Litter origin influences social rank in females only

We found a semi-linear hierarchy in the male group and a near-linear hierarchy in the female group. While the linearity index for the females is higher than those reported in the literature, the linearity index for the males is in accordance with Fels et al. (2012) and Puppe et al. (2008) who both described semi-linear hierarchies in groups of 10 and 12 pigs.

A litter-dependent dominance hierarchy was present in the female, but not the male group. Females were closer in social rank to their sisters than to a non-related group mate, whereas this effect was not seen among brothers. Fels et al. (2012) also detected a relationship between litter and social dominance in piglets, which they suggested was due

to cooperation between siblings. While they focused on mixed-sex groups, the pigs in the current study were sexually mature and so were housed separately by sex. An explanation for the sex-dependent litter effect may lie in the natural social structure of the pig. In feral populations, pigs live in family units that typically comprise a few mature sows and their offspring from that year. Males leave the group as sub-adults and live alone outside the mating season, while females remain with their dam's group or join an adjacent group (D'Eath and Turner, 2009). Thus, cooperation may be more likely to have evolved between sisters who live together, than among brothers who are adapted to lead a solitary lifestyle.

4.3. Males show greater consistency in hormone levels than females

Cortisol and CgA samples were repeatable within individual males but there was no apparent temporal consistency in all other hormonal response measures. However, repeatability estimates were consistently higher in the males than in the females. One other study has reported a sex difference in repeatability of hormone levels, although it revealed a pattern opposite to ours. Wada et al. (2008) found that the adrenocortical response was repeatable in female, but not male, zebra finches.

The lower repeatability that we found in the females may result from the modulation of the stress response across the estrous cycle. It is well established that estrogen influences stress-related physiology (Kajantie and Phillips, 2006; Ter Horst et al., 2009). In most contexts, estrogen attenuates HPA and sympathetic responsiveness (Kajantie and Phillips, 2006). The estrous cycle length in pigs averages 21 days, with

estrogen levels fluctuating across the four stages of the cycle (Henricks et al., 1972). Since samples were collected over a period of 8 days, cyclic changes in sex hormones likely increased the within-individual variation in female stress hormone levels, thereby reducing repeatability estimates for females. Receptiveness, or standing heat, was not recorded in the current study, so no attempts could be made to approximate the timing of the estrous cycle. The effects of estrogen on repeatability of the stress response should be further explored.

4.4. Sex differences in HPA axis and sympathetic responses

Perhaps our most interesting finding was the differential stress system activity between the sexes. We suggest that the sex differences we found collectively indicate a dysregulation of the stress systems in the female pigs. Most notably, the ratio of amylase over cortisol, which has been proposed as a marker of stress systems dysregulation (Ali and Pruessner, 2012), was much higher in the females than in the males. Increasing evidence suggests that chronic stress manifests as an asymmetry between HPA axis and sympathetic nervous system activity (Schommer et al., 2003; Gordis et al., 2008; Vigil et al., 2010).

The asymmetry in sAA and cortisol responses among the females in our study was due to an attenuated response in the HPA axis and heightened sympathetic activity. Females showed a lower overall cortisol output than the males, as measured by cortisol AUC_G. Females also demonstrated a decreased responsivity of the HPA axis relative to the males, as a change in cortisol in response to the stressor was seen only in the male

subjects. In contrast, the change in CgA from baseline was affected by the stressor in the females but not the males, suggesting elevated sensitivity of the sympathetic nervous system in females. Furthermore, changes in CgA were highly consistent with changes in cortisol in the males, while only a weak correlation was present in the females. This finding indicates that the sensitivity of both systems is more similar in animals with symmetrical sAA and cortisol output than in those without.

The nature of the relationship between the stress systems remains a topic of investigation. Bauer et al. (2002) proposed that the HPA axis and sympathetic nervous system perform complementary actions in the stress response. This interactive model is based on the hypothesis advanced by Munck and colleagues (1984), suggesting that glucocorticoids function to supress the initial activity of the sympathetic nervous system. Sapolsky and colleagues (2000) summarized research indicating that the actions of stress-induced glucocorticoid concentrations are predominantly suppressive in nature. A dissociation between the HPA and sympathetic nervous systems would therefore be problematic if optimal functioning requires the stress systems to act in coordination (Bauer et al., 2002). According to this model, activation asymmetries are associated with dysregulation of the stress systems and thus may act as a marker of chronic stress (Ali and Pruessner, 2012).

We are not sure why the female pigs in our study appear to have endured greater chronic stress than the males. Individuals of both sexes experienced nearly identical treatment since birth, were similar ages at the time of testing, and were housed with

conspecifics. It is possible that, similar to humans, the female brain in pigs is more susceptible to stress and stress-related disorders. Mounting evidence suggests that there is a biological basis for the increased incidence of stress-related psychiatric disorders in women (Bangasser and Valentino, 2012; Kudielka and Kirschbaum, 2005). Sexual dimorphisms in brain structures, circulating sex hormones, and corticotropin-releasing factor expression and function may predispose females to increased stress sensitivity (Bangasser and Valentino, 2012; Kudielka and Kirschbaum, 2005). Given the high anatomical and physiological similarity between humans and pigs (Lind et al., 2007), sexual dimorphisms in the stress response may also exist in pigs and place females at increased risk of developing chronic stress.

Our findings complement other pig studies that have found higher basal cortisol levels in barrows (castrated males) and boars (intact males) than in gilts (females who have not yet been bred) (Marple et al., 1974; Ruis et al., 1997). Moreover, following isolation stress, the amplitude of the circadian rhythm of salivary cortisol was increased in barrows, but remained unchanged in gilts (Ruis et al., 1997). The authors attributed this increase to a sensitized HPA axis in males; however, in light of our findings, we suggest that it could alternatively reflect a blunted cortisol response in the females as a result of chronic stress. In support of this interpretation, chronic stress in pigs has been shown to lead to suppressed plasma cortisol levels (Sutherland et al., 2006) and a flattened rhythm of plasma cortisol (Janssens et al., 1995). We are not aware of any studies other than ours that have investigated sex differences in sympathetic activity in pigs.

4.5. Rank differences in sympathetic activity are context-dependent

In the non-stressed condition, subordinates showed a greater decline in CgA with respect to baseline than did dominant individuals, whereas the pattern was reversed in the stressed condition, with high ranking animals tending to display a more negative AUC_I than their subordinates. The less negative change in CgA from baseline seen in the non-stressed dominants suggests that they experienced greater sympathetic sensitivity to the testing situation than the subordinates. This elevated sensitivity of the sympathetic nervous system may reflect the lack of control the dominant animal experienced as a result of separation from the group while undergoing testing. While isolated from their conspecifics, the dominant individual was unable to actively subjugate subordinates and thereby maintain their status. Conversely, the more negative change in CgA in the non-stressed subordinates may be in response to the opportunity to evade harassment by dominant individuals during the test. In the stressed condition the rank effect is erased, possibly because the subordinate individuals may react disproportionately more to the stressor than their dominant counterparts.

Interestingly, total sympathetic output, as measured by sAA AUC_G, was higher in subordinate compared to dominant pigs, suggesting that the lower ranks were more aroused overall during testing. This result differs from another pig study that found that plasma norepinephrine and epinephrine levels did not vary between dominants and subordinates (Fernandez et al., 1994).

5. Conclusions

In summary, our experiments showed: (1) pig personality varied along, at least, two dimensions; (2) a litter-associated dominance hierarchy was present in females, but not males; (3) hormone levels were more repeatable in males than in females; (4) females were more susceptible to chronic stress than males; and (5) social rank influenced sympathetic activity in a context-dependent manner.

We found two independent personality traits in pigs, which we labelled curiosity and timidity. The PCA results indicated that the initial novel object test and subsequent novel object test measured different traits. Thus, we recommend that care be taken when interpreting results of novel object tests, and other personality tests, as they may assay unintended traits. PCA is useful in this regard as it identifies unrelated traits captured by experimental assessments, thereby reducing confusion about over- or under-labelling traits (Carter et al., 2012).

This is one of the few studies to show that some components of the stress response are repeatable in a mammalian species. It is also the first time, to our knowledge, that a sympathetic marker has been shown to be repeatable within individuals. The results of the present study suggest that researchers should apply caution when making the assumption that a hormonal sample taken on one occasion is representative of the phenotype of the sampled individual. Our findings also emphasize the importance of considering sex effects when investigating the repeatability of stress hormones in mammals, as variability may be higher in females than in males due to cyclical reproductive hormones.

Our study is the first to have investigated sex differences in both HPA axis and sympathetic activation in pigs. Our results highlight the importance of evaluating both stress systems as it permits a more refined interpretation of physiological stress responses. A consideration of the interactions between these two systems can reveal far more about an individual's physiological state than an examination of each system in isolation.

The pervasiveness of sex effects throughout our study underscores the necessity of considering both sexes in behavioural and physiological studies. It has long been assumed that results from males generalize to females, with the consequence that females are neglected in both human and non-human biological research (Beery and Zucker, 2011). Animal and human studies have demonstrated that males and females react differently to stress – both physiologically and behaviourally. Dismissal of these well-documented sex differences and continued underrepresentation of female models in biological disciplines compromises our understanding of female biology and has profound implications for healthcare in women. We therefore encourage researchers to fully incorporate both sexes in their study and to analyze results by sex as this will undoubtedly increase the quality and impact of their work.

Table 1.1

Varimax rotated loadings for the first three components (n = 20), as well as their Eigenvalues and the percentage of the total variation they explain. HAT = human approach test, NOT = novel object test, GNOT = group novel object test.

	PC1	PC2	PC3
Duration exploring in HAT 1	0.44		-0.21
Duration exploring in HAT 2	0.51		0.12
Duration exploring in NOT 1		-0.63	
Duration exploring in NOT 2	0.53		
Duration exploring in GNOT 1		-0.63	
Duration exploring in GNOT 2	0.52		
Latency to explore in GNOT 1		0.18	0.82
Latency to explore in GNOT 2		0.41	-0.51
Eigenvalue	2.87	1.69	1.14
Percentage of variance	35.9	21.2	14.2

Table 1.2

Dominance hierarchy statistics for male and female groups based on the combined outcomes of two food competition tests.

	Males	Females
Number of dyads	45	45
Agonistic interactions	62	149
Unknown dyads (%)	33.3	17.8
One-way dyads (%)	60.0	71.1
Two-way dyads (%)	6.7	11.1
Landau index, *h'*	0.752 (p = 0.01)	0.952 (p = 0.0004)

Table 1.3

Repeatabilities of hormonal measurements. All repeatabilities have been adjusted for stress condition. Repeatabilities for individual samples were also adjusted for sampling time (0, 10, 35 min after stressor onset). The number of individuals (n(i)) and the number of measurements (n(m)) are indicated.

	Females			Males		
	r	CI	n(i), n(m)	r	CI	n(i), n(m)
sAA						
Individual samples	0	0, 0.18	10, 60	0.08	0, 0.21	10, 120
AUC_G	0	(0*)	10, 20	0.17	0, 0.47	10, 40
AUC_I	0.08	0, 0.68	10, 20	0.14	0, 0.48	10, 40
CgA						
Individual samples	0	0, 0.19	10, 60	0.51	0.27, 0.70	10, 60
AUC_G	0	(0*)	10, 20	0.48	0, 0.84	10, 20
AUC_I	0.21	0, 0.72	10, 20	0.36	0, 0.79	10, 20
Cortisol						
Individual samples	0.17	0, 0.35	10, 120	0.20	0.04, 0.36	10, 119
AUC_G	0.25	0, 0.55	10, 40	0.30	0, 0.57	10, 39
AUC_I	0	0, 0.27	10, 40	0.15	0, 0.46	10, 39
Testosterone						
35 min	0	(0*)	10, 20	-	-	-
sAA:Cortisol						
(AOC_G)	0	(0*)	10, 20	0.16	0, 0.50	10, 39

*the point estimate for adjusted *r* was exactly zero and no confidence interval was calculated.

