(Em)pathetic pigs?

The impact of social interactions on welfare, health and productivity

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Thesis

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Abstract

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The welfare, health and productivity of intensively raised pigs may be affected by routine management procedures and the physical environment they are housed in, but also by their social environment, i.e. by social interactions between pen mates. In this thesis, the effect of social interactions on pig welfare, health and productivity has been investigated in several ways. On the one hand, a new breeding method based on interactions, i.e. on heritable effects on the performance of pen mates, was investigated. The effect of divergent selection for a relatively positive or negative indirect genetic effect on growth of pen mates on pig behavior and physiology was studied. On the other hand, it was investigated whether pigs can be affected by (the emotional state of) their pen mates on the basis of two social processes, emotional contagion and social support. Pigs selected for a relatively positive indirect genetic effect on the growth of their pen mates seemed less fearful and less stressed in several novelty tests and they had lower leukocyte, lymphocyte and haptoglobin concentrations compared to pigs selected for a relatively negative indirect genetic effect on the growth of their pen mates. Moreover, it was found that pigs can indeed be affected by the emotional state of their pen mates either in a positive or negative way, which points to emotional contagion, a simple form of empathy, in pigs. Furthermore, evidence for social support has also been found. To conclude, this breeding method may be a strategy to improve the social environment of intensively raised pigs as pigs with relatively positive indirect genetic effects for growth may create a less stressful social environment for themselves. In addition, the welfare, health and productivity of pigs may not only depend on their own emotional state, but also on the emotional state of their pen mates.

Table of contents

CHAPTER 1

General Introduction

Animal welfare research is concerned with the well-being of captive animals of which feeling or emotions have become an important aspect (Désiré et al., 2002; Mendl and Paul, 2004; Boissy et al., 2007; Špinka, 2012). Emotions can be defined as intense, but brief subjective responses to a particular situation (Rolls, 2000; Spoor and Kelly, 2004; Boissy et al., 2007). These subjective responses cannot be measured in animals, but the behavioral, (neuro)physiological, and cognitive components which accompany the subjective responses can be measured (Désiré et al., 2002; Mendl and Paul, 2004; Paul et al., 2005). Intensively housed farm animals such as pigs (*Sus scrofa*) are generally subjected to several standard management procedures such as tail docking, castration, mixing with unfamiliar pigs, and relocation which have been shown to lead to fear and stress (e.g. Noonan et al., 1994; Stookey and Gonyou, 1994; Geverink et al., 1998; Von Borell, 2001; Dudink et al., 2006; Rault et al., 2011) which in turn have implications for pig welfare, health and productivity (Hemsworth, 2003; Held et al., 2009). Moreover, the expression of fear and stress by one pig could lead to fear and stress in other pigs if pigs are sensitive to each other's emotions. If so, pigs are not only negatively affected by the stressful events they are exposed to, but also by those imposed on other pigs in their environment. This could lead to repeated or even chronic stress which affects the welfare, health and productivity of the whole group. On the other hand, the spread of positive emotions would likely improve the welfare, health and productivity of pigs (Špinka, 2012). Therefore, it is not only important to study emotions in individual pigs, but also to what extent the emotional state of individual pigs affects the emotional state of other pigs.

In the project described in this thesis, several ways in which social processes could affect welfare in pigs, including the degree of positive and negative emotional states, were investigated. Firstly, it was investigated whether fear and stress could be reduced by a novel breeding method based on interactions, i.e. heritable effects on the performance of group members. As interactions between pigs may be affected by their environment and their coping style, effects of housing conditions and coping style on fear and stress were also investigated. Furthermore, pigs might be affected by the emotional state of other pigs through emotional contagion and social support. Therefore, it was investigated to what extent these two social processes occur in pigs. Hereto, the concepts of breeding, housing, coping style, emotional contagion and social support are first described in more detail in this chapter after which the aim and outline of this thesis are presented.

The art of breeding

From the first indications of pig domestication between 5000 and 10,000 years ago (Graves, 1984; Giuffra et al., 2000; Larson et al., 2005) to the highly efficient pig production systems of today, large changes have been made: pigs lived, for instance, in relatively small groups outdoors and were free to roam around under the protection of a swineherd early in domestication (D'Eath and Turner, 2009), whereas most pigs now live indoors in closed pens and at a high stocking density (Spoolder et al., 2000; Van de Weerd and Day, 2009). To illustrate this, about 12 million pigs were produced on farms in The Netherlands in 2012 alone (website Centraal Bureau voor de Statistiek). Such immense productivity is to a large extent made possible due to successful breeding programs, but other procedures such as reducing costs of housing and availability of high quality feed also contributed (Rauw et al., 1998; Stricklin, 2001; Prunier et al., 2010). Pig breeding is the process of selecting and mating a boar (father) and a sow (mother) to produce a next generation (Bijma, 2012). Which boars and sows are selected is based on which phenotypical traits are needed in the next generation. A phenotypical trait can be defined as an observable trait of an individual (Bijma, 2012) such as growth rate, the number of piglets in a litter, milk production, and back fat (Rauw et al., 1998; Merks, 2000). Simply said, a phenotypical trait - from here on referred to as trait - of an individual is the sum of a genetic component (A) and an environmental, non-genetic component (E) (Bijma, 2012):

$$P = A + E \tag{1}$$

According to the principles of inheritance, half of the genes of a piglet are maternal and half are paternal. Thus, by careful selection of the mother and father it is possible to obtain the desired traits in the offspring. Hereby, breeders make use of the breeding value which is the expected trait of an individual given its set of genes (Bijma, 2012). As shown by the model, however, a trait is not only determined by the genetic component. A pig may have, for instance, a high growth potential based on its breeding value, but if this pig does not get proper feed, an environmental, non-genetic component, its growth will not be as expected. This environmental component can, however, not be estimated by breeders. As there is also no one to one relationship between genes and traits (Porter, 1993; Rauw et al., 1998), the process of breeding is complicated, but not impossible thanks to very large databases in which all kinds of information on pigs has been stored (e.g. the database of the pig breeding company Topigs contains records of 20 million pigs (website Topigs)). Breeding companies decide which traits are important to select for. These are largely based on demands from producers in the sector who in turn are guided by market and efficiency (Porter, 1993).

Although this way of breeding has resulted in good quality meat for an affordable price, several disadvantages have also become apparent (Merks, 2000). For instance, selection on one particular trait (e.g. large litters) could lead to a negative effect on another trait (e.g. higher piglet mortality) (Rauw et al., 1998; Bijma, 2012). Moreover, pigs selectively bred for meat production are suggested to suffer from health problems such as leg weakness (Rauw et al., 1998; Prunier et al., 2010) and a higher susceptibly to stress (Prunier et al., 2010). Furthermore, selection for the best performing individuals may result in individuals that are (highly) competitive towards their group mates. If so, this may result in a lower performance of the group as a whole than expected based on the individual breeding values (Muir and Craig, 1998; Bijma, 2012) and may have negative consequences for welfare. In an experiment of Muir (2005), Japanese quail were housed in groups, but were selected on the highest individual body weights at six weeks of age. After 25 generations, the body weight of the quails had, however, not increased. This was caused by the fact that individual selection for a higher body weight resulted in a large increase in mortality at six weeks of age due to fighting and cannibalism (i.e. 24 % mortality in the 25th generation compared to 6 % mortality at the start of the selection experiment) (Muir, 2005; Rodenburg et al., 2010). This example illustrates that individual selection in group-housed animals could indeed lead to selection of traits (i.e. cannibalism) that are not optimal for the group as a whole and may instead lead to increased levels of negative social interactions and poor group performance (Rodenburg et al., 2010).

An option could be to select those animals that show desired behavior(s). But that means that behavioral observations have to be carried out for thousands of individuals which is both costly and labor-intensive. Behavior is, therefore, usually not taken into account in breeding programs and this is not likely to change in the near future (Turner, 2011; Bijma, 2012). Another solution may, however, be presented in group selection instead of individual selection and that may, indirectly, also affect behavior (Bijma, 2012). A model for group selection was already proposed by Griffing in the sixties (Griffing, 1967), but his model was not applicable for animal breeders and had therefore limited impact (Bijma et al., 2007a; Bijma, 2012). Muir (2005) and Bijma et al. (2007a,b) adapted Griffing's model in such a way that it was applicable for animal breeders. Muir and Bijma reasoned that a trait of an individual living in a group would not only be affected by the genes of that individual, but also by the other individuals in that group due to social interactions. Therefore, they adapted the current breeding model in the following way: in (1), the group members of an individual are considered to be part of the environmental component, but as these group members have a set of genes too they should not be part of that non-genetic, environmental component. Hence, in the adapted model, model (2), the genetic component of the group mates is represented by a third component. Accordingly, an environmental component of the group mates was included too.

$$P_i = A_{D,i} + E_{D,i} + \sum_{i \neq j}^{n-1} A_{S,j} + \sum_{i \neq j}^{n-1} E_{S,j} \tag{2}$$

With this model, the trait of each individual living in a group of *n* individuals can be modelled as the sum of the genetic ($A_{D,i}$) and environmental effect ($E_{D,i}$) of the individual itself and the summed genetic ($A_{S,j}$) and environmental effects ($E_{S,j}$) of its *n-1* group mates. These genetic effects of the group mates on the trait of an individual are referred to as indirect genetic effects (Moore et al., 1997; Wolf et al., 1998), but also as associative, social genetic, or competitive effects (Griffing, 1967; Arango et al., 2005; Muir, 2005; Bijma et al., 2007a) or social breeding values (SBV) (Bijma et al., 2007a,b). So, via indirect selection on each other's performance, animals can perform better as a group. Several studies have estimated that a pig's growth can substantially be affected by SBV (Chen et al., 2007; Bergsma et al., 2008, 2013), but at present it is not yet understood how pigs actually can affect each other's growth. Aggressive and competitive behavior towards pen mates and oral manipulations (e.g. tail biting) of pen mates are proposed as likely candidates (Rodenburg et al., 2010), but (non-behavioral) processes such as disease transmission may also be implicated (Yirmiya et al., 2000; Lipschutz-Powell et al., 2012). There is, however, another candidate that must be considered here. This candidate may be less tangible, but is therefore not less relevant. Pigs might also affect each other's growth by affecting each other's emotions. In a series of experiments Hemsworth and colleagues (e.g. Hemsworth et al., 1981; Hemsworth and Barnett, 1991; Hemsworth, 2003) showed that treating pigs aversively (i.e. light slapping or giving a mild electric shock) for 30 s to 2.5 min/d resulted in growth reductions of on average 25 g/d during the treatment period compared to pigs that were treated pleasantly (i.e. gentle stroking). Hemsworth (2003) proposed that the aversive handling made pigs fearful of humans which resulted in a (chronic) stress response and thereby diminished growth. Thus, if pigs are sensitive to the fear or stress expressed by their pen mates during standard management procedures for instance, they might become fearful or stressed themselves which then leads to a reduced group performance. Interestingly, changes in fear-related behaviors and stress sensitivity have already been found in laying hens selected on high group performance (e.g. group survival) as compared with laying hens subjected to mere common breeding practices (e.g. Cheng and Muir, 2005; Bolhuis et al., 2009; Rodenburg et al., 2009; Nordquist et al., 2011). Therefore, fearfulness or stress sensitivity could also be related to SBV for growth in pigs. If pigs with a relatively positive effect on the growth of their pen mates are found to be less sensitive to fear or stress, this breeding method could be a strategy to reduce fear or stress in intensively housed pigs and thereby improve their welfare.