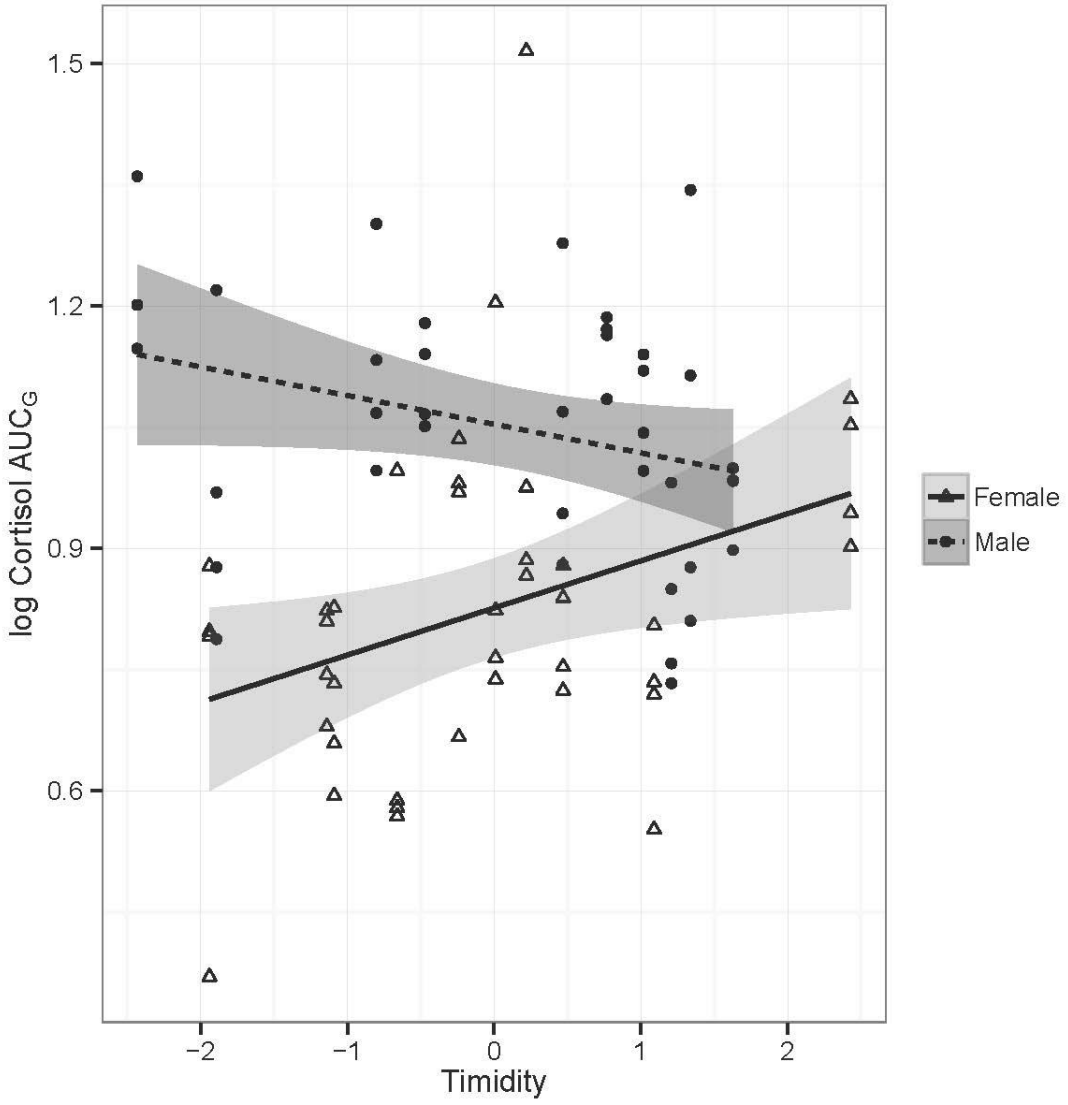

Figure 1.1. Log-transformed cortisol AUC_G as a function of an individual's timidity level. The slopes for males and females differed significantly from one another. The shaded regions indicate the 95% confidence intervals.

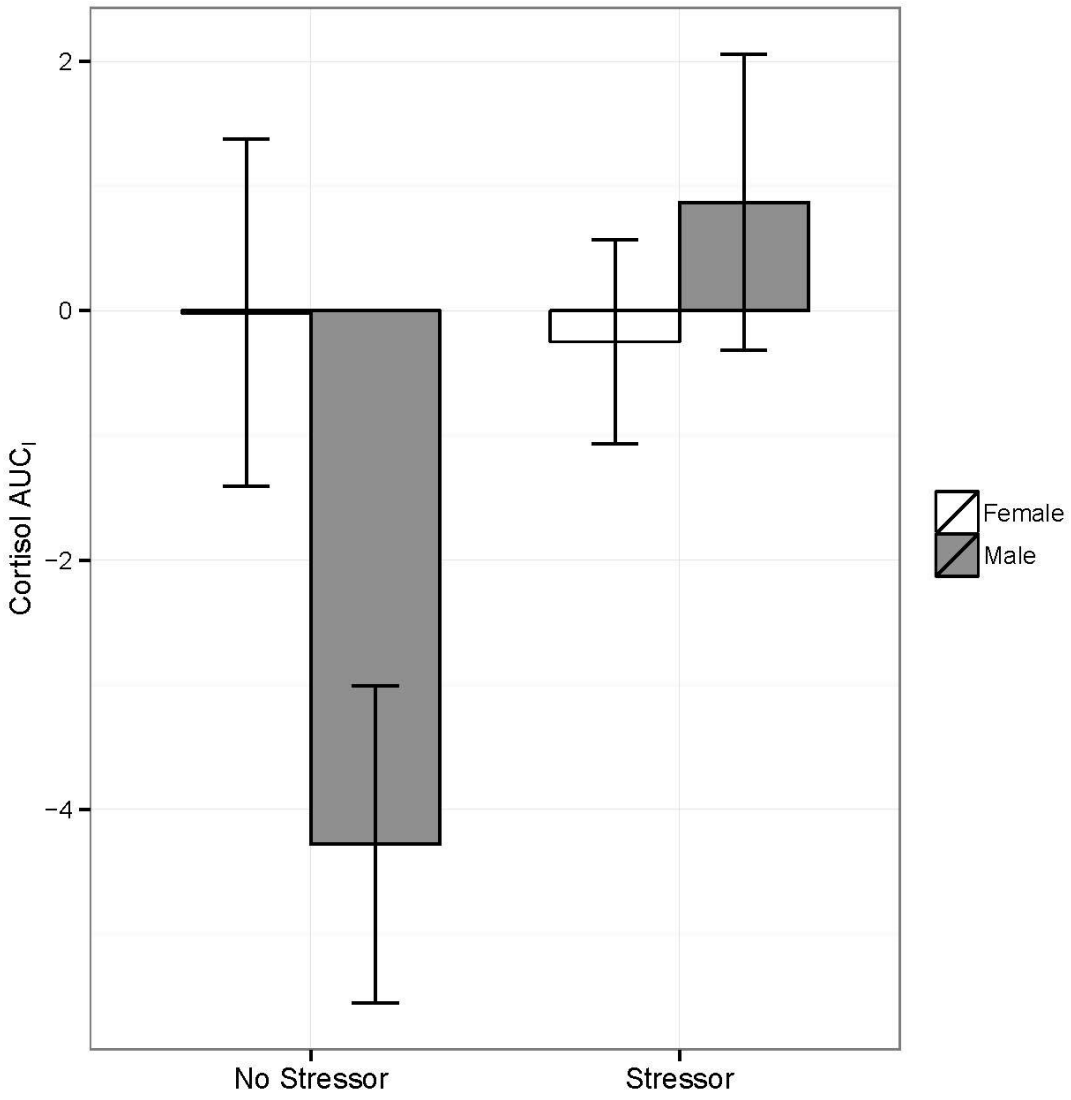

Figure 1.2. Cortisol AUC_I (mean ± standard error of the mean) for the stressed and non-stressed conditions. Stress caused an increase in males' cortisol levels with respect to baseline, while it did not affect females' cortisol levels in relation to baseline.

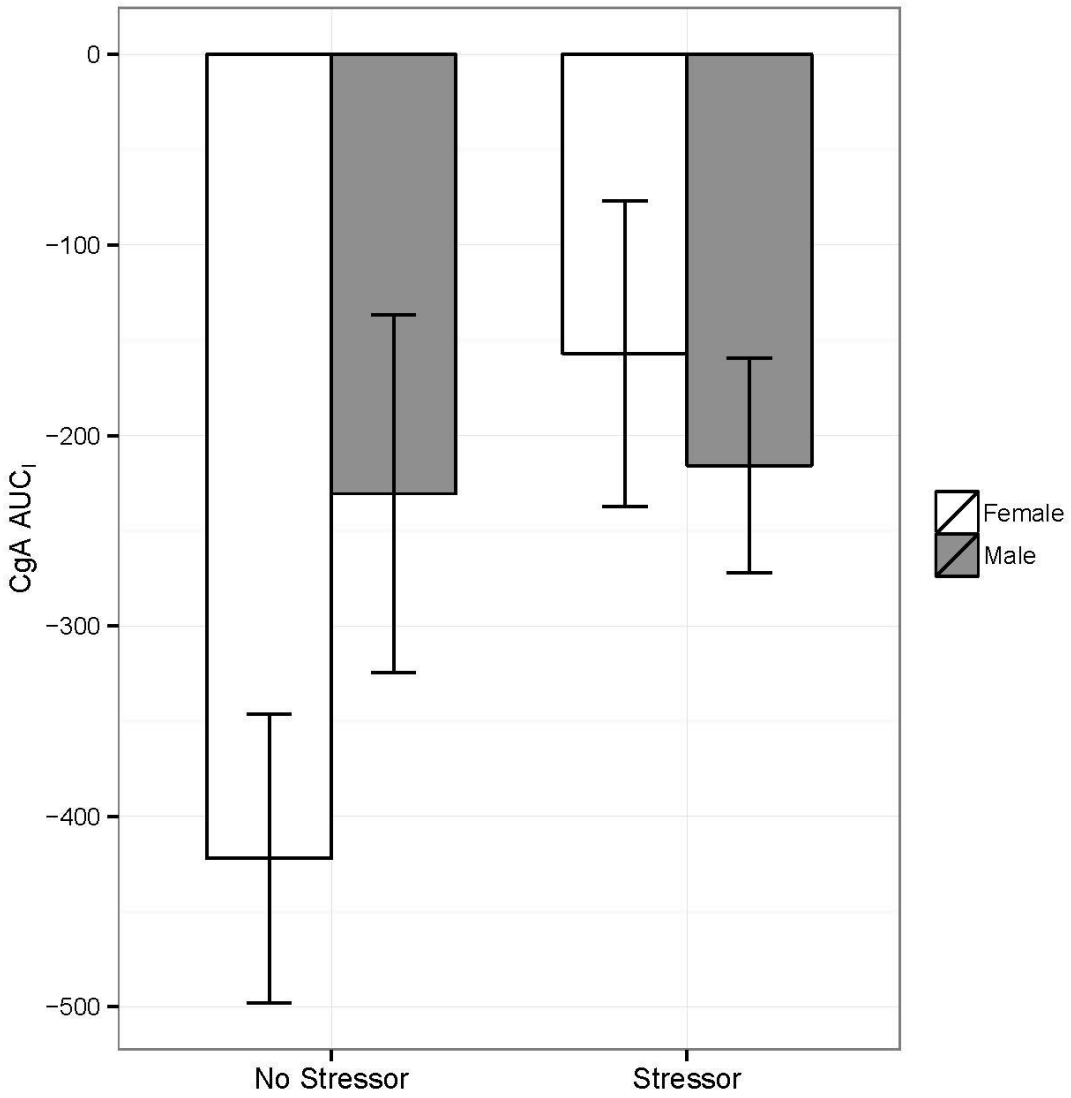

Figure 1.3. CgA AUC_I (mean ± standard error of the mean) for the stressed and non-stressed conditions. Stress caused a less negative decrease in females' CgA levels from baseline, while it did not affect males' CgA levels in relation to baseline.

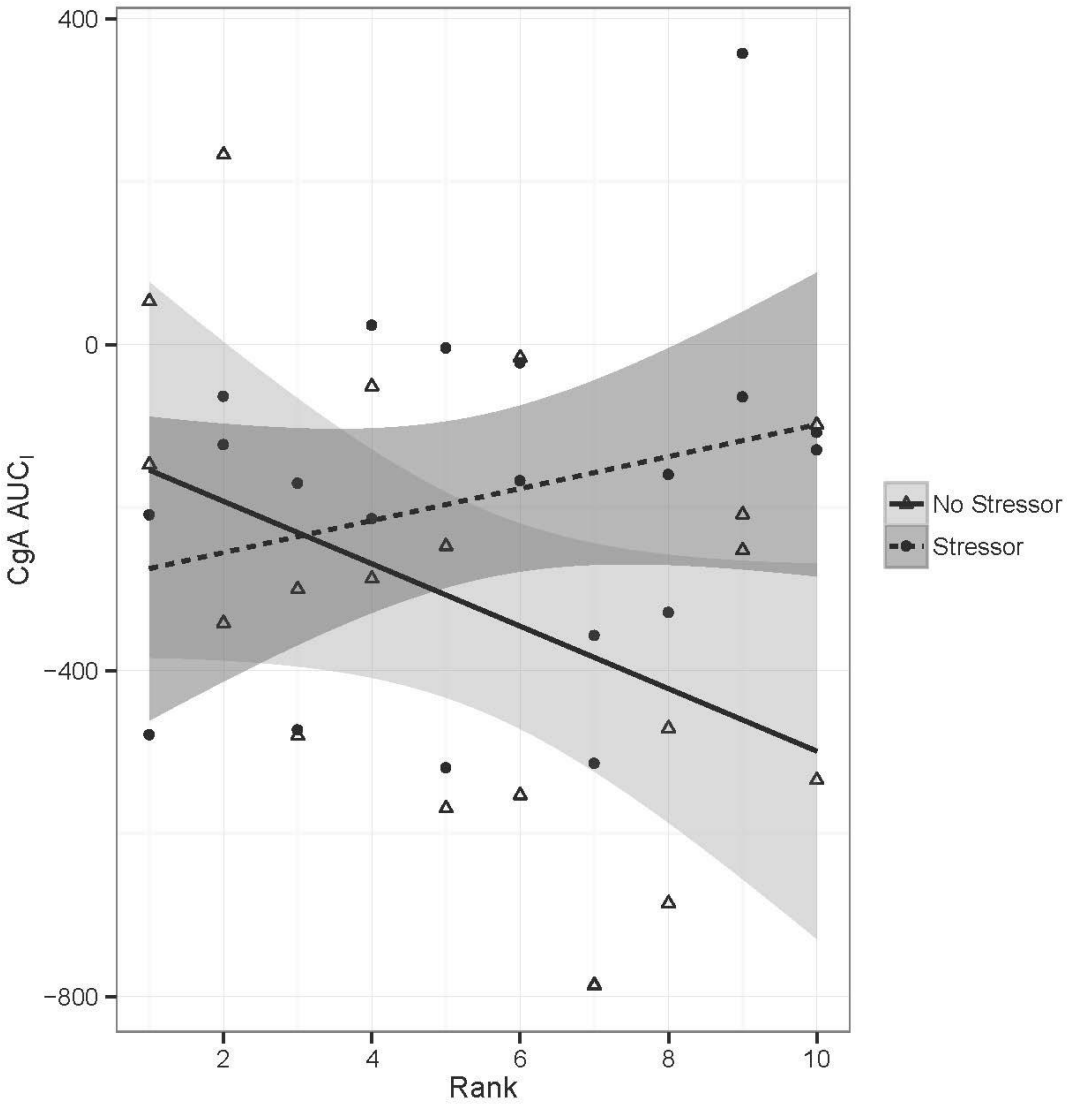

Figure 1.4. CgA AUC_I as a function of an individual's social rank (1 = most dominant, 10 = most subordinate). The slopes in the stressed and non-stressed conditions differed significantly from one another. The shaded regions indicate the 95% confidence intervals.

MANUSCRIPT 2

The effects of stress and individual differences on object location memory in Yucatan minipigs (*Sus scrofa*)

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Note: Manuscript 2 describes a parallel study conducted on the same pigs studied in Manuscript 1. The dependent variables that were the focus of Manuscript 1 (i.e., personality scores, social rank, and stress biomarkers) are considered as the predictor variables in Manuscript 2. To avoid having the reader flip back and forth between manuscripts, the methods and statistical analysis presented for personality, social rank, and the stress biomarkers in Manuscript 1 are repeated in Manuscript 2.

Abstract

Stress has selective effects on memories, enhancing some, while impairing others. The theory of arousal-biased competition states that arousal/stress will enhance perception and memory for high priority stimuli and impair it for low priority stimuli. In addition to the stress response, cognitive performance can be influenced by personality, and, in gregarious species, social rank. Using 20 Yucatan minipigs (*Sus scrofa*), we: (1) determined the effect of acute stress on memory for the locations of high and low priority items in an object location memory task; (2) objectively assessed stress levels using salivary biomarkers that include cortisol, alpha-amylase, and chromogranin A; and (3) examined whether cognitive performance is influenced by individual differences in personality and social rank. Our results indicate: (1) pigs can recall "what" object they saw "where", (2) enhanced memory for the location of high priority objects vs. that of low priority objects is independent of stressor presence; (3) stress negatively impacts object location memory in the males, but not the females; and (4) timidity may influence cognitive performance in a sex-dependent manner.

Keywords: Domestic pig (Sus scrofa), object location memory, stress, arousal biased competition, personality, social rank, sex, salivary alpha-amylase, cortisol, chromogranin A

1. Introduction

Studies report both enhancing and disruptive effects of stress on memory function. A recent model provides specific predictions about when arousal/stress will enhance or impair memory for particular information from an event (Mather and Sutherland, 2011). The theory of arousal-biased competition (ABC) holds that arousal will enhance perception and memory for high priority stimuli and impair perception and memory for low priority stimuli. Priority can be determined independently by bottom-up sensory influences (i.e., perceptual salience) or top-down cognitive factors (i.e., goal relevance), or by interactions between the two. Stimuli that contrast with their surroundings and/or that are relevant to current goals have priority over low contrast or irrelevant stimuli.

ABC theory builds on the notion that objects in the visual field compete for mental representation. A perceptually salient or goal-relevant object will dominate the competition at the expense of weaker representation of others (Beck and Kastner, 2009). According to ABC theory, arousal amplifies these existing biases in competition. Studies indicate that arousal affects both the initial perception and encoding of information as well as memory consolidation in ways consistent with the predictions of ABC theory. A stressor administered immediately before or during a task enhances the processing of goal-relevant or perceptually salient stimuli, even if the stimuli are not themselves arousal inducing (Chajut and Algom, 2003; Lee et al., 2014). Arousal induced shortly after encoding facilitates memory consolidation of goal-relevant information, even when

that information is not inherently emotional (Andreano and Cahill, 2006; Roozendaal et al., 2008).

In response to a perceived threat to bodily homeostasis (stressor), the brain initiates a number of physiological responses to cope with the stressor. In particular, stress activates the sympathetic nervous system and hypothalamus-pituitary-adrenal (HPA) axis, which respond by stimulating secretion of catecholamines (epinephrine and norepinephrine) and glucocorticoids (corticosterone in amphibians, reptiles, rodents and birds; cortisol in fish and most mammals), respectively (Romero and Butler, 2007). Catecholamines are secreted within seconds of the stressor onset and will induce rapid, transient changes in neuronal activity. Over the course of minutes, glucocorticoids are secreted and exert rapid, non-genomic actions as well as slow genomic changes in cellular excitability. Catecholamines and glucocorticoids are known to affect hippocampal-dependent memory processes by influences on limbic brain structures (Roozendaal et al., 2006, 2009). It is well established that catecholamines enhance consolidation of new memories in a dose-dependent manner, even when the information to be remembered is not itself arousal inducing (Cahill and Alkire, 2003; McIntyre et al., 2002; Segal and Cahill, 2009; Segal et al., 2012). Glucocorticoid effects on memory depend largely on the different memory phases investigated. Glucocorticoids typically facilitate memory consolidation in a dose-dependent manner, while impairing retrieval processes (Roozendaal, 2002).

Salivary biomarkers of the sympathetic nervous system and HPA axis provide a non-invasive, stress-free way of evaluating the stress response. Salivary alpha-amylase

(sAA), a starch-digesting enzyme, is secreted by acinar cells in the salivary glands and is well-established as a sensitive biomarker for endogenous norepinephrine activity in humans and other primates (Granger et al., 2007; Nater and Rohleder, 2009). More recently, chromogranin A (CgA) has been proposed as a reliable index of sympathetic activity. The release of CgA from the salivary gland is mediated by the secretion of catecholamines, and previous studies have reported elevations in salivary CgA in response to psychological stressors (Nakane et al., 1998, 2002; Tanaka et al., 2010). Thus, sAA and CgA may serve as useful tools for monitoring the activity of the sympathetic nervous system. To assess the HPA axis response, salivary cortisol is a widely used and reliable surrogate for unbound free cortisol levels in plasma and serum (Hellhammer et al., 2009).

In addition to the physiological stress response, individual behavioural characteristics influence cognitive performance (reviewed in: Carere and Locurto, 2011; Sih and Del Giudice, 2012; Thornton and Lukas, 2012). While individual variation has received much attention in human psychology, animal cognition studies focus largely on species-level cognitive capacity, dismissing individual differences as noise around an adaptive mean (Thornton and Lukas, 2012). However, this 'cognitive capacity' perspective overlooks the fascinating possibility that variation in learning and memory is related to personality differences. Personality is thought to influence cognition at three different stages of the learning process: (1) encountering a new situation; (2) assessing changes in the environment; and (3) altering behaviour in response to processed new information (Sih and Del Giudice, 2012). Bolder, more explorative personality types are

thought to encounter new stimuli more quickly and, consequently, should be faster at learning a cognitive task, but are less sensitive to environmental changes, and therefore should perform more poorly than their shyer, less explorative counterparts in tasks that require adjusting their behaviour to the changing environment. A recent study by Titulaer and colleagues (2012) suggests a sex-dependent relationship between personality and cognitive performance. They found that, in great tits, performance on a reversal learning task improved with increasing exploratory behaviour in males, whereas females showed the opposite pattern with the slow-explorers outperforming the fast-explorers.

Social rank is another biologically important axis of behavioural variation, as dominance hierarchies occur in a range of species, with the dominant individuals in a group controlling access to preferred resources. An individual's position in the hierarchy may either facilitate or inhibit behaviours, such as cognitive performance. Humphrey's (1976) "social intelligence hypothesis" proposed that an animal's social success depends on his or her cognitive ability. Individuals that are superior at calculating the consequences of their own and others' behaviour are more likely to acquire a high rank. An individual's aptitude in the social intelligence domain may generalize to broader cognitive domains, such as learning and memory. In male starlings, for example, high-ranking individuals mastered a foraging task faster than low-ranking conspecifics (Boogert et al., 2006). Dominant chickadees also performed significantly better than subordinates on a spatial memory task (Pravosudov et al., 2003).

Pigs (*Sus scrofa*) have been increasingly recognized as an ideal model for studying learning and memory (reviewed in: Gieling et al., 2011; Held et al., 2002).

These gregarious, inquisitive animals demonstrate well-developed cognitive abilities (Broom et al., 2009; Kouwenberg et al., 2009), as well as sophisticated social behaviour (Held et al., 2010; McLeman et al., 2008). Moreover, pigs are an excellent species for studies of individual differences in cognition, as they show high inter-individual variation in behavioural responses to a task (Forkman et al., 1995; Janczak et al., 2003; Spoolder et al., 1996). From a comparative perspective, pigs share many physiological similarities with humans. In particular, the anatomy, growth and development of the pig brain resemble that of the human brain more closely than do the brains of rodents and other small laboratory animals (Lind et al., 2007). Such parallels between their species and ours make pigs a promising model for investigating learning and memory. In addition, salivary cortisol has been used extensively as a HPA axis marker in pigs (Escribano et al., 2012; Geverink et al., 2002; Merlot et al., 2011) and, recently, pigs have been shown to produce measurable increases in sAA (Fuentes et al., 2011) and salivary CgA (Escribano et al., 2013) in response to a stressor.

In this study we used an object location memory task to assess hippocampal-dependent memory in Yucatan miniature pigs. The object location memory task is a one-trial, reinforce-free animal model that was first developed in rats (Ennaceur et al., 1997) and is critically dependent on hippocampal structures (Barker and Warburton, 2011; Lee et al., 2005). This task takes advantage of the pig's innate tendency to preferentially explore novel situations (Kouwenberg et al., 2009; Moustgaard et al., 2002; Wood-Gush and Vestergaard, 1991). In the task, animals are presented with two identical, familiar objects, one of which is in its previous location while the other is in a new location.

Animals who remember the previous location of the object spend more time investigating the object in the novel location. In the present study, we modified the paradigm so that we could evaluate how an object's relative priority affects the pig's ability to remember its previous location.

The aims of this study were to: (1) test the theory of arousal-biased competition by investigating the effect of acute stress on memory for the location of high and low priority items; (2) objectively assess stress levels using salivary biomarkers that include cortisol, alpha-amylase, and chromogranin A; and (3) examine whether cognitive performance is influenced by individual differences in personality and social rank.

2. Methods

2.1. Subjects

The subjects were twenty Yucatan miniature pigs (10 males, 10 females) born from six litters between September 23 and October 7, 2012 at the Memorial University of Newfoundland pig breeding facility (see Appendix A for birth records of subjects). Within the first two weeks after birth, the pigs were injected with iron and had their pin teeth clipped. Around two months of age, the pigs were ear-tagged, injected with *Erysipelothrix rhusiopathia* vaccine, and dewormed. Males in this study were not castrated. All procedures and daily husbandry practices were carried out according to guidelines set out by the Canadian Council of Animal Care. Male pigs were 28 weeks of age, and females 32-34 weeks of age, at the start of our experiment.