Housing conditions

Apart from breeding, changes to the housing conditions of pigs have also been proposed to reduce fear and stress. Pigs are highly motivated to perform explorative behaviors as rooting, sniffing, grazing and chewing, because that is their natural way to find food (Studnitz et al., 2007; De Jonge et al., 2008; D'Eath and Turner, 2009). Intensively kept pigs do not have to search for their food, but the need to perform foraging and explorative behaviors has not been lost during years of selection and breeding (Stolba and Wood-Gush, 1989; Price, 1999). Therefore, farmers are obliged by law to provide sufficient substrate such as straw, hay, wood, sawdust, mushroom compost and peat to the pigs to fulfill this need (EU directive 2001/93EC (Studnitz et al., 2007)). At present, these substrates are, however, hardly provided to pigs, because they block the liquid-slurry system (Van de Weerd and Day, 2009). Instead, pigs are usually provided with a chain or other objects (Bracke et al., 2013). Unfortunately, pigs can become quickly habituated to these objects as they are not edible or destructible and therefore do not stimulate foraging and explorative behaviors (Day et al., 2002; Studnitz et al., 2007). To still fulfil their exploration needs, pigs may then use their pen mates as substrate leading to harmful behaviors such as ear and tail biting (e.g. Fraser et al., 1991; Beattie et al., 1995), with all the consequences which that entails (Zonderland et al., 2008; Taylor et al., 2010). In addition, barren environments have also shown to lead to cognitive impairment (De Jong et al., 2000; Sneddon et al., 2000; Bolhuis et al., 2013) and to fearful and even chronically stressed pigs (Beattie et al., 2000; De Jong et al., 2000; Wemelsfelder et al., 2000). That housing conditions can affect pigs emotionally has also been found by Douglas et al. (2012), who showed that barren housed pigs respond more pessimistically in a judgment bias task than enriched (i.e. substrate provided) housed pigs. Changes to the current housing conditions with respect to enrichment may therefore profoundly improve the quality of life for pigs, but it is not easily implemented in current practice (Te Velde et al., 2002; Bock and Van Huik, 2007). This may be overcome with the development of completely new housing systems (e.g. De Greef et al., 2011), but that is a difficult and time consuming process (Grin et al., 2004; Elzen et al., 2011).

Compared to housing condition changes, the breeding method described earlier is more easily implemented in practice, but likely less accepted by society. This is because morally it may feel wrong to adapt an animal to its environment instead of adapting the environment to the animal (Sandøe et al., 1996; Millet et al., 2005) and because there is no consensus as to how far adaptions to the animal may go (Star et al., 2008). Thus, both breeding and environmental enrichment have the potential to improve pig welfare by reducing fear and stress. The potential of breeding to reduce fear and stress by taken into account SBV for growth is, however, largely unknown. In addition, it is also not known whether pigs diverging in SBV for growth respond differently to different housing conditions. Therefore, the separate and interactive effects of SBV for growth and housing conditions on fear and stress were investigated in this thesis (Swanson, 1995; Muir and Craig, 1998).

Coping styles

In many different animal species, the existence of different coping styles has been demonstrated (Gosling, 2001; Carere et al., 2010). Coping styles, which are also referred to as personalities, temperaments, or behavioral syndromes (Koolhaas et al., 2010), can be defined as individual differences in behavior with underlying differences in (neuro)physiology that are consistent across time and across situations (e.g. Sih et al., 2004a; Koolhaas et al., 2010). Two coping styles are usually described in animals: a proactive and a reactive coping style. Generally, proactive animals are characterized as aggressive, active, bold and prone to take risks. Reactive animals, on the other hand, are less aggressive, more cautious and avoid taking risks (Sih et al., 2004b; Coppens et al., 2010; Koolhaas et al., 2010). With the knowledge that animals have different coping styles, researchers in the field of ecology and evolution are now making progress in discovering the functional significance of different coping styles (e.g. Réale et al., 2010; Stamps and Groothuis, 2010).

Coping styles have also been described in pigs. Different methods have been used to study coping styles in pigs (Forkman et al., 1995; Spoolder et al., 1996; Erhard et al., 1999), of which the backtest (Hessing et al., 1993) has been used most frequently. Pigs are usually subjected to the backtest between one and three weeks of age. In this test, a piglet is put on its back on a surface (e.g. a table) outside its home pen and manually restrained for 1 min during which the number of struggles and vocalizations and their latencies are recorded. Piglets that struggle (and vocalize) relatively much are classified as having a high-resisting (HR) or proactive coping style and piglets that hardly struggle (and vocalize) are classified as having a low-resisting (LR) or reactive coping style. From its first use by Hessing et al. (1993), the backtest has been used in numerous other studies as a means to classify pigs and to see whether HR and LR pigs also differ in a consistent way in other situations later in life (e.g. thesis of Hessing, 1994; Ruis, 2001; Van Erp-van der Kooij, 2003; Bolhuis, 2004). Although the use of the backtest as a means to assess a pig's coping style has been questioned on a methodical, functional, and predictive level (Jensen, 1995; Jensen et al., 1995; D'Eath and Burn, 2002), most studies have shown that a pig's backtest response is to a certain extent predictive of its behavioral, (neuro)physiological and immunological response to other situations later in life, up to adulthood (e.g. Hessing et al., 1994a; Schrama et al., 1997; Bolhuis et al., 2000, 2003; Geverink et al., 2002; Karman, 2003).

The coping style of the pigs was taking into account in this thesis for three reasons. Firstly, pigs with different coping styles respond differently to a stressful or challenging situation. Generally, HR pigs respond to such situations with more active behaviors such as locomotion, escape attempts and vocalizations (Hessing et al., 1994a; Bolhuis and Schouten, 2002; Geverink et al., 2002; Jansen et al., 2009), whereas LR pigs respond with more passive behaviors such as standing alert (Bolhuis and Schouten, 2002; Jansen et al., 2009). Without information on the coping style of the animal under study, inappropriate conclusions might be drawn about the level of fear or stress of individual pigs in a particular situation. Secondly, concerns have been raised that the breeding method investigated here could affect the distribution of coping styles (Rodenburg et al., 2010). If for instance SBV for growth would be related to aggressive behavior, selection of pigs with a relatively positive effect on the growth of their pen mates would then lead to less aggressive pigs, but also to more LR pigs as these pigs are suggested to be less aggressive towards their pen mates after mixing (Hessing et al., 1994b; Bolhuis et al., 2005a) and under stable conditions (Bolhuis et al., 2005b, 2006a). If so, keeping pigs of only one coping style could then have further implications for their health (Hessing et al., 1995; Bolhuis et al., 2003), behavior (Bolhuis et al., 2005b, 2006a) and productivity (Hessing et al., 1994b). Hence, it is important to find out whether including SBV for growth into the breeding program has consequences for the (distribution of) coping style of pigs. Thirdly, pigs with different coping styles have been found to respond differently to different housing conditions (Bolhuis et al., 2003, 2004, 2005b, 2006a; Melotti et al., 2011). For example, HR and LR pigs did not differ in play behavior in barren housing, but LR pigs played more than HR pigs in enriched housing. On the other hand, oral manipulation of pen mates was not different between HR and LR pigs in enriched housing, but LR pigs showed more of this behavior than HR pigs in barren housing (Bolhuis et al., 2005b). This suggests that when investigating the effects of different housing conditions, the presumably existing individual differences of the animals under study should not be ignored (Bolhuis, 2004).

Emotional contagion

Thus, it can be hypothesized that pigs may affect the growth of their pen mates if pigs are sensitive to each other's emotional state. To be affected by and to share the emotional state of another individual is termed emotional contagion (Preston and De Waal, 2002; De Waal, 2008). Emotional contagion is regarded as the most simple form of empathy, because it occurs without any kind of cognitive perspective taking (Preston and De Waal, 2002; De Waal, 2008). This is often explained with the example of crying infants: upon hearing an infant cry, other infants usually start crying too, but these infants do not (yet) understand why that infant started to cry in the first place (e.g. Miller, 2006; Panksepp and Lahvis, 2011). Some authors argue that, particularly in humans, emotional contagion goes hand in hand with behavioral mimicry (i.e. the automatic synchronization of facial expressions, vocalizations, postures and movements with those of another individual (e.g. Hatfield et al., 1993; Panksepp and Lahvis, 2011)), but others do not make this association per se (De Waal, 2008; Edgar et al., 2012; Špinka, 2012). In this thesis, emotional contagion is viewed in accordance with the latter. Emotional contagion starts with registering the emotional state of the other individual (Edgar et al., 2012). This emotional state can be expressed through a combination of behavior, vocalizations and/or pheromones (Amory and Pearce, 2000; Paul et al., 2005; Špinka, 2012) which is then accordingly seen, heard and/or smelled by the observer. Thereafter, an emotional response is generated in the observer individual (Edgar et al., 2012). Preston and De Waal (2002) provide evidence that the emotional response in the observer is generated through a perception action mechanism in which neural representations of the emotional state – in all its components, e.g. behavioral, physiological and subjective components (Bastiaansen et al., 2009) – are automatically and unconsciously activated. In this mechanism, many brain areas are proposed to be involved (Preston and De Waal, 2002; Singer, 2006; Bastiaansen et al., 2009; Shamay-Tsoory, 2011) which may be interconnected through oxytocin (Shamay-Tsoory, 2011). Oxytocin is a peptide of nine amino acids which is traditionally known for its role in the process of giving birth and lactation (Uvnäs-Moberg, 1998). Research has shown, however, that oxytocin is also involved in quite some other biological processes (e.g. Uvnäs-Moberg et al., 2005; Neumann, 2008; Heinrichs et al., 2009; Rault et al., 2013), including emotional contagion (Domes et al., 2007; Guastella et al., 2010; Hurlemann et al., 2010; Lane et al., 2012). Hurlemann et al. (2010) showed, for instance, that human male subjects that were given an intranasal administration of oxytocin were emotionally more affected by photos of other humans expressing a range of emotions, positive and negative, than subjects that received a placebo. Emotional contagion is considered as the phylogenetically earliest form of empathy (Preston and De Waal, 2002; De Waal, 2008) and, therefore, likely to occur in many different animal species (Špinka, 2012). Indeed, emotional contagion has been described to occur in primates, birds, rats and mice (reviewed in De Waal, 2008; Panksepp and Lahvis, 2011; Edgar et al., 2012).

Intensively housed pigs are kept in close proximity to each other with often no possibilities to escape from the emotional state of their group mates during stressful events. It is, therefore, very likely that pigs are exposed to and subsequently affected by the distress of their group mates. The extent to which they are affected, i.e. the extent to which emotional contagion occurs, depends on their capacity for empathy (Edgar et al., 2011). At present, there are only two studies that have investigated emotional contagion in pigs (Anil et al., 1997; Düpjan et al., 2011). Both studies, however, found no evidence for emotional contagion in pigs, but that could have been due to their experimental set up. Anil et al. (1997) investigated whether pigs were affected by witnessing their conspecifics being slaughtered by measuring their heart rate, packed cell volume, cortisol, ß-endorphin, vocalizations and struggle behavior. They found no evidence that the witnessing pigs were affected by the slaughter of a conspecific. However, the transport to the slaughterhouse, handling and sampling techniques, and the fact that the witnessing pigs were restrained in a harness could have made them already so stressed that any additional stress from seeing a conspecific being slaughtered could not be shown anymore, i.e. a ceiling effect was reached (Edgar et al., 2012). In addition, as pigs were not habituated to the test procedures and no control treatment was applied, the conclusions drawn by Anil and coworkers are limited (Edgar et al., 2012). In the study of Düpjan et al. (2011) pigs were individually exposed to the playback of distress calls (i.e. screams) of a conspecific or to artificial noise in a test room.

They measured locomotive and eliminative behavior, vocalizations, and heart rate and heart rate variability responses to both sounds. Based on their results, Düpjan and coworkers concluded that the exposed pigs did not share the stress of the caller pig and, thus, no evidence of emotional contagion was found. The exposed and caller pigs were, however, unfamiliar to each other which may have prevented emotional contagion to occur (Langford et al., 2006). In addition, hearing another pig, even when that pig is distressed, while being socially isolated from its pen mates could have actually made the exposed pigs less stressed. Therefore, it cannot be concluded from their study that emotional contagion just does not occur in pigs.

Most emotional contagion studies in animals, including the two pig studies described above, focussed on emotional contagion of negative emotional states (De Waal, 2008; Edgar et al., 2012; Špinka, 2012). For the welfare of animals, however, emotional contagion of positive emotional states is just as important to investigate (Held and Špinka, 2011; Špinka, 2012). Hence, emotional contagion during negative as well as positive situations is studied in more detail in this thesis.

Social support

During emotional contagion as introduced above, an individual shares the emotional state - positive or negative - of another individual. It could, however, happen that when the other individual is in a negative emotional state, the observer individual is able to separate the stress of the other individual from its own emotional state and, thereby, does not become stressed itself (Edgar et al., 2012). Moreover, the observer individual might even be able to lower the stress experienced by the other individual through its behavior, i.e. the observer individual provides social support to the stressed individual (Edgar et al., 2012). This example shows that emotional contagion and social support are closely linked. Social support or social buffering can, thus, be defined as the ability of one or more social partner(s) to lower the stress response of an individual that is subjected to a stressful experience (Kikusui et al., 2006; Hennessy et al., 2009). The value of social support is clearly seen in people with HIV/AIDS (Rault, 2012) and cancer (e.g. Kroenke et al., 2006), or generally in disease outcome (Uchino et al., 1996). For instance, Leserman et al. (1999) found that the progression of HIV over a period of 5 years was much faster in men that received no social support compared to men that received social support. Social support is not a phenomenon that only occurs in humans, because it has also been described to occur in other animal species such as squirrel monkeys, guinea pigs, dogs, sheep and even zebra finches (reviewed in Kikusui et al., 2006; Hennessy et al., 2009; Rault, 2012).

As pigs undergo various stressful situations during their life, providing social support in one way or another during and after these situations could be beneficial for their health and welfare. Indeed, several studies have suggested or shown that pigs are able to benefit from social support (Fraser, 1974; Arnone and Dantzer, 1980; Geverink et al., 1998; Ruis et al., 2001; Hameister et al., 2012) and the provider does not have to be a conspecific, because a familiar person is able to provide support just as well (Bolhuis et al., 2006b). However, before social support can be applied in practice, we need to know exactly in what circumstances social support would be beneficial and would work and who should provide the support (Kikusui et al., 2006; Hennessy et al., 2009; Rault, 2012). Social support in pigs might, for instance, be dependent on their coping style. Research has shown that the (behavioral) differences between HR and LR pigs, as described previously, could stem from a difference in use of environmental stimuli. Pigs with a HR coping style seem to act primarily on the basis of previous experience, i.e. they develop routines more easily, whereas pigs with a LR coping style seem to act primarily on the basis of actual environmental information (Bolhuis et al., 2004; Koolhaas et al., 2010). If so, it can be hypothesized that in times of stress LR pigs might profit more from social support, because they are more attentive to environmental cues than HR pigs.

Aim and outline of the thesis

The aim of this thesis was to investigate to what extent pig welfare, including emotional states, could be affected by social processes. Firstly, the level of fear and stress of a first generation of pigs, divergently selected to genetically affect the growth of their pen mates during the finishing phase (i.e. from 25 kg to 110 kg (slaughter weight)) either relatively positively (+SBV) or negatively (–SBV), was studied before weaning (chapter 2) and after weaning (chapter 3) by subjecting the pigs to a group-wise novel object test, a group-wise human approach test and an individual novel environment test. The coping style of these pigs was taken into account in chapter 2 and 3 as well, because selection on SBV for growth might affect a pig's coping style and it is known that pigs with different coping styles express fear and stress in a different way. From weaning to slaughter, half of the pigs were housed in barren pens and the other half in pens enriched with a deep litter bedding of straw and wood shavings. In this way, it could be studied whether the behavior of pigs diverging in SBV for growth and coping style is different in different environments (chapter 3). Moreover, the salivary cortisol response to the individual novel environment test was measured in order to get a first idea of whether +SBV and –SBV pigs also differ physiologically with respect to fearfulness and stress (chapter 3). In chapter 4, the effects of SBV for growth, coping style and housing on immune parameters and (stress)physiology were studied. Therefore, three blood samples were taken from the pigs at 8, 9 and 22 weeks of age. Between 8 and 9 weeks of age, pigs were subjected to a 24 h regrouping test which induces acute stress.

As emotional contagion could be a mechanism underlying SBV for growth, but also because of the implications for welfare, health and productivity, emotional contagion was studied in more detail in chapter 5 and 6. In chapter 5, emotional contagion was studied during anticipation and during a positive and negative treatment. To this end, one must first understand how a pig expresses a certain emotional state. Indicators for positive and negative emotional states were thus also studied in chapter 5. The results of chapter 5 indicated that emotional contagion can indeed occur in pigs. Hence, emotional contagion was further studied in chapter 6 by exploring the role of oxytocin in emotional contagion. Instead of sharing emotions, emotions may also be buffered. Therefore, social support and coping style effects were studied in chapter 7. In chapter 8, the general discussion, the major findings of these studies are discussed. This outline is graphically presented in Fig. 1.

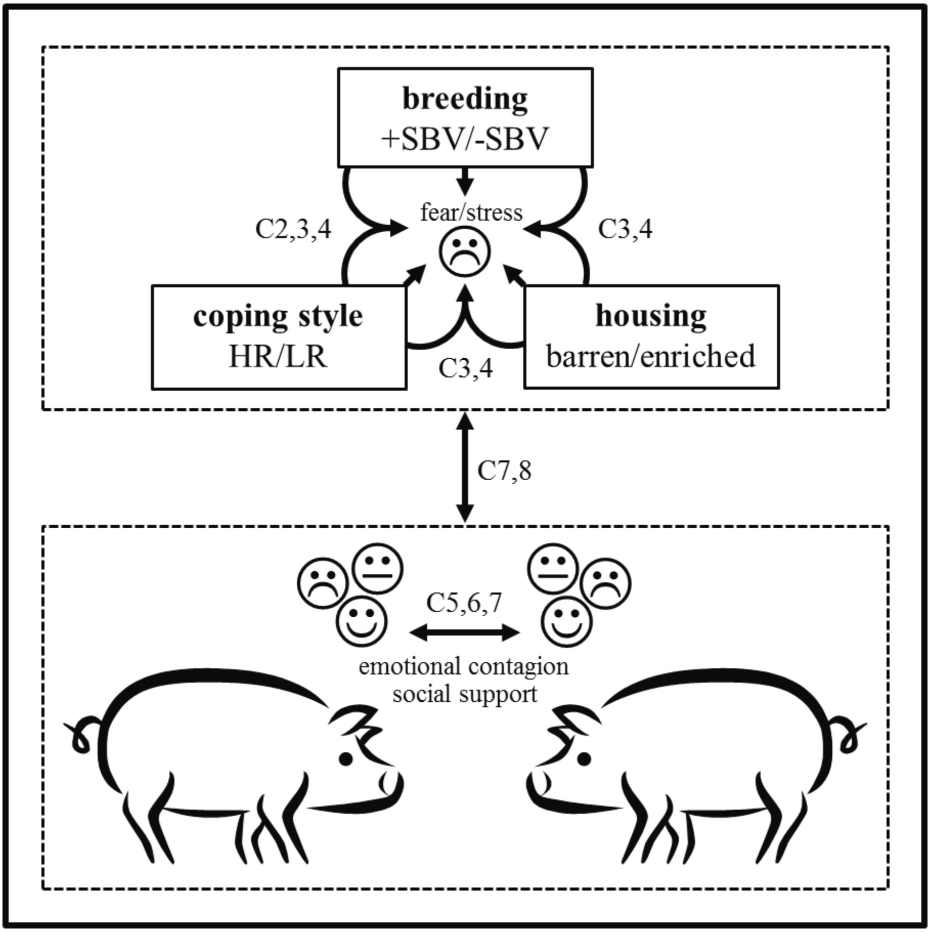

Fig. 1. Schematic overview of the outline of the thesis. The C's followed by two or more numbers refer to the different chapters. In chapters 2, 3 and 4, the effect of SBV for growth, housing conditions and coping style on fear and stress were studied. In chapters 5, 6 and 7, the extent to which pigs can be affected by the emotional state of their pen mates through emotional contagion and social support was studied.