2.2. Housing

Prior to the experiments, the subjects were housed in an indoor room (5.8 x 6.7 m) divided into four pens separated by chain-link fencing. They shared the room with approximately fifteen other pigs who were not used in experiments. Males and females were kept in separate pens. For our study, the subject pigs were transferred to an adjacent room (4.5 x 6.7 m) where they stayed for the duration of the experimental period. Since the animals had reached sexual maturity, males and females were tested as separate groups between April 23 and May 12, 2013, and May 20 and June 8, 2013, respectively. The test room consisted of a main pen with three side pens along one side (Appendix B). When tests were not being carried out the pigs had access to all pens. The floor consisted of red tiles covered partially with black rubber mats. The room was maintained under a 14:10 h light:dark cycle, with lights on at 6 a.m. Temperature was maintained between 17 and 21 ºC. Pigs were fed Co-op Pig Grower around 9:30 am and 4:00 pm every day throughout the experimental period. All animals had continual access to water. The floor of the pen was washed twice daily with a hose. Heavy rubber balls and hanging chains were provided as environmental enrichment objects for the pigs.

2.3. Object location memory task

The object location memory task was used to assess the effect of an acute stressor on perception and memory for the location of high and low priority items in pigs. The task took place in a test box (1.5 m L x 1.5 m W x 1.0 m H) with fixtures to attach objects. The siding of the test box consisted of white plastic panels with a door on one side that opened and closed with a slide latch.

2.3.1. Habituation

During the first 8 days of the experiment, the pigs underwent daily habituation trials in the test box. The test box was wheeled into the room each morning, following which the pigs received part of their feed in it. They were free to enter and leave the test box over a period of 20 min. In the early afternoon, the animals underwent individual trials consisting of 5 min in the test box with the door closed and two objects present. If needed, we used food to entice the animals to enter the box for the first few days of habituation. At the end of each day, the remaining portion of feed was tossed in the test box. The test box was removed from the room once all the food was eaten. After the habituation period, the pigs appeared relaxed in the test box and no escape behaviours (i.e. reaching up on the sides, jumping) were observed. A wooden coat hanger, plastic sieve, and leather tool belt were used as objects only in the habituation trials.

2.3.2. Priority

Object priority was defined by the physical characteristics and relative familiarity of the objects. In all the trials, the low priority object was a metal spoon that was simple in form and uniform in texture and colour and was found in a pilot study to be of little interest to pigs. The high priority objects were selected for characteristics favoured by pigs (i.e., deformable, chewable, odourous; Van de Weerd et al., 2003). As pigs will preferentially explore a novel object, an object's priority will decrease with increasing familiarity. Thus, the spoon was present in the test room throughout the habituation

period to further discriminate it from the high priority objects, which the pigs had not encountered prior to the trials.

2.3.3. Trials

The object location memory task began on the eleventh day of the experimental period (Days 9 and 10 were allocated to the personality tests and food competition test, described below). The pigs were divided randomly into two groups of five pigs. The groups were tested on alternate days over 8 consecutive days. Each pig underwent one trial per day, for a total of 4 trials over the 8 day period. After the animals had eaten in the test box in the morning, each group was confined to separate side pens. The test box was positioned in the middle of the main pen. A subject was let out of the side pen when it was their turn to be tested and, upon completion of the trial, was transferred to the third, unoccupied side pen. The order of pigs tested within each group was kept constant across days. We restricted testing to between 12:00 pm and 4:00 pm, in order to control for the diurnal rhythm of the salivary analytes.

A trial consisted of three phases in the test box: (1) 5 min exposure phase; (2) 5 min test phase A; and, (3) 5 min test phase B. In between the phases the pig was allowed to roam around the main pen for 5 min. A trial lasted 25 min. In the exposure phase, a low priority object and a high priority object were placed in the far left and the far right (relative to the door) corners of the test box for the subject to explore (Appendix F). The location of the low and high priority objects in the exposure phase varied across trials. In test phase A the pig was shown two copies of one of the objects in the exposure phase,

one on the left and the other on the right. If the animals remembered the location of the object in the exposure phase, they should allocate their exploration time preferentially towards the novel object/location configuration during the test phase. Test phase B was a repeat of test phase A, but with the other object seen in the exposure phase. For example, if the pigs saw two copies of the low-priority object in test phase A, then in test phase B they would see two copies of the high-priority object (Appendix G).

We used four high priority objects, one for each of the 4 trials that each pig experienced. The high priority objects were: a metal rake with a wooden handle, a spiral rope toy with a tennis ball on one end, a rubber plunger with a plastic handle, and an orange, plastic traffic cone (Appendix H). The order of the high priority objects presented was constant within groups, but differed between the two groups.

A stressor was applied immediately before the exposure phase in two out of the four trials for each individual. The stressor involved social isolation in a crate for 10 min. The crate with the pig inside was moved just outside the test room to augment the experience of social isolation as well as to prevent the other animals from observing their crated pen-mate. The crate was kept outside the room when not in use. Animals in the non-stressed condition were let out of the side pen and allowed to wander around the main pen for 10 min before the exposure phase.

Each trial varied along two factors: (1) stress condition, and (2) the order of the test phases. Thus, the four trials were: (1) stressed condition, low priority in test phase A; (2) stressed condition, high priority in test phase A; (3) non-stressed condition, low

priority in test phase A; and, (4) non-stressed condition, high priority in test phase A. The order of trial presentation was randomized across individuals.

Two female observers stood on each side of the door of the test box while testing was underway. One person video recorded the trial, while the other took notes. We analyzed the video files using the logger.app (© A. Earle, Memorial University, online version: http:play.psych.mun.ca) to record the time the pig spent exploring each object in the exposure and test phases. Duration spent exploring an object was defined as either the pig touching the object with their snout or holding their snout within 5 cm of the object.

In order to control for odour cues on objects, we used three copies of an object in each trial. Thus, in the test phases of the trial, the objects were separate copies of the same object shown in the exposure phase. The test box and the objects were hosed down at the end of each phase.

2.4. Saliva sampling

Three saliva samples were taken for each trial: 0 min (baseline; immediately before pig was crated), 10 min (post-stress; immediately upon removal from the crate), and 35 min (post-test) after stressor onset. In the non-stressed condition, saliva samples were collected with the same time-interval without the stressor present. A total of twelve samples were collected per subject. We used Salimetrics Children's Swabs (Salimetrics, LLC, Pennsylvania, USA) to obtain saliva. The pig was enticed to chew on the swab by placing it in front of his or her snout. If the pig was not interested, then the swab was gently inserted into the back of the animal's mouth to stimulate chewing. The pig was

allowed to chew on the swab until it had become saturated (20-30 s). Saliva samples were immediately placed in storage tubes and frozen at -20 ºC. We shipped samples on dry ice to Salimetrics LLC for analysis via enzyme immunoassay for sAA, cortisol, CgA, and testosterone. Saliva flow rate was not measured, as valid measurements of our analytes of interest can be obtained without the need for assessing flow rate (sAA: Rohleder et al., 2006; cortisol: Vining and McGinley, 1987; CgA: Escribano et al., 2013).

2.5. Personality tests

Three personality tests adapted from Janczak et al. (2003) and Spake et al. (2012) were performed 1-2 days before and 1-2 days after the object location memory task: a solitary human approach test, a solitary novel object test, and a group novel object test. In the solitary human approach test and novel object test, each pig was guided individually into a side pen in the test room. Depending on the test, a human or an object was positioned against the wall opposite the pen entrance prior to the pig's arrival. The amount of time the pig spent exploring the human or object during a 5 min period as well as the latency to approach the object were recorded. Individuals who did not make contact with the human or object were given an exploration time of 0 sec. In the human approach test, the test person wore rubber boots similar to those worn by animal care staff, but was unfamiliar to the pigs. The pen was hosed down following each test. The group novel object test took place in the main pen. An object was attached to a pole using a bungee cord and the pigs were allowed 20 min to explore the object. A knobby ball and a football were used as the novel objects in the solitary novel object tests, and a red truck

and green tractor were used as the novel objects in the group novel object test (Appendix C). All tests were recorded using a Sanyo VPC-HD1010 (Sanyo Electric Co. Ltd., Osaka, Japan) handheld video camera at 60 fps.

2.6. Food competition test

After a hierarchy is established, pigs drastically reduce agonistic behavior, making it difficult to calculate a general 'aggressive order' (Langbein and Puppe, 2004). In stable groups in which overt agonistic interactions are rare, a common approach is to force agonistic encounters by restricting resource availability (e.g., food competition test), resulting in a 'competitive order'. Outcomes of social ranking fights during hierarchy formation and later food competition tests are highly correlated in pigs (Hessing et al., 1994). Since both the male and female groups in our study had established stable hierarchies prior to the experiment, we determined the social rank of each individual by means of a food competition test, conducted 1-2 days before and 1-2 days after the object location memory task. Food was provided in a kennel bowl (21 cm L x 17 cm W x 6 cm H) attached to the chain-link of the middle side pen. The size of the bowl allowed only one animal to feed at a time. All pigs were familiarized with eating from the bowl prior to the tests. Each test lasted 70 min and was video recorded. Feed was replaced as needed. To ensure that the bowl was not monopolized by the dominant animals, different combinations of individuals were allowed to feed at a time, with the aim of maximizing encounters between all individuals. However, agonistic interactions were rare for less aggressive individuals or dyads with large discrepancies in rank, and so

some unknown dyads were inevitable. We recorded the occurrence and outcome of all agonistic interactions in a dyadic interaction matrix with the rows labelled as wins and the columns as defeats (Appendix D). An agonistic interaction was defined as a fight or displacement initiated by one individual and followed by submissive behavior (i.e., turning away from an attack, fleeing, or displacement from the food bowl) displayed by the opponent (Langbein and Puppe, 2004).

3. Statistical analyses and results

All analyses were conducted using the statistical package R, version 3.0.2 (R Core Team 2013). High inter-observer reliability for all test measures was observed (see Appendix E for intraclass correlation coefficients).

3.1. Personality

3.1.1. Statistical analysis

Since we were interested in a general personality measure, we used principal components analysis (PCA) to reduce the number of variables measured in the personality tests (exploration duration in the first and second human approach test, novel object test, and group novel object test, and latency in the group novel object test). Since the variables measured have different ranges, we used the correlation matrix to calculate the principal components rather than the covariance matrix. Varimax rotation was performed on the components with eigenvalues greater than 1 in order to clarify the structure of the loadings matrix.

3.1.2. Results

PCA yielded three components with eigenvalues larger than 1 that explained a total of 71% of the overall behavioural variation (Table 2.1). The first component, PC1, which accounted for 36% of the variation explained, showed positive loadings exceeding 0.4 on four out of the six exploration duration measures. Therefore, increasing values of PC1 indicate more extensive exploratory behaviour. We subsequently refer to PC1 as a curiosity measure. The second principal component explained 21% of the variation and showed negative loadings exceeding 0.6 for duration spent exploring in the first novel object test and the first group novel object test. Animals scoring high on PC2 can therefore be said to be more neophobic than animals with low scores. Thus, PC2 was considered a measure of timidity. The third principal component was less readily interpretable, as it showed positive and negative loadings for latency to explore in the first and second group novel object tests, respectively. Thus, pigs who were slower to explore in the first group novel object test were quicker to approach the novel object in the second test. PC1 and PC2 were retained for subsequent analysis because they have clear behavioural meaning and together represent 57% of the variation.

3.2. Social rank

3.2.1. Statistical analysis

We calculated sociometric indices with the R package *DyaDA* (Leiva et al., 2010). As males and females were not housed together, separate indices were computed for both sexes. Landau's improved linearity index *h'* (de Vries, 1995) measures the

degree of linearity in a social hierarchy containing unknown or tied relationships. In order to assign each pig an individual rank position, linearity must be statistically significant. Since near-linear hierarchies for both sexes were found, we applied the I & SI method described in de Vries (1998) to rank individuals in an order that is most consistent with a linear hierarchy. Given the high repeatability of social rank between the food competition tests (males: $r = 0.770$, $p = 0.003$; females: $r = 0.915$, $p < 0.0001$), data from both tests were combined and overall indices for the males and females were subsequently calculated.

3.2.2. Results

A total of 149 agonistic interactions were observed over the two food competition tests for females. We observed fewer interactions for the males ($n = 62$). The improved Landau's index h' for males and females was 0.752 ($p = 0.01$) and 0.952 ($p = 0.0004$), respectively, indicating that both males and females established near-linear hierarchies. Individuals were subsequently assigned ranks from 1 (most dominant) to 10 (most subordinate).