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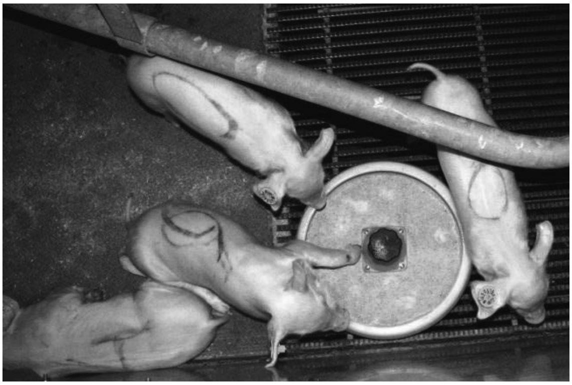
CHAPTER 2

Backtest and novelty behavior of female and castrated male piglets, with diverging social breeding values for growth



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Abstract

Pigs housed together in a group influence each other's growth. Part of this effect is genetic and can be represented in a social breeding value. It is unknown, however, which traits are associated with social breeding values. The aim of this study was, therefore, to investigate whether personality and response to novelty could be associated with social breeding values for growth in piglets. Female and castrated male piglets from 80 litters, with either an estimated relative positive or negative social breeding value (+SBV or –SBV) for growth, were individually tested in a backtest and novel environment test, and group-wise in a novel object (i.e. a feeder with feed) test and human approach test. All tests were performed during the suckling period. No differences between +SBV and –SBV piglets were found for the frequency and latency of struggling and vocalizing in the backtest (at least, $P > 0.30$). In the novel object test, piglets with a +SBV for growth touched the feeder faster than piglets with –SBV for growth ($P = 0.01$) and were more frequently present near the person in the human approach test ($P < 0.01$). No behavioral differences between +SBV and –SBV piglets were found in the novel environment test (at least, $P > 0.40$), but piglets that struggled more in the backtest walked more in this test ($P = 0.02$). Behavior was affected by gender in each test. Female piglets were faster than castrated male piglets to start struggling in the backtest ($P = 0.047$). In the novel object test, females were faster than males to touch the feeder and sample the feed. In the human approach test, they were also faster than male piglets to touch a person (all, $P < 0.001$). Females were also more frequently present near the feeder ($P < 0.001$) and person ($P = 0.03$). In the novel environment test, female piglets explored the floor more ($P = 0.046$), produced less low- ($P = 0.04$) and high-pitched vocalizations ($P = 0.02$), and defecated ($P = 0.08$) and urinated less than male piglets ($P < 0.01$). It was concluded that +SBV and –SBV piglets do not differ in their response to the backtest, and only subtle differences were found in their response to novelty. More research is warranted to identify the traits underlying SBV for growth in pigs. Moreover, castrated male piglets seemed to react more fearfully to each test than female piglets.

Key words: backtest, gender differences, indirect genetic effects, novelty behavior, pigs, social breeding values

1. Introduction

Social interactions between individuals have largely been ignored in animal breeding, where selection traditionally targets individual performance (Bijma, 2012). There is increasing evidence, however, that performance of an individual animal is not only affected by its own genes but also by genes of the other individuals in its social group (Griffing, 1967; Muir, 1996, 2005; Bijma et al., 2007a,b; Chen et al., 2008; Ellen et al., 2008). This effect, which is referred to as associative effect, social genetic effect, or indirect genetic effect (e.g. Wolf et al., 1998; Bouwman et al., 2010; Duijvesteijn et al., 2012), is represented in a social breeding value (Bijma et al., 2007a). In pigs, social breeding values have been calculated for growth and it has been estimated that pigs have a substantial heritable effect on the growth of their pen mates during the finishing period (Bergsma et al., 2013).

At present, it is, however, not clear how pigs could heritably affect their pen mates' growth, but this could be related to their personality and behavior (e.g. Rodenburg et al., 2010). Being fearful, for instance, can have negative consequences for performance (Hyun et al., 1998; Hemsworth, 2003; Jones and Boissy, 2011) and the presence of fearful hens in a group has been found to affect their group mates' ability to cope with fear and stress (De Haas et al., 2012). Moreover, in laying hens, social breeding values for survival were found to coincide with differences in fear-related behavior (Bolhuis et al., 2009; Rodenburg et al., 2009; Nordquist et al., 2011). This study, therefore, aims to investigate the personality and fearfulness of pigs selected for diverging social breeding values for the growth of their pen mates by submitting piglets to a backtest (Bolhuis et al., 2005a), and a novel object test, human approach test, and novel environment test (Forkman et al., 2007).

2. Materials and methods

2.1. Animals and housing

In this experiment, the personality and fear-related behavior of piglets of 80 litters, equally divided over 5 batches, were studied. For this purpose, Topigs-20 sows (64 in total) and Tempo boars (24 in total) with the most extreme positive or negative estimated social breeding value (SBV) for growth, i.e. estimated heritable effect a pig has on the growth of its pen mates during the finishing period (~25 to 110 kg), available within a batch, were mated (for details see Camerlink et al., 2013). Averaged over 5 batches, this resulted in 40 litters with an estimated positive social breeding value (+SBV) of +1.96 ± 0.13 g/d and 40 litters with an estimated negative social breeding value (–SBV) of –1.60 ± 0.11 g/d. During the finishing period, growth of a pig is affected by each of its pen mates, which means that the total effect on that pig's growth is obtained by multiplying the estimated SBV with n – 1 pigs in that pen. The genetic effect each piglet has on its own growth (i.e. its direct breeding value) was kept as similar as possible for both SBV classes (Camerlink et al., 2013).

Per batch, 16 sows were divided over 2 farrowing rooms with 8 sows per room, balanced for SBV class (i.e. +SBV and –SBV). Sows were fed according to standard procedures of the farm and water was available ad libitum. Piglets were born in standard farrowing pens (Fig. 1). On the day of birth, piglets received an ear tag. At 3 d of age, piglets received an iron injection and male piglets were castrated. Tails and teeth were kept intact. If a litter consisted of >14 piglets, the surplus piglets were placed with another experimental sow within the same SBV class or to a foster sow not included in the experiment. Lights were on between 0700 and 1600 h. Temperature in the farrowing rooms was kept between 25 and 26 ºC. A heating lamp was provided for piglets during the first days after birth.

2.2. Behavioral tests

2.2.1. Testing order

During the lactation period, piglets were exposed group-wise to a novel object test and human approach test, and individually to a backtest and novel environment test. On the day before each test, piglets received a number on their back for individual recognition. In each of the 4 tests, litters were tested in blocks of 4 consecutive tests. In each block, 2 +SBV and 2 –SBV litters were tested in random order, alternating between farrowing rooms. In addition, in the individual tests, piglets from the same litter were tested in a consecutive order. The group-wise tests were performed on 1 d and individual tests on 2 consecutive days. If a litter was suckling at the beginning of the test, the test was postponed. If suckling started during the group-wise tests that were performed in the farrowing pens, this was recorded.

2.2.2. Novel object test

On the farm, creep feed in a round, open feeder (diameter of 24 cm) was provided to the piglets (n = 1,009) for the first time at 1 wk of age. This feed and the feeder were, therefore, used as a novel object in this test. Before starting the test, all piglets were placed behind a wooden partition that was placed in a corner of the farrowing pen (Fig. 1). Thereafter, the feeder with creep feed was attached to the floor of the pen (Fig. 1). The test started as soon as the wooden partition was withdrawn and lasted for 10 min. For each piglet, latency to touch the feeder and latency to sample the feed was recorded. If a piglet did not touch the feeder or did not sample the feed, the maximum test time (i.e. 600 s) was given. In addition, every 30 s it was scored which piglets were within 10 cm of the feeder with their heads directed to it and from this the percentage of time present near the feeder was calculated.

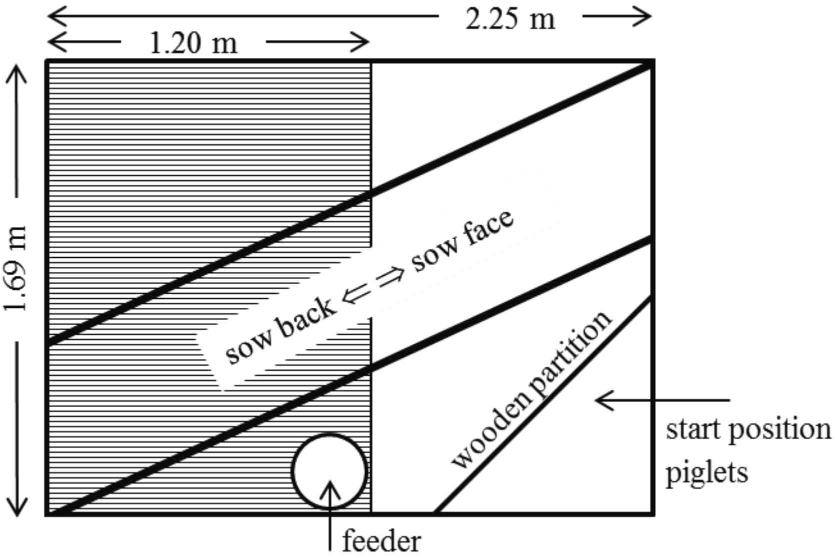

Fig. 1. A layout of a farrowing pen in which the position of the sow, position of the feeder, and start position of the piglets for the novel object test have been indicated.