3.3. Stress biomarkers

3.3.1. Statistical analysis

sAA, cortisol, and CgA levels were analysed separately using linear mixed models. In each analysis, we fitted hormone levels as the response term. Fixed effects included stressor (present or absent), day, sex, and time (0, 10, 35 min following stressor

onset). Two-way interactions between stressor and sex, and stressor and time were included. Pig identity was entered as a random effect. sAA, cortisol and CgA data were positively skewed and so were $\log_{10}(Y+1)$-transformed for analysis. Visual inspection of residual vs. fits plots confirmed that all models met the assumption of homogeneous variances. We removed one outlier from the cortisol data due to its improbably high value (>4.0 ug/dL). The input variables were standardized following Gelman (2008). For visual representation, we present data as non-transformed values to allow biologically meaningful interpretation.

In order to determine the magnitude of the stress response, area under the curve (AUC; Preussner et al., 2003) was computed for the non-transformed values of sAA, cortisol, and CgA. We used the formula for 'AUC with respect to ground (AUC_G)' described by Preusner and colleagues (2003) as AUC_G measures total hormonal output.

3.3.2. Results

Figure 2.1 shows the profiles for sAA, cortisol, and CgA in response to the stressor over the three sampling times. *Stressor + Day + Sex + Time + Stressor*Sex + Stressor*Time* linear mixed models for sAA, cortisol, and CgA revealed a Sex effect and Stressor effect for sAA, a Sex effect and Stressor*Time effect for cortisol, and a Time effect for CgA.

sAA: Females had higher levels than males (Standardized parameter estimate ± S.E.; Sex: -0.10 ± 0.05, t_{18} = -2.20, p = 0.04; females (geometric mean (CI)):1.2 (0.9, 1.5); males (geometric mean (CI)): 0.7 (0.5, 0.8)). Levels tended to be higher in the

stressed condition than in the non-stressed condition (Stressor: 0.06 ± 0.03, t_{155} = 1.91, p = 0.06) (Fig. 2.1A).

Cortisol: Males had higher levels than females (Sex: 0.05 ± 0.01, t_{18} = 4.28, p = 0.0005; females (geometric mean (CI)): 0.17 (0.15, 0.20); males (geometric mean (CI)): 0.31 (0.28, 0.34)). Levels decreased across sampling time in the non-stressed condition, but did not change across samples in the stressed condition (Stressor*Time: 0.03 ± 0.01, t_{214} = 2.77, p = 0.006). Post-hoc analysis revealed that cortisol levels taken 35 min after stressor onset were significantly higher than cortisol levels in the non-stressed condition from the same time point (mean log cortisol level at 35 min ± CI; stressor present: 0.091 ± 0.016; stressor absent: 0.067 ± 0.008) (Fig. 2.1B).

CgA: Levels decreased across the three samples, regardless of whether the stressor was applied (Time: -0.34 ± 0.04, t_{95} = -9.64, p < 0.0001) (Fig. 2.1C).

3.4. Factors influencing total exploration time in the object-recognition task

3.4.1. Statistical analysis

It is reasonable to assume that performance in the object location memory task will be influenced by the amount of time the animal spends exploring the objects in the exposure phase and test phase. Therefore, in order to determine whether differences in exploration times are driving the effects of different variables on cognitive performance, we investigated the effects of stressor, object priority, sex, and their interactions on object exploration time in the exposure phase and the test phase using linear mixed models. Identity was included as a random effect. Exploration times in both the exposure phase

and test phase were positively skewed and so were $\log_{10}(Y+1)$-transformed for analysis. Visual inspection of residual vs. fits plots confirmed that both models met the assumption of homogeneous variances.

3.4.2. Results

The median total exploration time of both objects in the exposure phase was 103.0 s (interquartile range, IQR: 64.8 – 158.5 s). As expected, both males and females spent more time exploring the high priority object than the low priority object in the exposure phase (parameter estimate ± SE; Priority: -0.54 ± 0.13, t_{134} = -4.31, $p < 0.0001$; high (geometric mean (CI)): 62.5 (48.9, 79.9); low (geometric mean (CI)): 15.5 (12.6, 19.0)). The duration spent exploring in the exposure phase did not differ between the sexes (Sex: 0.14 ± 0.14, t_{18} = 1.03, p = 0.31) or the stressed and non-stressed conditions (Stressor: -0.22 ± 0.13, t_{134} = -1.72, p = 0.09). Thus, we can rule out the possibility that sex or stressor effects on cognitive performance are the result of different levels of exploration in the exposure phase.

The median total exploration time of both objects in the test phases was 64.5 s (IQR: 26.0 – 80.2 s). Overall, males and females engaged in a similar amount of exploration during the test phases (Sex: -0.17 ± 0.17, t_{18} = -1.02, p = 0.32). However, females, but not males, spent less time exploring during the test phases in the stressed condition than in the non-stressed condition (Sex*Stressor: 0.44 ± 0.19, t_{134} = 2.30, p = 0.02; data not shown). Total exploration time during the test phase was not affected by object priority (Priority: -.10 ± 0.14, t_{134} = -0.75, p = 0.45), indicating that our construct

of priority is relevant only when objects are competing for the animal's attention (i.e., in the exposure phase).

3.5. Factors influencing cognitive performance in the object-recognition task

3.5.1. Statistical analysis

We used a linear mixed model to examine the effects of the independent variables on the proportion of time spent exploring the novel object/location configuration out of the total time spent exploring both configurations in the memory test. Since the response variable is a non-binomial proportion, we applied a logit-transformation (Warton and Hui, 2011). Visual inspection of the residuals vs. fits plot confirmed that the transformed values satisfied the homogeneity assumption. An a priori, full model was selected according to factors that we found to be important in the literature. Fixed effects included stressor, object priority, test phase, curiosity, timidity, social rank, sex, sAA AUC_G and cortisol AUC_G. CgA AUC_G was not included in the full model, as it was significantly negatively correlated with sAA AUC_G (rho=-0.38, p=0.016) and, of the two analytes, sAA is better established as a marker of sympathetic activity. Two-way interactions between object priority and stressor, object priority and sAA, object priority and cortisol, stressor and sex, curiosity and sex, and timidity and sex were entered in the model. Pig identity was included as a random effect to account for repeated measures on the same individuals. An information-theoretical approach based on Akaike's information criterion corrected for small sample size (AIC_C) was used to rank all possible subsets of the full model and weigh the relative support for each one (Burnham and Anderson, 2002).

Given the high model selection uncertainty as quantified by Akaike weights, we used model averaging to obtain robust parameter estimates. Model averaged parameter estimates were calculated from the 95% confidence set of models that included all models whose cumulative Akaike weights summed to 0.95 (Burnham and Anderson, 2002). Since each parameter does not appear an equal number of times in the model, the model set was averaged using the natural average method (Burnham and Anderson, 2002). We standardized the input variables in the full model using Gelman's (2008) approach to facilitate interpretation of the parameter estimates (Grueber et al., 2011).

Linear mixed models were fitted using the R package *nlme* (Pinheiro et al., 2013). Information-theoretic model selection was carried out with the R package *MuMIn* (Barton, 2013).

3.5.2. Results

The final averaged model was based on the 95% confidence set of 585 models (see Table 2.2). The models are ordered most to least supported by the data based on the value of the Akaike information criterion corrected for small sample size (AIC_C). Table 2.3 shows the model-averaged parameter estimates for the 95% confidence set. Predictors and interactions that have parameter estimates with 95% confidence intervals excluding 0 are considered important. Object priority, the interaction between stressor and sex, and the interaction between timidity and sex were the best predictors to explain performance on the memory task (Table 2.3).

Priority effect: pigs spent a greater proportion of time with the novel configuration when the object was high priority than when it was low priority. Post-hoc analysis of this result showed that the pigs spent significantly more than 50% of their time with the novel configuration only when they were being tested on high-priority objects ((back-transformed proportion of time spent with novel configuration (CI); high priority: 0.72 (0.60, 0.82); low priority: 0.50 (0.38, 0.62)).

Stressor*Sex effect: males spent a greater proportion of time with the novel configuration when they were in the non-stressed condition than when they were in the stressed condition, but this effect was absent for females (Fig. 2.2). Post-hoc analysis revealed that males in the non-stressed condition and females in the stressed condition spent significantly more than 50% of their time with the novel configuration (back-transformed proportion (CI); male/stressor present: 0.43 (0.25, 0.62); male/stressor absent: 0.77 (0.62, 0.88); female/stressor present: 0.70 (0.54, 0.83); female/stressor absent: 0.53 (0.35, 0.71)).

Timidity*Sex effect: Less timid males tended to perform better on the memory task, while the opposite pattern was found in females with performance improving with increasing timidity (Fig. 2.3).

4. Discussion

See MS1 for a discussion of the personality and social rank results.

4.1. sAA and cortisol levels respond to the stressor, but CgA levels do not

Social isolation and confinement in a crate for 10 min elicited an increase in pigs' salivary cortisol concentrations and sAA activity, but CgA levels remained unchanged from the non-stressed condition. In agreement with our finding, Filaire et al. (2009) examined stress in professors delivering a lecture to students and reported that salivary CgA did not change in response to teaching, while sAA and cortisol did. Another study found that sAA activity increased in response to a mental arithmetic task, but salivary CgA and cortisol did not (Noto et al., 2005). Our study suggests that the stressor did alter HPA axis and sympathetic activity, as indicated by an increase in salivary cortisol and sAA, respectively, but this change was not strong enough to be detected by salivary CgA.

sAA activity was elevated in the samples taken at 0 and 10 min in the stressed condition compared to those taken in the non-stressed condition. At 35 min after stressor onset sAA activity in both conditions was the same. However, sAA responses among and within individuals were highly variable. High variability in sAA values among pigs was also observed by Fuentes et al. (2011). This variability is widely reported in human studies, as well, and is thought to reflect individual differences in sensitivity to environmental stimuli (Out et al., 2013; Segal et al., 2012).

An increase in salivary cortisol in the stressed condition relative to the non-stressed condition was detected 35 min following stressor onset. This delayed cortisol response is expected, as the HPA axis stress response is slower to activate and secrete stress hormones than the fast-acting sympathetic response. Salivary cortisol changes in response to a stressor have been well documented in pigs. Immobilization with a nasal snare for 1 min (Escribano et al., 2012) or 5 min (Geverink et al., 2002; Merlot et al.,

2011) has been shown to elicit an increase in salivary cortisol at 15 min following stressor application. Salivary cortisol was also found to be elevated in pigs after transportation for 25 min (Geverink et al., 1998), 30 min (Escribano et al., 2012) or 120 min (Schönreiter and Zanella, 2000).

Interestingly, when the stressor was absent, cortisol levels declined relative to baseline concentrations. The same pattern was observed for CgA, even when the stressor was applied. A similar result was reported by Toda et al. (2013), who found that cortisol and CgA secretion decreased in their control group. They suggest this decrease reflects the expected circadian decline in cortisol and CgA levels. Although possible, we think it is unlikely that the circadian decline explains our results, as our samples were collected over a much shorter time interval (35 min) than those of Toda et al.'s study (85 min). Another possibility is that the decrease we see in CgA in the stressed and non-stressed conditions and cortisol in the non-stressed condition reflects the possible stress the pigs experience from being kept in the side pen prior to test commencement. The confined space, separation from the other half of the group, as well as lack of access to their usual defecation area likely contributed to the high initial levels and subsequent decline upon release from the side pen. Despite this, our stress manipulation was effective at eliciting a stress response, as evidenced by the increase seen in sAA and cortisol concentrations in response to the stressor.

A slightly puzzling finding was the negative correlation between CgA and sAA total ouput. As they are both considered potential markers of sympathetic activity, we expected to observe a positive correlation between the two salivary analytes. Although

the physiological role of CgA is still under investigation, CgA is the precursor of a number of other proteins, including the sympathoinhibitory peptide, catestatin, which may inhibit sAA release, thereby potentially explaining the negative correlation between CgA and sAA (Gaede and Pilowsky, 2012). Intriguingly, Kawada et al. (2009) also found a negative relationship between CgA and sAA changes in response to psychological stress. Robazza et al. (2012) reported no correlation between CgA and sAA, but a correlation did exist between CgA and cortisol. Similarly, salivary CgA and sAA respond differently to high-intensity exercise (Gallina et al., 2011). Taken together, these findings imply that sAA and CgA are regulated differently by the sympathetic nervous system and suggest that both should be assessed simultaneously in order to attain more extensive and accurate knowledge of the physiological stress response.

4.2. Enhanced memory for location of high priority objects vs. that of low priority objects is independent of stressor

ABC theory proposes that arousal will improve perception and consolidation of high priority stimuli while weakening perception and consolidation of low priority stimuli. Our results showed that pigs remembered the location of high-priority objects, but not the location of low-priority objects, regardless of whether they were tested in the stressed or non-stressed condition. This result provides only partial support for the theory of arousal-biased competition, as we did observe a bias towards memory processes for high priority stimuli, but this bias was not amplified under stress as the theory predicts. One reason that would explain why we did not see the predicted interaction between

priority and stressor is the absence of object location memory for low priority stimuli in the non-stressed condition. Since the pigs did not remember the location of low priority objects when non-stressed, an impairing stressor effect could not be observed.

The absence of object location memory for the low priority object likely reflects suppressed perception and encoding of the low priority information in favour of enhanced processing of the high priority stimulus. Pigs demonstrated an attentional bias towards the high priority item in the exposure phase, which would result in a gain of mental representation for the high priority object at the expense of weakened processing of the low priority stimulus (Beck and Kastner, 2009). It is also possible that the habituation period to the low priority object prior to testing interfered with processing its location in the test box during the exposure phase. Defining object priority by a characteristic other than its relative familiarity would be one way of overcoming this limitation in future studies.

It should be noted that poor performance on the object location memory task may reflect impaired memory for the previous location of the object or the object itself. While the test does not allow us to differentiate between the "what" and "where" components of episodic-like memory, we think it is most likely that pigs who performed poorly could recall the "what", but not the "where", of the past event. Several studies have demonstrated that pigs' ability to recognize an object can persist for several days (Gifford et al., 2007; Kouwenberg et al., 2009), suggesting that it is unlikely our pigs failed to remember an object they had seen 5-15 min ago. Moreover, in the case of the low-

priority object, the pigs had been exposed to the spoon throughout the habituation and testing period. Gifford et al. (2007) showed that longer exposure times facilitate object recognition at longer delays, indicating that our pigs should have had no problem recognizing the low-priority object. Thus, their poor performance when tested with low-priority objects likely reflects an inability to remember where the object was located in the exposure phase, rather than an inability to remember the object per se.

Alternatively, it is also possible that the pigs could remember the location of the low-priority object, but because they spent relatively little time with the spoon in the exposure phase, they perceived both locations in the test phase as being relatively equal in regards to novelty. Thus, as is the case with many cognitive studies, it is uncertain exactly what a performance deficit reflects. In our study, poor performance on low-priority objects may be attributed to impaired memory for the location of the object or insufficient time to habituate to the object in the context of the test box.

4.3. Stress negatively impacts cognitive performance in the males, but not the females

The application of a stressor immediately before the object location memory task impaired subsequent performance, but only in the males. Males in the non-stressed condition remembered where the object was previously placed, but failed to do so in the stressed condition. Object location memory in the females, on the other hand, was not affected by the stressor. The female pigs did not discriminate between novel and familiar configurations in the non-stressed condition, thus one logical interpretation of the sex-specific stressor effect is that the females' performance could not decline any further

under stress. However, females demonstrated an ability to remember the previous location of an object in the stressed condition. Therefore, we cannot attribute the absence of an impairing stressor effect on the females' performance to a lack of object location memory.

It is possible that the males were less attentive to the objects in the exposure phase after the stressor was applied and this resulted in their impaired performance in the test phases. However, the pigs spent an equal amount of time exploring in the exposure phase regardless of whether the stressor had been applied immediately beforehand. Thus, we can rule out an attention deficit as the cause for the males' impaired performance in the stressed condition.

Males had higher cortisol concentrations than females and this may have contributed to their performance decline following administration of the stressor. Our parallel study on the same pigs also showed that cortisol reactivity in response to the stressor only occurred in the males. Many studies report an inverted-U dose-response curve for glucocorticoid effects on declarative memory, with moderate levels inducing optimum levels of performance, and performance falling away at lower or higher levels (reviewed in Lupien and McEwen, 1997). However, if high cortisol levels were the primary reason behind the cognitive deficit in the stressed males then we would expect cortisol to predict cognitive performance in our model, but this is not the case. One likely possibility for why there is no such cortisol effect is that glucocorticoid effects on memory are regulated by another variable, such as catecholamine concentrations. Indeed,

it is well established that glucocorticoid modulation of memory requires noradrenergic activation in the basolateral amygdala (McIntyre et al., 2012; Roozendaal et al., 2006, 2009). It would be interesting in a future investigation to examine how hormonal and behavioural measures interact with cortisol concentrations to mediate cognitive function.

A complementary explanation for the sex-specific cognitive impairment under stress is that the females were chronically stressed. Evidence from our parallel study suggests dysregulation of the stress systems in the female subjects, as reflected by a blunted cortisol response and heightened sympathetic activity. Thus, the diminished responsiveness of the HPA axis in chronically stressed females may account for the absence of a stressor effect on their cognitive performance. Chronic stress may also be a factor in the females' poor performance in the non-stressed condition, in which they spent equal amounts of time with the novel and familiar configuration. Prolonged exposure to stress produces numerous changes in hippocampal structure and is frequently associated with declarative memory impairments (Conrad, 2010; McEwen, 2000; McEwen and Sapolsky, 1995; Roozendaal, 2002).

It is also possible that sex differences exist in cognition and may explain why the males in the non-stressed condition remembered the location of the objects while the females did not. Sex differences in spatial cognition that are in favour of males are well-documented in several mammalian species (Jones et al., 2003). In line with these observations, Kouwenberg (2008) failed to find episodic-like memory in the female pigs. However, in human studies, when tested on other aspects of spatial abilities, notably

object location memory, women typically outperform men (Voyer et al., 2007). Moreover, in the current study, the females in the stressed condition spent more than 50% of their time with the novel configuration, indicating that a male advantage in object location memory does not exist. Thus, we think the most probable explanation for the sex-differences in cognitive performance is the differential reactivity of the two stress systems between the sexes.

4.4. Timidity may influence cognitive performance in a sex-dependent manner

The effect of timidity on cognitive performance differed between the sexes, with performance tending to be better among less timid males and more timid females. While previous studies have linked personality traits to cognition (Dugatkin and Alfieri, 2003; Guillette et al., 2011; Lind and Moustgaard, 2005), ours is only the second study to find a sex-specific effect. A similar result was found by Titulaer et al. (2012), with fast-exploring male great tits performing better on a learning task than slow-exploring males, whereas slow females outperformed fast females. The sex-specific relationship between personality and memory suggests that males and females gain different cognitive advantages from similar personality traits and that sex-dependent selection processes may act as a mechanism by which personality differences are maintained (Pruitt and Riechert, 2009). It is also worth considering that differences in cognitive ability can impact the development of an individual's personality (Sih et al., 2004), and may do so in a sex-dependent way. Further studies are needed to more clearly characterize the sex-dependent

personality effects on learning and memory, as well as the ongoing feedback between personality and cognition.

5. Conclusions

Our study demonstrates that pigs can recall "what" object they saw "where", which is in accordance with Held et al. (2005) and Kouwenberg et al. (2009) who also reported the existence of what/where memory in pigs. We further showed that this ability is dependent at least in part on stress levels and the relative priority of the object. Moreover, individual differences in personality, but not social rank, influence performance in a sex-dependent manner.

Male and female pigs responded to the acute stressor differently, with cognitive function being impaired in the males, but not the females. In parallel with these results, males, but not females, showed increased cortisol levels in response to the stressor. Our findings extend a growing literature in humans and rodents that suggests sex influences stress-memory interactions (Conrad et al., 2004; Felmingham et al., 2012; Shors, 1998; Wolf et al., 2001). Sexual dimorphisms in the stress response may contribute to the differential effects of acute stress on cognition. Thus, in order for our understanding of stress effects on memory to progress, both sexes demand equal consideration in studies.

Table 2.1

Varimax rotated loadings for the first three components (n = 20), as well as their Eigenvalues and the percentage of the total variation they explain. HAT = human approach test, NOT = novel object test, GNOT = group novel object test.

	PC1	PC2	PC3
Duration exploring in HAT 1	0.44		-0.21
Duration exploring in HAT 2	0.51		0.12
Duration exploring in NOT 1		-0.63	
Duration exploring in NOT 2	0.53		
Duration exploring in GNOT 1		-0.63	
Duration exploring in GNOT 2	0.52		
Latency to explore in GNOT 1		0.18	0.82
Latency to explore in GNOT 2		0.41	-0.51
Eigenvalue	2.87	1.69	1.14
Percentage of variance	35.9	21.2	14.2

Table 2.2

95% confidence set of best-ranked models (the 585 models whose cumulative Akaike weight ≤ 0.95).

	Explanatory variables	df	AICc	Δ_i	ω_i
1	Priority + Sex + Stressor + Timidity + Sex:Stressor + Timidity:Sex	9	294.341	0.000	0.033
2	Phase + Priority + Sex + Stressor + Timidity + Sex:Stressor + Timidity:Sex	10	294.582	0.241	0.029
3	Priority + Sex + Stressor + Sex:Stressor	7	295.027	0.686	0.024
4	Phase + Priority + Sex + Stressor + Sex:Stressor	8	295.124	0.783	0.022
5	Priority + sAA + Sex + Stressor + Sex:Stressor	8	295.247	0.906	0.021
6	Priority + sAA + Sex + Stressor + Timidity + Sex:Stressor + Timidity:Sex	10	295.270	0.929	0.021
7	Phase + Priority + sAA + Sex + Stressor + Sex:Stressor	9	295.327	0.986	0.020
8	Phase + Priority + sAA + Sex + Stressor + Timidity + Sex:Stressor + Timidity:Sex	11	295.508	1.167	0.019
9	Cortisol + Priority + Sex + Stressor + Timidity + Sex:Stressor + Timidity:Sex	10	296.104	1.762	0.014
10	Cortisol + Phase + Priority + Sex + Stressor + Timidity + Sex:Stressor + Timidity:Sex	11	296.404	2.063	0.012
…					
584	Phase + Priority + Rank + Timidity	7	305.213	10.872	0.000
585	Cortisol + Curiosity + Phase + Priority + Rank + sAA + Sex + Stressor +Curiosity:Sex + Sex:Stressor	13	305.217	10.876	0.000

Table 2.3

Model averaged parameter estimates for the 95% confidence set of models.

Parameter	Estimate*	Unconditional SE	Adjusted SE	Lower CI	Upper CI
Intercept	0.452	0.172	0.176	0.107	0.797
Phase	-0.465	0.312	0.319	-1.090	0.160
Priority	**-0.900**	**0.312**	**0.319**	**-1.525**	**-0.274**
Sex	0.053	0.392	0.422	-0.773	0.879
Stressor	-0.424	0.324	0.331	-1.072	0.225
Rank	-0.052	0.468	0.496	-1.025	0.921
Curiosity	-0.220	0.377	0.408	-1.020	0.580
Timidity	0.174	0.337	0.365	-0.542	0.890
Cortisol	-0.198	0.506	0.516	-1.209	0.812
sAA	0.465	0.354	0.362	-0.245	1.175
Sex:Stressor	**-2.077**	**0.624**	**0.638**	**-3.328**	**-0.826**
Sex:Curiosity	0.667	0.721	0.784	-0.870	2.204
Sex:Timidity	**-1.658**	**0.705**	**0.763**	**-3.153**	**-0.163**
Priority:Cortisol	0.679	0.626	0.641	-0.577	1.934
Priority:sAA	-0.301	0.647	0.661	-1.597	0.994
Priority:Stressor	0.357	0.627	0.642	-0.901	1.614

*Effect sizes have been standardized on two SD following Gelman (2008).
Predictors in bold have confidence intervals that do not include zero, implying these factors have an important effect on performance in the memory task.