2.2.3. Backtest

At 2 wk of age, piglets (n = 993) were subjected individually to the backtest, because this test can be used to determine a pig's personality (Bolhuis et al., 2003). A litter was placed in a cart and brought to the test area. There, each piglet was tested out of earshot of the rest of the piglets. In short, a piglet was put on its back and manually restrained for 60 s (see Melotti et al. (2011) for details). During the test, the number of struggles, latency to first struggle, number of vocalizations, and latency to the first vocalization were recorded.

2.2.4. Human approach test

At 2.5 wk of age, piglets (n = 995) were subjected group-wise to a 10 min human approach test. Before the start of the test, sleeping piglets were awoken by hand clapping. Thereafter, all piglets were driven behind the front bar of the pen (Fig. 2), using a wooden partition. A person (same for all 5 batches) entered the pen and stood idle while facing the wall (Fig. 2). Clothing of the person was different from that of the animal caretakers (i.e. white coverall, instead of blue). The test started as soon as the wooden partition was withdrawn. For each piglet, latency to touch the person was recorded. If a piglet did not touch the person, a latency of 600 s was given. In addition, every 30 s it was scored which piglets were within 10 cm of the person with their heads directed to her and from this the percentage of time present near the person was calculated.

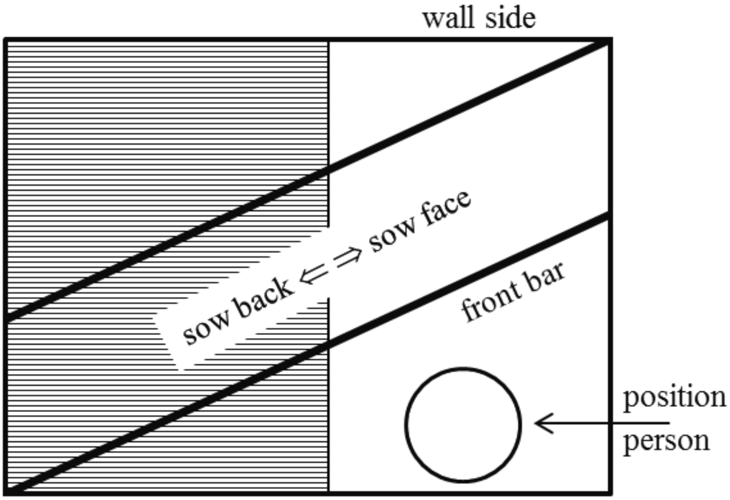

Fig. 2. A layout of a farrowing pen in which the position of the sow, front bar of the pen, and position of the person for the human approach test have been indicated.

2.2.5. Novel environment test

At 3.5 wk of age, piglets (n = 543) were individually subjected to a 2.5 min novel environment test. These were piglets (on average, 6 to 7 piglets per litter) that were selected (96 piglets per batch) for further experiments (not reported here) and ~12 extra piglets per batch. The novel environment was an arena with wooden walls of 125 × 125 × 62.5 cm (length × width × height), which was built in the corridor adjacent to both farrowing rooms. A heating lamp was placed above the arena. Piglets were brought individually to the corridor and placed in the middle of the arena, after which the test started. The postures, locomotion, and other behaviors were scored in 2 mutually exclusive classes (Table 1), using focal sampling and continuous recording on a Psion handheld computer with the Observer software (Noldus Information Technology, Wageningen, The Netherlands). After each test, the arena was cleaned from feces and urine.

Table 1
Ethogram used for the individual novel environment test

Behavior	Description
Postures and locomotion (states)	
Walking	Moving in a forward or backward direction or turning around at the same location
Standing alert	Standing motionless with head fixed (up or down) and ears upright
Standing	Standing with all 4 paws on the floor
Behaviors (states)	
Exploring floor	Exploring the floor by sniffing, nosing, rubbing, licking or rooting it with the rooting disc. Rooting disc is either in contact or very close to surface.
Exploring wall	Exploring the walls of the arena by sniffing, nosing, rubbing, licking or rooting it with the rooting disc. Rooting disc is either in contact or very close to surface.
Behaviors (events)	
Low-pitched vocalizations	Short or long grunts
High-pitched vocalizations	Screams, squeals or grunt-squeals
Defecating	Defecating
Urinating	Urinating
Jumping	Jumping in air or against the wall apparently trying to escape

2.3. Statistical analyses

The SAS (SAS Inst. Inc., Cary, NC) was used for statistical analyses. Preliminary analyses showed no effects of farrowing room, testing order, and in case of the individual tests, day of testing. Therefore, these factors were not included in the final models.

2.3.1. Backtest

Possible differences between +SBV and –SBV piglets for their behavior in the backtest were analyzed with a mixed linear model, with SBV class (+SBV, –SBV), gender, and batch as fixed effects, and pen as random effect, nested within SBV class and batch.

2.3.2. Novelty tests

The effect of SBV class in the other behavioral tests was tested in a mixed linear model (see below). As it has been shown that the behavioral response to novelty may be related to the response in the backtest (Hessing et al., 1994a; Ruis et al., 2000, 2001; Bolhuis et al., 2004; Jansen et al., 2009), the response of the piglets in the backtest (BTR) was included as covariate in this model. As BTR, the number of struggles was used, because this variable has most frequently been used by others (Van Erp-van der Kooij et al., 2000; Cassady, 2007; Velie et al., 2009; Spake et al., 2012) and, moreover, strongly correlates with the other variables measured ($|r| > 0.50$, $P < 0.001$, this study; see also Bolhuis et al., 2003). Several piglets were omitted from statistical analysis due to missing records or impaired health: 34 piglets in the novel object test, 20 piglets for latency in the human approach test, and 33 piglets for presence near the person in the human approach test. During the novel environment test, jumping hardly occurred (mean 0.07 ± 0.03 times) and was therefore not analyzed statistically. The other behaviors of the novel environment test (Table 1) that were recorded as states were expressed as percentages of time and behaviors that were scored as events were expressed as absolute frequencies. Latencies to touch the feeder and the person in the group-wise tests, and frequencies of high-pitched vocalizations and defecating in the individual test were log and square root transformed, respectively, to obtain normality of residuals. Latencies and percentages of time present near the feeder and near the person in the group-wise tests were analyzed, using a mixed linear model, which included SBV class, gender, suckling occurrence (i.e. whether or not a suckling bout occurred during the 10 min test), and batch as fixed effects. The BTR and its interaction with SBV class were included as covariates. Litter (nested within SBV class, batch, and suckling occurrence) was included as random effect. Behaviors of the individual novel environment test were analyzed with a similar model but without suckling occurrence. Urinating during the novel environment test was analyzed as a 0/1 trait, using a generalized linear mixed model with a logit link and binary distribution, and with the same fixed and random effects as the other variables of the novel environment test. Data are presented as means ± SEM.

3. Results

3.1. Backtest

No differences between +SBV and –SBV piglets were found for the number of struggles, latency to start struggling, number of vocalizations, and latency to start vocalizing in the backtest (Table 2). Female piglets were faster to start struggling than male piglets (33.5 ± 1.0 vs. 37.0 ± 1.0 s, P = 0.047). No other gender effects were found.

Table 2
Mean, SEM, and between brackets the range for the variables recorded during the backtest for piglets with a positive (+) or negative (–) social breeding value (SBV) for growth

Variable	+SBV piglets	–SBV piglets	P-value
Number of struggles	1.5 ± 0.1 (0-8)	1.6 ± 0.1 (0-7)	0.31
Latency to first struggle (s)	35.9 ± 1.8 (0-60)	34.5 ± 1.7 (0-60)	0.50
Number of vocalizations	16.9 ± 1.3 (0-69)	17.8 ± 1.4 (0-91)	0.63
Latency to first vocalization (s)	26.7 ± 2.0 (0-60)	25.4 ± 1.8 (0-60)	0.64

3.2. Novel object test

Piglets with a +SBV for growth touched the feeder faster than piglets with a –SBV for growth (P = 0.01; Fig. 3). In addition, the interaction between SBV class and BTR for latency to touch the feeder was found to be significant (P = 0.04). The more +SBV piglets struggled during the backtest, the later they tended to touch the feeder in the novel object test [β = 0.039 (95% confidence interval (CI)), –0.003 – 0.082), P = 0.07], whereas for –SBV piglets, latency to touch the feeder was independent of BTR [β = –0.023 (95% CI, –0.066 – 0.020), P = 0.30]. There was no effect of SBV class (Fig. 3), BTR, or their interaction on latency to sample the feed and presence near the feeder (all, at least P = 0.34; data not shown).

Female piglets were faster than male piglets to touch the feeder (162.8 ± 9.3 vs. 209.2 ± 10.0 s, P < 0.001) and sample the feed (454.0 ± 9.3 vs. 502.3 ± 7.9 s, P < 0.001). In addition, female piglets were also more frequently present near the feeder than male piglets (21.3 ± 0.8 vs. 17.2 ± 0.7%, P < 0.001). If suckling occurred during the test (in 47% of the litters and equally divided among +SBV and –SBV litters), piglets were later to sample the feed (484.5 ± 11.9 vs. 470.4 ± 11.0 s, P = 0.048) and piglets were less frequently present near the feeder, compared with when suckling did not occur (15.4 ± 1.0 vs. 22.6 ± 1.2%, P < 0.001).

3.3. Human approach test

Latency to touch the person was not different between +SBV and –SBV piglets (P = 0.93), but +SBV piglets were more frequently present near the person than –SBV piglets (P < 0.01; Fig. 4). In addition, the interaction between SBV class and BTR was found to be significant for latency to touch the person (P = 0.04) and presence near the person (P = 0.02). For +SBV piglets, latency to touch the person [β = 0.021 (95% CI, –0.013 – 0.054), P = 0.23] and presence near the person were independent of BTR [β = –0.009 (95% CI, –0.027 – 0.008), P = 0.31]. In contrast, the more –SBV piglets struggled in the backtest, the faster they tended to touch the person [β = -0.031 (95% CI, –0.065 – 0.004), P = 0.08] and the more time they spent near the person [β = 0.020 (95% CI, 0.002 – 0.038), P = 0.03].

Female piglets were faster than male piglets to touch the person (198.0 ± 9.7 vs. 229.0 ± 10.0 s, P < 0.001) and they were also more frequently present near the person than male piglets (43.8 ± 1.3 vs. 40.4 ± 1.3%, P = 0.03). If suckling occurred during the test (in 18% of the litters and equally divided among +SBV and –SBV litters), piglets were later to touch the person compared with when suckling did not occur (284.7 ± 25.5 vs. 196.2 ± 11.7 s, P < 0.01).