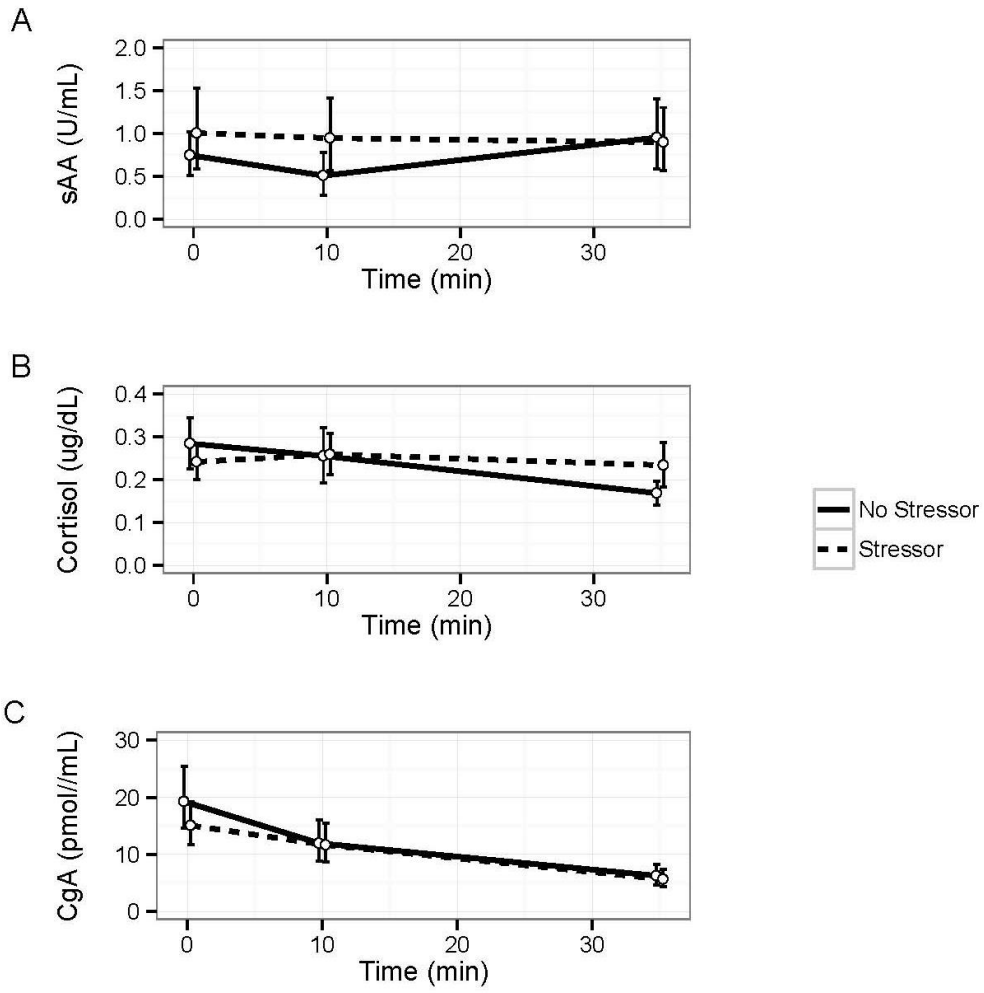

Figure 2.1. A) Salivary alpha-amylase (sAA), B) salivary cortisol, and C) salivary chromogranin A (CgA) levels before (0 min) and 10 min and 35 min after onset of the stressor (social isolation in crate). Graphs show geometric mean ± 95% confidence intervals.

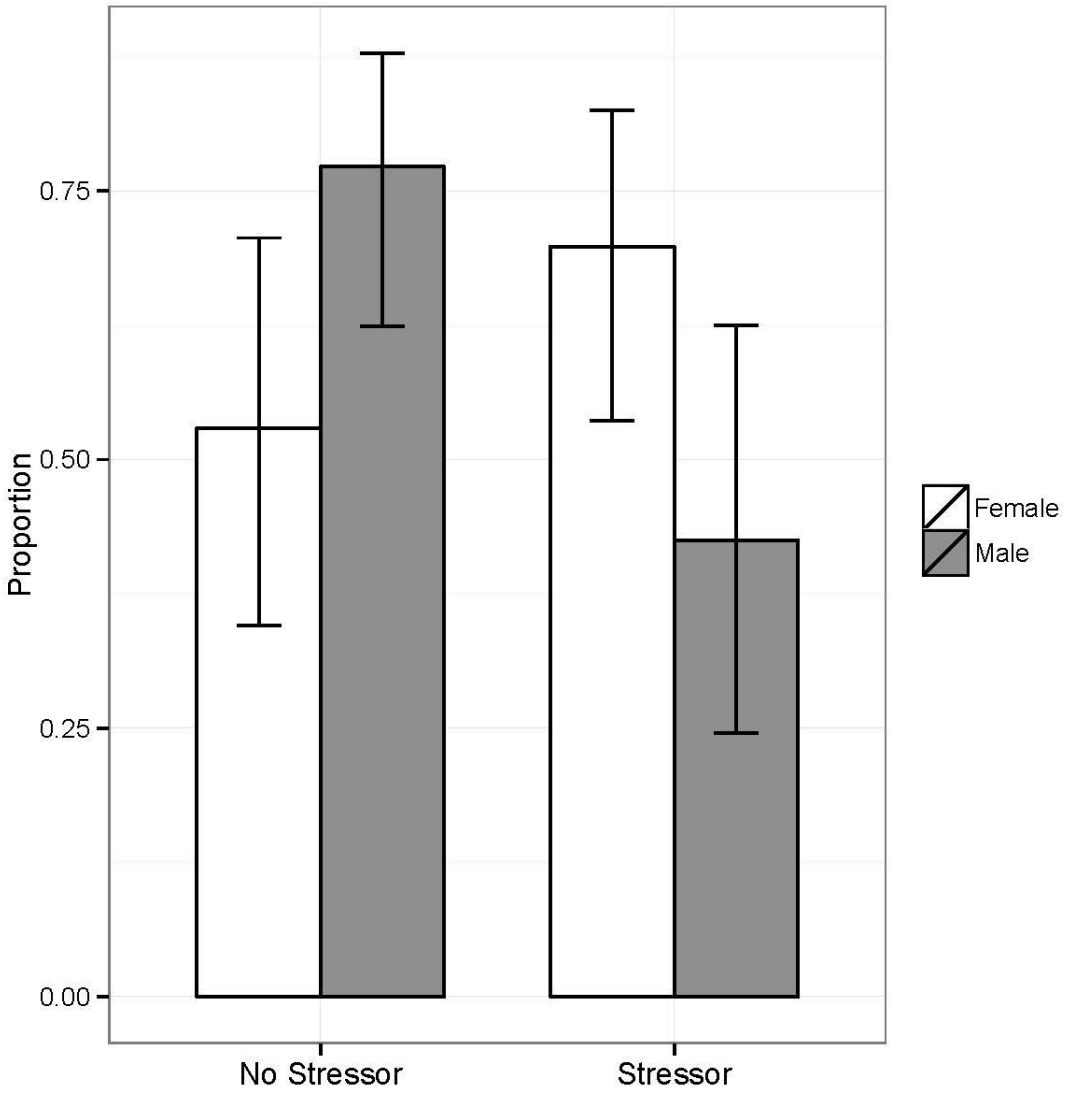

Figure 2.2. The proportion of time spent with the novel configuration (back-transformed mean ± 95% confidence intervals) for the stressed and non-stressed conditions. Stress decreased males' performance on the memory task, while it did not affect females' performance.

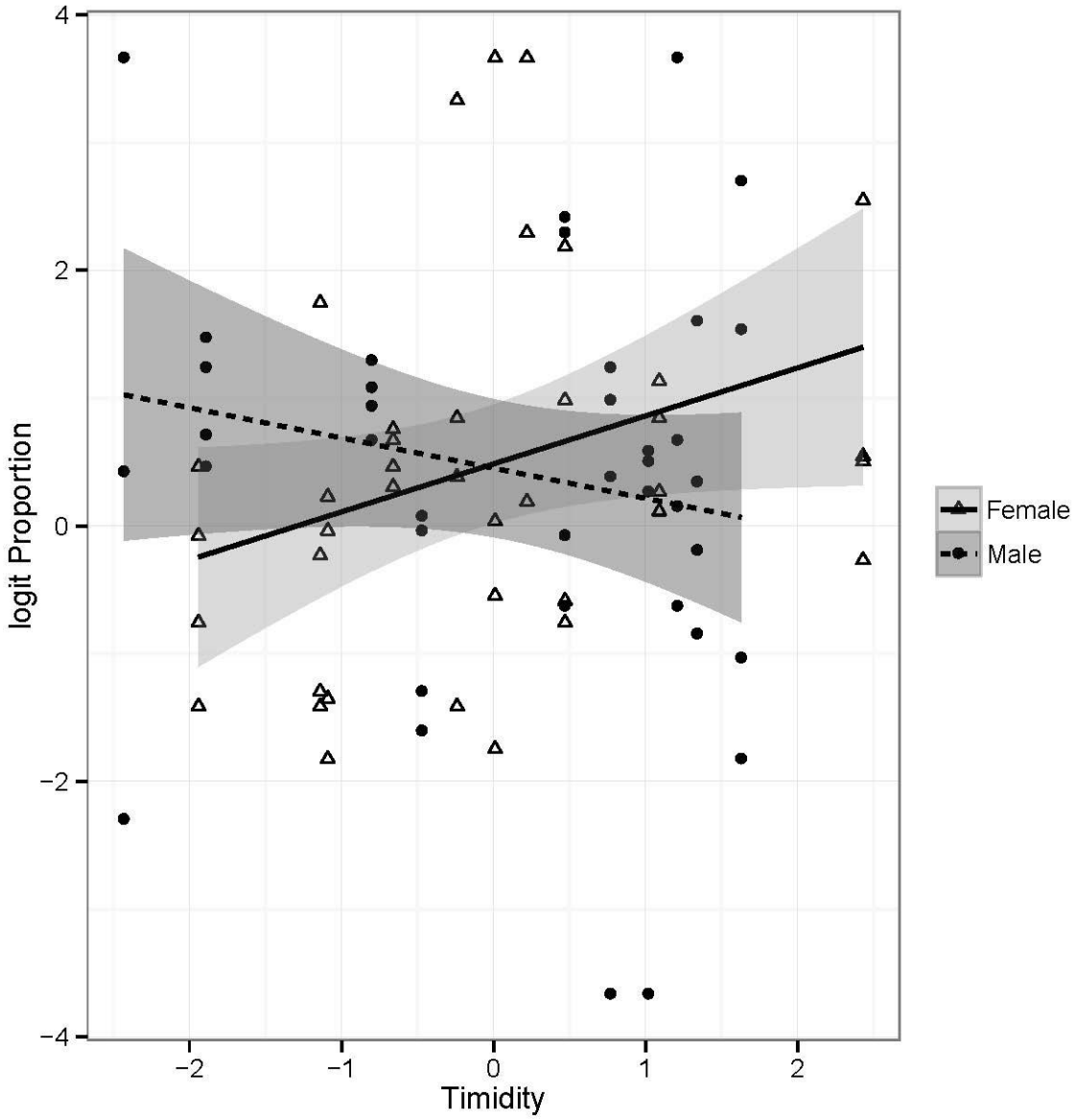

Figure 2.3. The logit-transformed proportion of time spent with the novel configuration as a function of an individual's timidity level. The slopes for males and females differed significantly from one another. The shaded regions indicate the 95% confidence intervals.

SUMMARY

To our knowledge, this is the first study to investigate the relations between cognitive performance, the stress response, social rank, and personality in a socially living species. As expected, pigs demonstrated personality, which varied along two dimensions labeled curiosity and timidity. Males and females formed semi-linear and near-linear social hierarchies, respectively. Also as expected, the pigs demonstrated object location memory, but only for the high priority items. We observed sex differences in: (1) litter origin influences on social rank; (2) inter-individual consistency of stress hormone levels; (3) HPA axis and sympathetic nervous system (re)activity and; (4) stress and personality effects on object location memory.

The widespread prevalence of sex differences in our experiments challenges the generally implicit assumption that sex matters little, if at all, in physiological and behavioural studies. In a review of the sex bias in research on mammals, Beery and Zucker (2011) reported that a male bias exists in the vast majority of biological fields, including neuroscience, endocrinology, zoology, behavioural physiology, and behaviour. In the studies that do enroll both males and females, data are often not analyzed by sex. The inclusion of female subjects in both human and non-human animal research in conjunction with sex-specific analysis would greatly advance progress in these disciplines. Studies of both sexes will yield a more complete understanding of the trait in question and its underlying mechanisms.

Our results attest to the importance of measuring the concurrent actions of both the HPA axis and sympathetic nervous system. A multisystem approach affords insights into an individual's physiological state that would be overlooked in a study of one stress system. For example, analysis of only the HPA axis would have led us to conclude that the females were unresponsive to the stressor, when in fact their sympathetic reactivity was greater than that of the males. An analysis of the associations between the two systems is also necessary in fully characterizing stress disorders. The ratio of sAA to cortisol is particularly useful in quantifying the asymmetry between the stress systems, which in turn is systematically associated with chronic stress.

The findings from the current study can be applied to pig welfare and husbandry issues. Our results suggest that, under near identical conditions, females are more susceptible to developing chronic stress than males. Heightened sensitivity to stressors in female pigs should therefore be considered when designing and implementing husbandry protocols that aim to maximize the animals' well-being. It should be noted, however, that the males in the current study were intact (boars), while most commercial pigs are castrated shortly after birth (barrows). Castration is known to induce acute pain and stress (Hay et al., 2003), as well as alterations in stress responsiveness (Gaskin and Kitay, 1971; Seale et al., 2004). Thus, barrows and boars likely differ in their susceptibility to developing chronic stress.