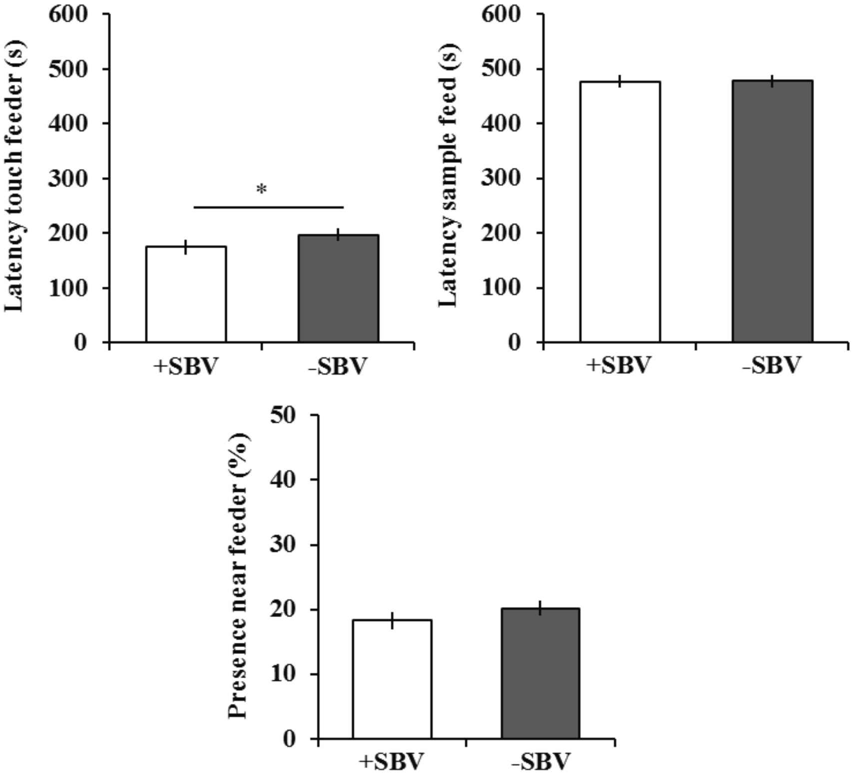

Fig. 3. Latency to touch the feeder (s), latency to sample feed (s), and presence near the feeder (%) during the novel object test for piglets with a positive (+) or negative (–) social breeding value (SBV) for growth. Differences between means are indicated by an asterisks (* $P \leq 0.05$).

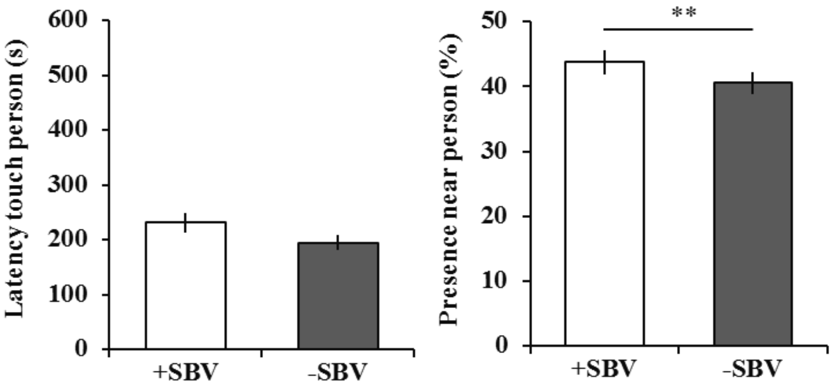

Fig. 4. Latency to touch the person (s) and presence near the person (%) during the human approach test for piglets with a positive (+) or negative (–) social breeding value (SBV) for growth. Differences between means are indicated by 2 asterisks (** $P \leq 0.01$).

3.4. Novel environment test

During the novel environment test, no effect of SBV class (Table 3) or its interaction with BTR were found (all interactions, at least $P > 0.15$; data not shown). The BTR affected time spent walking ($P = 0.02$). The more piglets struggled in the backtest, the more time they spent on walking in the novel environment test [$\beta = 0.73$ (95% CI, 0.08 – 1.37), $P = 0.03$].

Female piglets spent more time exploring the floor than male piglets (44.1 ± 1.1 vs. 41.0 ± 1.2% of time, $P = 0.046$). Moreover, female piglets produced less low- and less high-pitched vocalizations than male piglets (low-pitched vocalizations: 52.8 ± 1.9 vs. 57.0 ± 1.9 times, $P = 0.04$; high-pitched vocalizations: 3.0 ± 0.5 vs. 4.7 ± 0.8 times, $P = 0.02$). In addition, they also tended to defecate less (0.6 ± 0.06 vs. 0.7 ± 0.06 times, $P = 0.08$) and were much less likely to urinate than male piglets (4.8 vs. 11.8% of pigs, $P < 0.01$). No other effects of BTR or gender on the behaviors during the novel environment test were found (data not shown).

Table 3
Mean and SEM for the behaviors scored during the individual novel environment test for piglets with a positive (+) or negative (–) social breeding value (SBV) for growth

Behavior	+SBV piglets	–SBV piglets	P-value
Walking (% of time)	18.0 ± 1.3	19.3 ± 1.1	0.81
Standing alert (% of time)	32.3 ± 1.5	32.1 ± 2.0	0.69
Standing (% of time)	49.3 ± 1.1	48.9 ± 1.3	0.60
Exploring floor, (% of time)	42.5 ± 2.1	43.6 ± 2.2	0.68
Exploring wall (% of time)	3.3 ± 0.2	3.7 ± 0.3	0.76
Low-pitched vocalizations (freq.)	52.5 ± 3.4	55.7 ± 3.6	0.62
High-pitched vocalizations (freq.)	3.5 ± 0.9	3.9 ± 0.9	0.90
Defecating (freq.)	0.6 ± 0.1	0.6 ± 0.1	0.72
Urinating (% of time)	9.7	6.8	0.41

4. Discussion

Piglets with either a +SBV or –SBV for growth did not differ in their response in the backtest. Some behavioral responses to the novelty tests were, however, affected by SBV class or its interaction with the backtest response. Interestingly, female and castrated male piglets behaved differently in all tests.

4.1. Social breeding value for growth and backtest

In this study, it was hypothesized that SBV for growth in pigs could be related to personality traits. The results of this study show, however, that aspects of a pig’s personality, as reflected by its response to a backtest at young age, are not affected by SBV for growth. Several studies have demonstrated that piglets that struggle relatively often in the backtest (high-resister (HR) pigs) show a different behavioral, (neuro)physiological, and immunological response to an array of challenging situations later in life, compared with piglets that show hardly any struggles in the backtest (low-resister (LR) pigs) (e.g. Hessing et al., 1994a; Bolhuis et al., 2000, 2003; Ruis et al., 2000). For instance, HR pigs have been found to respond to a change in situation with more active behaviors, such as locomotion (Jansen et al., 2009) or escape behavior (Hessing et al., 1994a; Bolhuis and Schouten, 2002), which resembles a more proactive personality (Koolhaas et al., 2010), whereas LR pigs responded to the same change with more passive behaviors as standing alert (Bolhuis and Schouten, 2002; Jansen et al., 2009), reflecting a more reactive personality (Koolhaas et al., 2010). Research has shown that this difference could stem from a difference in cue dependency. Proactive animals act primarily on the basis of previous experience, i.e. they develop more easily routines, and hence are more likely to be successful in a stable environment (Bolhuis et al., 2004; Koolhaas et al., 2010). Reactive animals, on the other hand, act primarily to actual environmental information and hence are more likely to cope successfully with unpredictable situations (Bolhuis et al., 2004; Koolhaas et al., 2010). We wanted to investigate the relationship between SBV for growth and personality, because growth and other performance parameters of pigs have been found to be related to their response in a backtest (Ruis et al., 2000; Van Erp-van der Kooij et al., 2000, 2003; Geverink et al., 2004; Cassady, 2007; Velie et al., 2009; Spake et al., 2012). These relationships do not always seem consistent but are at least partly in line with the hypothesis that HR pigs thrive best in a stable environment. The HR pigs have been reported to perform better before weaning (Ruis et al., 2000; Cassady, 2007) and during fattening (Ruis et al., 2000; Van Erp-van der Kooij et al., 2003), whereas LR pigs cope more successfully with unpredictable situations, as they perform better after relocation (Geverink et al., 2004) and after weaning (Cassady, 2007). In addition, the performance of pigs could also be influenced by the personality of their pen mates. Receiving aggressive behavior, for instance, has been found as a factor limiting growth (Stookey and Gonyou, 1994; Hyun et al., 1998) and pigs that struggle relatively often in the backtest were found to be more aggressive toward their pen mates after mixing (Hessing et al., 1994b; Ruis et al., 2002; Bolhuis et al., 2005b; Melotti et al., 2011) and under stable conditions (Bolhuis et al., 2005a; Bolhuis et al., 2006), but not in a resident-intruder test (D'Eath and Burn, 2002; Cassady, 2007; Velie et al., 2009; Spake et al., 2012). On the other hand, pigs that show relatively few struggles in the backtest have been reported to show more oral manipulative behaviors directed at pen mates, such as ear and tail biting (Bolhuis et al., 2005a; Bolhuis et al., 2006), and receiving this type of behavior has been found to negatively affect growth (e.g. England and Spurr, 1967; Camerlink et al., 2012). These studies show that SBV for growth and personality, as assessed with a backtest, could thus be related. If so, genetic selection for +SBV piglets would have major implications for pig behavior in the long term. No differences between +SBV and –SBV piglets in their response to the backtest were, however, found. These results suggest, therefore, that including SBV for growth in genetic selection programs would not immediately result in large personality changes as assessed with a backtest. In addition, these results are beneficial for the performance of pigs as well, because Hessing et al. (1994b) found that performance was better in groups of pigs that consisted of a mixture of personalities (i.e. HR and LR pigs), compared with groups that consisted only of one personality type (either HR or LR pigs).

Several studies have shown that the response of pigs in a novelty test is related to their response in a backtest (Hessing et al., 1994a; Ruis et al., 2000; Ruis et al., 2001; Bolhuis et al., 2004; Jansen et al., 2009). For instance, pigs that struggled frequently during the backtest have been described to explore a novel object fast and superficially, whereas pigs that show hardly any struggles during the backtest explore a novel object more slowly and thoroughly (Hessing et al., 1994a). In this study, no effect of BTR was found in the group-wise novel object test and human approach test. These results are in line with other studies (Van Erp-van der Kooij et al., 2002; Velie et al., 2009) and could indicate that group-wise tests in the home pen are not sufficiently novel and challenging for pigs to lead to different ways in coping with the test situation. It could also be that personality differences are masked by social facilitation in, for instance, the approach of an object or person.