Since our study was limited by the relatively small sample size (n=20), further study is required to confirm our findings. Another limitation of our study design was that sex was confounded with age and time. Due to the necessity for testing the sexes as

separate groups, females were tested immediately following completion of the male study and were therefore one month older than the males at the time of testing. However, we think it is unlikely that age or time account for the plethora of sex differences observed in the current study. All pigs had reached sexual maturity at the time of testing and had experienced the same routine management by animal care staff. Moreover, no noticeable changes (e.g., in personnel, feed, housing) or unforeseen events (e.g., a power outage) occurred in the period between the males and females being tested, and the experimental protocol was identical for both sexes. Thus, differences in age and time of testing likely played a minimal, if any, role in the observed sex differences. We also did not control for estrus cycle phase in the females. Future studies should take the fluctuation of sex hormones into consideration, as this will provide deeper insights into the mechanisms underlying sexual dimorphisms in the hormonal stress response and its effects on declarative memory. Another exciting avenue for further research that has received little attention to date is the investigation of possible evolutionary mechanisms mediating the variation in the stress response between the sexes.

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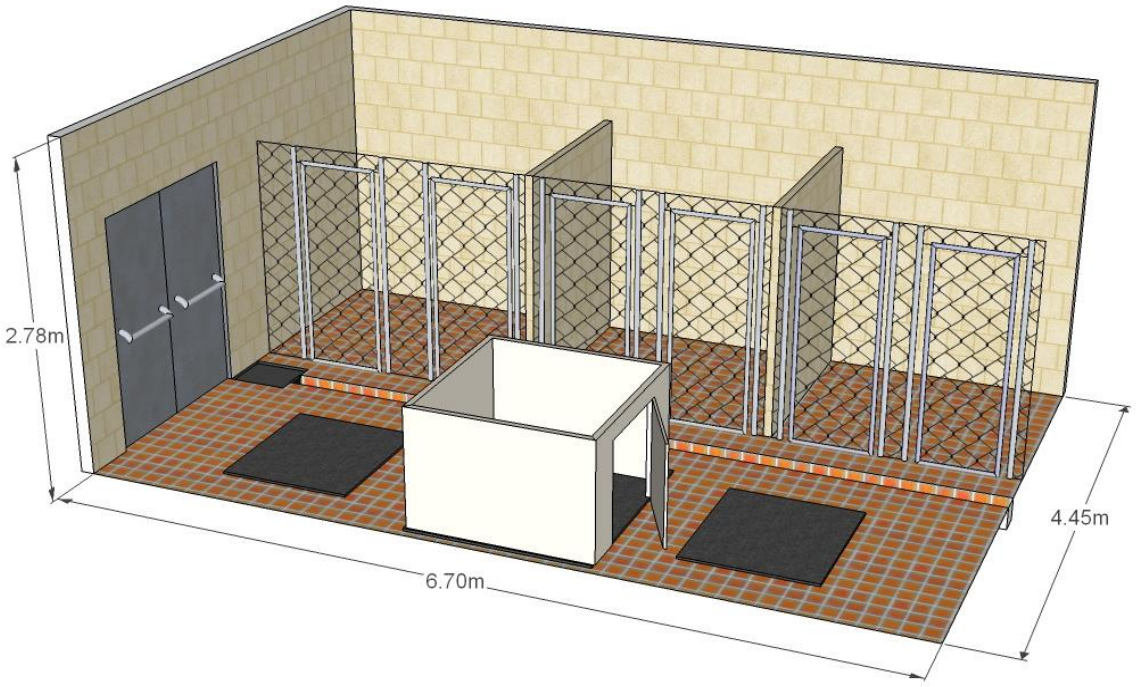
APPENDIX A: Sibling relationships between subjects

Sow	Boar #	Pig ID	Name	Sex	Birthdate
		676 Orange	Tabitha	Female	Sept 23 2012
		677 Orange	Matilda	Female	Sept 23 2012
Piggy Sue	118 White	303 White	Rupert	Male	Oct 4 2012
		304 White	Benjamin	Male	Oct 4 2012
		678 Orange	Charlotte	Female	Oct 4 2012
		679 Orange	Olivia	Female	Oct 4 2012
		680 Orange	Ruth	Female	Oct 4 2012
Olive	120 White	305 White	Ebenezer	Male	Oct 7 2012
Rosie	129 White	306 White	Cuthbert	Male	Oct 4 2012
		307 White	Wesley	Male	Oct 4 2012
		682 Orange	Isabel	Female	Oct 4 2012
		683 Orange	Judith	Female	Oct 4 2012
		684 Orange	Lucy	Female	Oct 4 2012
532 Yellow	126 White	308 White	Archibald	Male	Oct 4 2012
		310 White	Harold	Male	Oct 4 2012
		685 Orange	Harriet	Female	Oct 4 2012
		686 Orange	Alice	Female	Oct 4 2012
535 Yellow	129 White	311 White	Theodore	Male	Oct 7 2012
		312 White	Oliver	Male	Oct 7 2012
		313 White	Isaac	Male	Oct 7 2012

APPENDIX B: Diagram of the room in which pigs were housed throughout the experimental period.



APPENDIX C: Objects used in the solitary and group novel object tests.

Table 1.

Dominance hierarchy matrix for female pigs. Rows represent winners and the columns the losers in agonistic encounters. The order of pigs from left to right and top to bottom is most dominant to most subordinate.

	Harriet	Alice	Matilda	Lucy	Judith	Isabel	Tabitha	Ruth	Charlotte	Olivia
Harriet		5	5	7	4	•	2	•	7	8
Alice	2		2	2	2	•	2	4	4	1
Matilda	•	•		2	1	5	3	•	1	2
Lucy	•	•	•		12	3	4	6	2	8
Judith	•	•	•	•		5	3	•	2	1
Isabel	•	•	•	•	3		1	•	•	•
Tabitha	•	•	1	•	1	•		3	6	6
Ruth	•	•	•	•	•	•	•		7	2
Charlotte	•	•	•	•	•	•	•	1		1
Olivia	•	•	•	•	•	•	•	•	•	

Table 2.

Dominance hierarchy matrix for male pigs. Rows represent winners and the columns the losers in agonistic encounters. The order of pigs from left to right and top to bottom is most dominant to most subordinate.

	Archibald	Benjamin	Oliver	Theo	Ebenezer	Isaac	Rupert	Harold	Cuthbert	Wesley
Archibald		1	2	3	•	•	•	1	•	•
Benjamin			1	3	2	•	2	•	•	1
Oliver	•	•		3	3	2	1	3	2	•
Theo	•	1	•		4	1	2	•	1	•
Ebenezer	•	•	•	3		3	•	3	1	2
Isaac	•	•	•	•	•		2	•	2	1
Rupert	•	•	•	•	•	1		2	•	1
Harold	•	•	•	•	•	•	•		1	•
Cuthbert	•	•	•	•	•	•	•	•		1
Wesley	•	•	•	•	•	•	•	•	•	

APPENDIX E: Inter-observer reliability values

Table 1: Intraclass correlation coefficients for two-way mixed models (absolute agreement) obtained during inter-observer reliability analysis of data collected during Memory Test.

Observation type	Single measures intraclass correlation	p-value
Exploration duration of left-hand side object	.995	< 0.0001
Exploration duration of right-hand side object	.995	< 0.0001

Table 2: Intraclass correlation coefficients for two-way mixed models (absolute agreement) obtained during inter-observer reliability analysis of data collected during Novel Object Test (Solo)

Observation type	Single measures intraclass correlation	p-value
Object exploration duration	0.988	< 0.0001

Table 3: Intraclass correlation coefficients for two-way mixed models (absolute agreement) obtained during inter-observer reliability analysis of data collected during Novel Object Test (Group)

Observation type	Single measures intraclass correlation	p-value
Object exploration duration	0.998	< 0.0001

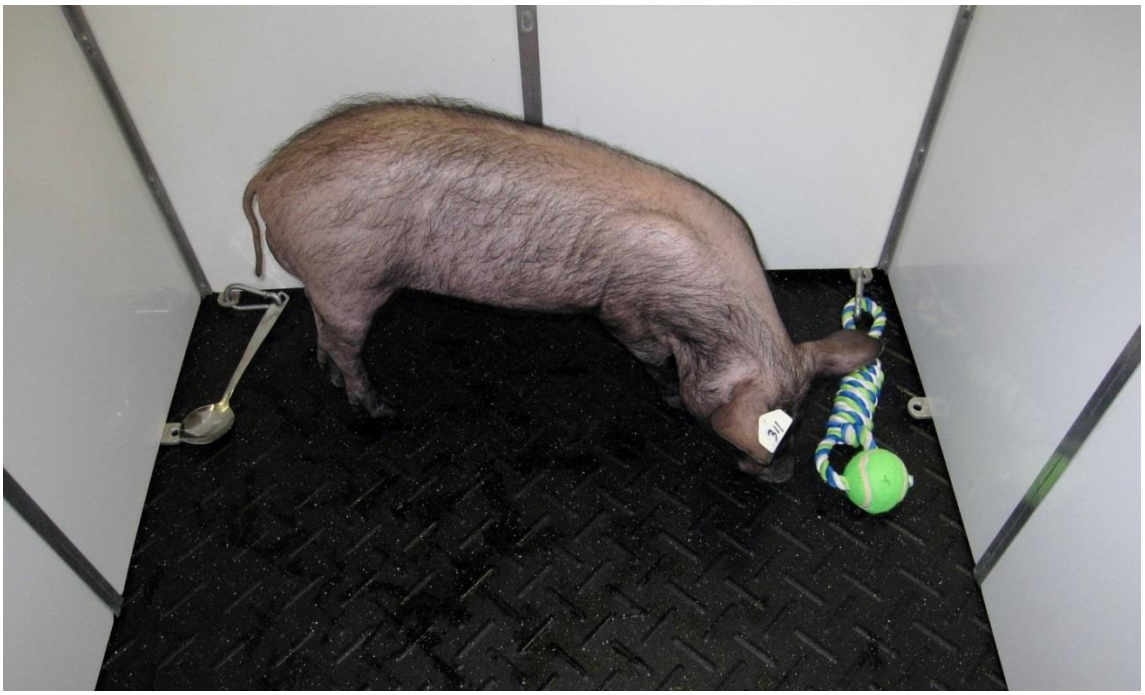
Table 4: Intraclass correlation coefficients for two-way mixed models (absolute agreement) obtained during inter-observer reliability analysis of data collected during Human Approach Test (Solo)

Observation type	Single measures intraclass correlation	p-value
Object exploration duration	0.966	< 0.0001

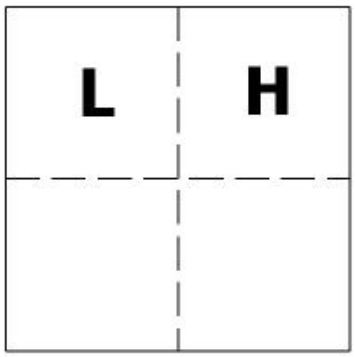
Table 5: Intraclass correlation coefficients for two-way mixed models (absolute agreement) obtained during inter-observer reliability analysis of data collected during Food Competition Test

Observation type	Single measures intraclass correlation	p-value
Dyadic wins and losses	0.882	< 0.0001

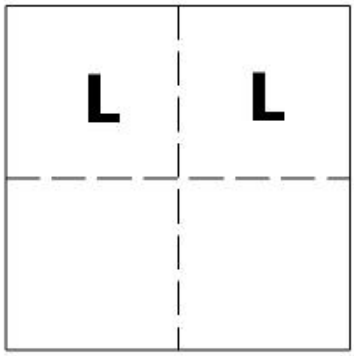
APPENDIX F: Theo in the test pen with the low priority object (i.e. spoon) and a high-priority object (i.e. rope toy).



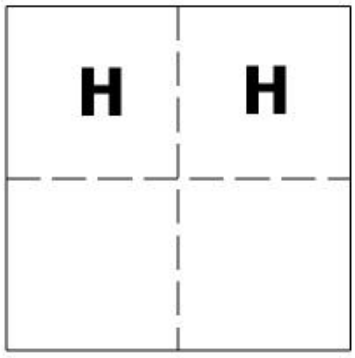
APPENDIX G: Example trial of the object location memory task. L=low priority object; H=high priority object.



Exposure Phase
0 min after stressor



Phase A
10 min after stressor



Phase B
20 min after stressor

APPENDIX H: Objects used in the object location memory task.