In the individual novel environment test, an effect of BTR was found on the time spent walking. Piglets that struggled relatively often in the backtest, walked more in the novel environment test than piglets that hardly struggled in the backtest. This result is consistent with Jansen et al. (2009), who found HR pigs to be more active (i.e. running and walking) than LR pigs in a novel maze test. To be socially isolated is very stressful for a piglet (Kanitz et al., 2009), which was also visible from the behavior (e.g. standing alert, defecations, and urination; Boissy, 1995; Mendl et al., 1997) of piglets in the test. From these studies, it can be concluded that the response of a piglet in the backtest seems to be predictive of its response in tests later in life when those tests are sufficiently novel and challenging for the pig, but for other tests the backtest has limited predictive value.

4.2. Social breeding value for growth and novelty behavior

Fear and stress have been proposed as factors limiting growth in pigs (Hyun et al., 1998; Hemsworth, 2003; Jones and Boissy, 2011). The expression of fear or stress by animals (Boissy, 1995; Jones and Boissy, 2011) could, in turn, lead to fearful or stressed conspecifics as well (De Haas et al., 2012), through a process called emotional contagion (the most simple form of empathy; Edgar et al., 2012; Špinka, 2012; Reimert et al., 2013). If so, the growth of these conspecifics may, then, also be reduced. Fearfulness could, therefore, be an underlying trait for social breeding values. Laying hens that have a +SBV for the survival of their cage mates indeed showed less fear-related behavior in an individual manual restraint test and group-wise human approach test than control hens (Bolhuis et al., 2009). We found, however, no clear differences in fear-related behavior between piglets with diverging SBV for the growth of their pen mates, except that +SBV piglets touched the feeder in the group-wise novel object test faster than –SBV piglets, and they were more frequently present near the person in the group-wise human approach test than –SBV piglets. In addition, we found a significant interaction between SBV class and BTR for latency to touch the feeder, latency to touch the person, and presence near the person, which indicates that SBV and BTR differently affect the motivation of pigs to explore or avoid novel stimuli. Considering these results and taking into account the rather small absolute differences, we conclude that piglets with a +SBV or –SBV for growth do not appear to differ much in fear-related behaviors in the lactation period. This conclusion is supported by Bouwman et al. (2010), who found no evidence for SBV for growth during the lactation period. This does, however, not mean per se that fearfulness is not an underlying trait of SBV. The genetic effect pigs have on the growth of their pen mates was estimated on the finishing phase. It could be that behavioral differences between pigs with diverging SBV for growth emerge during this period (Reimert et al., 2014). If so, this explains why in the lactation period fear-related behaviors were not clearly different between +SBV and –SBV piglets. On the other hand, other (behavioral) traits, such as aggressiveness or biting behavior, could be underlying SBV, as well (Camerlink et al., 2012, 2013). More research will, therefore, help us better understand the biological background of social breeding values for growth in pigs and what we are selecting for when we use this method of genetic selection.

4.3. Gender differences

While not the main aim of this study, we found many differences between female and castrated male piglets. In the backtest, female piglets were faster than male piglets to start struggling, which is in line with Van Erp-van der Kooij et al. (2000), who found that castrated male piglets struggled less than female piglets in a backtest, while e.g. Velie et al. (2009) found no effect of gender in this test. In the novel object test on d 7, female piglets were faster than male piglets to touch the feeder and sample the feed, and they were also more frequently present near the feeder. These results are in line with Kuller et al. (2007), who found that female piglets ate more creep feed than castrated male piglets, which might partly be explained by differences in deciduous dental development (Tucker and Widowski, 2009). Tucker et al. (2010) did, however, not find that gender affected feeding behavior before weaning. In the human approach test, female piglets were also faster to touch the person and more frequently present near the person. Taking into account these results, it could also be that female pigs have a higher motivation to explore novel stimuli or are less fearful (Brown et al., 2009). The latter is supported by results of the novel environment test in which female piglets expressed less fear-related behaviors than male piglets, such as high-pitched vocalizations (e.g. Düpjan et al., 2008) and urinating and defecating (Mendl et al., 1997). Also Chaloupková et al. (2007) found that castrated male piglets squealed more than female piglets in an individual novel environment test. Castration has been proposed as a cause of these gender differences due to a difference in being handled or difference in behavior as a consequence of pain (e.g. Van Erp-van der Kooij et al., 2000; Chaloupková et al., 2007; Siegford et al., 2008; Rault et al., 2011). Kranendonk et al. (2006) found, however, that female piglets also vocalized more than noncastrated male piglets in a novelty test. Moreover, female piglets have been reported to be less susceptible to stress than (castrated) male piglets because of a difference in energy allocation to different physiological systems

(Lay, Jr. et al., 2002; Baxter et al., 2012). In contrast, Jensen et al. (1995), Docking et al. (2008), Brown et al. (2009), and Rutherford et al. (2012) found no differences between female and (castrated) male pig(let)s in various novelty tests. More research into gender differences in pigs is, therefore, warranted, because more knowledge about factors underlying gender differences could contribute to management practices that are harmonized with the needs of both genders and thereby improve animal welfare (Vandenheede and Bouissou, 1993).

5. Conclusions

Limited effects of diverging SBV for growth were found for tests on personality and fear-related behaviors in piglets performed during the suckling period. Further research is needed to identify the traits underlying differences in pigs with a +SBV or –SBV for growth of their pen mates. Moreover, castrated male piglets seemed to react more fearfully to each test than female piglets.

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CHAPTER 3

Responses to novel situations of female and castrated male pigs with divergent social breeding values and different backtest classifications in barren and straw-enriched housing

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Abstract

The growth of a pig is not only affected by its own genes, but also by the genes of its pen mates. This indirect effect on a pig's growth is represented as social breeding value (SBV) in a newly developed breeding model. It has been hypothesized that pigs could affect their pen mates' growth through their behavior. We investigated whether pigs selected for a relatively positive (+SBV) or negative genetic effect (–SBV) on the growth of their pen mates and kept in either barren or straw-enriched pens differ in fearfulness. Effects of coping style, assessed in a backtest, and gender were also investigated. Pigs (n = 480) were subjected to a group-wise novel rope test and human approach test and individually to a novel environment test in which after 5 min a bucket was lowered from the ceiling. In the novel rope test +SBV pigs were faster than –SBV pigs to touch a rope ($P < 0.01$) and in the novel environment test +SBV pigs showed less locomotion than –SBV pigs after introduction of the bucket ($P < 0.05$). Furthermore, straw-enriched pigs were faster than barren housed pigs to touch a rope in the novel rope test ($P < 0.10$) and faster to approach ($P < 0.05$) and touch a person ($P < 0.05$) in the human approach test, suggesting that they are less fearful or more curious than pigs in barren housing. Straw-enriched pigs also had lower salivary cortisol concentrations than barren housed pigs ($P < 0.001$). Pigs classified as high-resisting in the backtest spent more time near the person in the human approach test ($P < 0.10$) and showed more locomotion ($P < 0.10$) and vocalizations ($P < 0.001$) after introduction of the bucket in the novel environment test than low-resisting pigs. Gilts appeared less fearful than barrows, because they were faster to touch a rope in the novel rope test ($P < 0.05$) and faster to approach ($P < 0.05$) and touch a person ($P < 0.10$) in the human approach test. In addition, in the novel environment test, gilts were more calm ($P < 0.05$) in the period before the bucket was introduced, paid more attention to the bucket once it was lowered ($P < 0.10$) and were overall more active ($P < 0.01$). Gilts also had lower basal cortisol concentrations than barrows ($P < 0.001$). Overall, these results suggest that +SBV pigs might be less fearful than –SBV pigs. Furthermore, the response of pigs in novelty tests seems to depend also on their housing conditions, coping style, and gender.

Keywords: backtest, enrichment, gender differences, novelty, pigs, social breeding values

1. Introduction

Social interactions between pigs may profoundly affect their welfare, health and growth performance, both in a negative and positive way. There has, however, been a lack of attention for social interactions in genetic selection of animals, as, in current breeding programs, selection is typically based on individual performance (Muir, 2005). A disadvantage of these breeding programs might be that they result in selection of competitive individuals with potentially negative effects on the performance of other individuals. Muir (2005) showed, for instance, that selection based on individual performance led to more feed competition in Japanese quail, resulting in poor group performance. To resolve this disadvantage, the behavior of individuals could be taken into account in breeding programs, but as this is very labor-intensive it has, at present, been ignored by breeding companies (Turner, 2011; Bijma, 2012). Several researchers therefore advocated to consider group performance in breeding programs (Griffing, 1967; Muir, 2005; Bijma et al., 2007a,b). This has led to the development of new breeding models in which not only individual performance with respect to production traits is included, but also the (genetic) effect on the performance with respect to production traits of group mates. This effect, which is also referred to as associative effect, social genetic effect, or indirect genetic effect (e.g. Wolf et al., 1998; Muir, 2005; Camerlink et al., 2012) is represented in a social breeding value (SBV) (Bijma et al., 2007a).

Recently, it has been estimated that the effect of a pig on the growth of its group mates during the finishing period is heritable (Chen et al., 2007; Bergsma et al., 2008, 2013). This indirect genetic effect for growth in pigs could well be related to behavior (Rodenburg et al., 2010), as, for instance, the growth of a pig may be reduced when it is subjected to harmful oral manipulations such as tail biting (e.g. Camerlink et al., 2012; Sinisalo et al., 2012), whereas non-harmful nosing seems to be beneficial for growth (Camerlink et al., 2012). Thus, selection for pigs that have a positive effect on the growth of their pen mates might be an indirect way to improve both the group performance and the welfare of pigs. The behavior of pigs with diverging SBV for the growth of their pen mates has, however, hardly been investigated.

Fear is a very strong emotion which can drive the way animals respond to their social and physical environment (Jones and Boissy, 2011). For instance, fearful pigs might have more difficulties with coping with environmental changes which, in turn, could lead to the expression of harmful behaviors in these pigs (Turner, 2011; Zupan et al., 2012). Also, fear is a major factor limiting growth in animals (Boissy, 1995; Hemsworth, 2003) and via a process called emotional contagion negative affective states can be transferred from one animal to another (Edgar et al., 2012; Špinka, 2012; Reimert et al., 2013a) which could then negatively affect the group as a whole. Moreover, laying hens which were selected with the same new breeding model but for a relatively positive social effect on the survival of their cage mates showed less fear-related behaviors than hens from a control line in several stressful situations (Bolhuis et al., 2009; Rodenburg et al., 2009; Nordquist et al., 2011). Fearfulness might, therefore, be underlying to SBV for growth in pigs as well. We hypothesize that pigs with a relatively positive indirect genetic effect (+SBV) on the growth of their pen mates are less fearful than pigs with a relatively negative indirect genetic effect (–SBV) on the growth of their pen mates. This hypothesis has been studied by subjecting the pigs to several novelty tests, because these tests are the conventional way to measure fear in animals (e.g. Forkman et al., 2007; Brown et al., 2009; Zupan et al., 2012). From literature it is known that both the housing conditions of animals and their coping style or personality influence their responses to novelty. Beattie et al. (2000), for instance, found that pigs housed in pens with straw and peat were less fearful of a novel object than barren housed pigs and Hessing et al. (1994) showed that pigs with a proactive coping style react to a novel stimulus by exploring it fast and superficially, whereas pigs with a reactive coping style explore a novel stimulus more slowly and thoroughly. To find out how indirect genetic effects for growth interact with housing and coping style, the +SBV and –SBV pigs of this study were either housed in standard barren pens or in pens enriched with a deep litter bedding of straw and wood shavings and their coping style was assessed with the backtest (e.g. Bolhuis et al., 2005a; Reimert et al., 2013b).

2. Materials and methods

This study was approved by the Animal Care and Use Committee of Wageningen University.

2.1 Animals and housing

For this study, a total of 480 Tempo x Topigs-20 pigs, equally divided over five batches, were used. Half of these pigs had a relatively positive indirect genetic effect on the growth of their pen mates (here referred to as +SBV) and the other half a relatively negative indirect genetic effect on growth (–SBV). A more elaborate explanation of how pigs diverging in indirect genetic effects on growth were obtained, can be found in Camerlink et al. (2013). Briefly, based on information about growth rates, group composition and pedigrees, the indirect genetic effects on growth, i.e. heritable effects that pigs have on the growth rate of their pen mates during the finishing phase (from app. 25 to 110 kg), were estimated (latest estimations show a heritability of 0.3 for indirect genetic effects (Bergsma et al., 2013)). By mating boars and sows with the most extreme estimated indirect genetic effects on growth available per batch, we created +SBV and −SVB offspring (n = 80 litters in total). The contrast for the estimated indirect genetic effect on growth between +SBV and –SBV offspring was 3.62 g/day, which, as all pigs had five pen mates (see below) during the finishing phase, corresponds to an expected growth difference of 5 × 3.62 = 18 g/day. Direct breeding values for growth were kept as similar as possible for both SBV classes.

Pigs were born at the experimental farm of Topigs Research Center IPG in Beilen, The Netherlands (for details see Reimert et al., 2013b), where they were housed until weaning in standard lactation pens (3.8 m^2). Piglets' teeth were not clipped and tails were not docked, but the male piglets were surgically castrated at 3 days of age. On the day of weaning, which was at 26 days of age, 96 piglets were selected per batch (see Camerlink et al., 2013) and transported to the experimental farm 'de Haar' of Wageningen University, The Netherlands, where they were housed until slaughter (week 23) in 16 pens (6-7 m^2) located in one room per batch. Each pen consisted of six unrelated pigs, three females and three castrated males. All pigs within a pen had either a +SBV or a –SBV for the growth of their pen mates. In addition, there were at least two pigs with a high-resisting and two pigs with a low-resisting backtest classification in each pen (see section 2.2.). In each pen a toy was present in the form of a metal chain with a ball. At the age of 8 weeks a jute sack was attached to the wall of each pen and replaced if needed. Half of the pigs were housed in barren pens with a partially slatted floor. Barren housed pigs received two hands of wood shavings every day from 6 weeks of age. The other half of the pigs were housed in pens which were enriched with 1.5 kg of straw and 12 kg of wood shavings. All pens were cleaned daily and afterwards 3 kg of fresh wood shavings and fresh straw (250 g at the start of the experiment and then gradually increased to 1.5 kg) were added to the enriched pens. A heating lamp was provided for the pigs during the first week after weaning. Feed (a standard commercial diet for growing pigs) and water were available ad libitum. Lights were on from 07:00 to 19:00 h and temperature was set at 25 ºC during the first week after weaning and then the set temperature gradually decreased with approximately 1 ºC per week until 20 ºC. Pigs could be individually recognized by an ear tag and by a number that was sprayed on their backs. Not all pigs participated in all behavioral tests (section 2.3.), because they had died or were taken out of the experiment due to health reasons.

2.2. Backtest

At approximately 2 weeks of age, piglets were subjected to a backtest to determine their coping style or personality (Hessing et al., 1994; Bolhuis et al., 2003). In short, a piglet was put on its back for 60 s and manually restrained (for details see Reimert et al., 2013b). During the test, the number of struggles, the latency to struggle, the number of vocalizations, and the latency to vocalize were recorded. Piglets that showed two struggles and produced at least 25 vocalizations, or showed at least three struggles were classified as high-resisters (HR). Piglets that showed zero or one struggle, or two struggles and produced less than 25 vocalizations were classified as low-resisters (LR). Previously, we found no differences between +SBV and –SBV piglets in their backtest response (Reimert et al., 2013b).

2.3. Behavioral tests

2.3.1. Novel rope test (n = 480 pigs)

At the age of 6 weeks, pigs were subjected group-wise to a 15 min novel rope test. Before the start of the test, lying pigs were approached to make them rise and stand. Thereafter, two cotton ropes (50 cm long of which 40 cm was available for chewing, and 1 cm thick) were attached to the middle of the left and right wall of a pen with a distance of 30 cm between the end of the rope and the floor of the pen, and the test was started. For each pig the latency to touch the left and right rope was recorded. If a pig did not touch one or both ropes within the 15 min a latency of 900 s was given. In addition, every 30 s it was scored which pigs were present at which rope either touching or chewing it and from this the percentage of time present at each rope was calculated. The pens were tested in an order balanced for SBV class and housing.

2.3.2. Human approach test (n = 478 pigs)

At the age of 7 weeks, pigs were subjected group-wise to a 10 min human approach test. Before the start of the test, a person (the same for all five batches) alerted the pigs by hammering five times with a hammer on the middle of the front wall of the pen after which she stepped into the corner of the pen and the test was started. She wore different clothes (i.e. a red coverall) than normally worn by the animal caretakers. Most pigs reacted to this person by going to the opposite part of the pen. Therefore two latencies were recorded for each pig. Firstly, if a pig approached the person at approximately 1.5 m, crossing an imaginary line, this was recorded as the approach latency. If a pig was already within this area, an approach latency of 0 s was given. Secondly, for each pig the latency to touch the person was recorded. If a pig did not approach or touch the person a latency of 600 s was given. In addition, every 30 s it was scored which pigs were within 50 cm of the person with their heads directed to her and from this the percentage of time present near the person was calculated. The pens were tested in an order balanced for SBV class and housing.

2.3.3. Combined novel environment and novel object test (n = 467 pigs)

At the age of 13 weeks, pigs were individually subjected to a 10 min combined novel environment and novel object test (Ursinus et al., 2013). This test was carried out in an arena of 5 x 5 x 1.2 m (length x width floor x height walls) in a testing room adjacent to the room with the pens. The walls and floor of the arena were dark grey. The test started when a pig was brought into the arena and the door was closed. After 5 min, a metal bucket was slowly lowered from the ceiling until it touched the floor which, consequently, resulted in a noise. Thereafter the bucket was left on the floor for another 5 min. Between pigs, the arena was cleaned with water and cleanser and dried thereafter. The test was divided over 5 days with no more than two pigs from the same pen tested on the same day and at least 1 h apart. In addition, the testing order was also balanced for SBV class, backtest classification, housing, and gender.

2.3.3.1. Behavioral observations

During the 10 min novel environment test, postures and locomotion, and various behaviors (Table 1) were scored with a Psion hand-held computer with the Observer software (Noldus Information Technology, Wageningen, The Netherlands). Postures, locomotion, and various behaviors were scored in two mutually exclusive classes using focal sampling and continuous recording. One observer not visible to the pigs scored the states (Table 1) and another observer scored the events (Table 1) and lowered the bucket from the ceiling. This second observer therefore needed to be close to the arena, but pigs hardly seemed to pay attention to her. Locomotion patterns were scored from video recordings using the automated tracking system EthoVision XT 8.5 (Noldus Information Technology, Wageningen, The Netherlands) (Fig. 1). Two zones were distinguished in the arena: a door zone and a bucket zone (Fig. 1). Per pig, two tracks of 280 s were made of which the first started as soon as a pig was present in the arena and the door was closed and the second started as soon as the bucket touched the floor of the arena. The middle of the pig was used as tracking point. For each track, the following variables were extracted: total distance moved (m), time spent in, and frequency of visiting the door zone and bucket zone.

Table 1
Ethogram used for the combined novel environment and novel object test

Behavior	Description
Postures and locomotion (states)	
Walking	Moving in a forward or backward direction or turning around at the same location
Standing alert	Standing motionless with head fixed (up or down) and ears upright
Standing	Standing with all four paws on the floor
Behaviors (states)	
Exploring floor	Exploring the floor by sniffing, nosing, licking, rubbing, or rooting it with the rooting disc. Rooting disc is either in contact or very close to surface.
Exploring wall	Exploring the walls of the arena by sniffing, nosing, licking, rubbing, or rooting it with the rooting disc. Rooting disc is either in contact or very close to surface.
Behaviors (events)	
Low-pitched vocalizations	Short or long grunts
High-pitched vocalizations	Screams, squeals or grunt-squeals
Defecating	Defecating
Urinating	Urinating
Jumping	Jumping in air or against a wall of the arena trying to escape
Bucket related behaviors (states)	
Approaching bucket	Approaching the bucket within 1 m distance either slowly (step by step) or quickly (easy walking or running)
Drawing back from bucket	Drawing back from the bucket with or without the head directed to it
Exploring bucket	Exploring the bucket by nosing, sniffing, rooting, licking, or chewing it

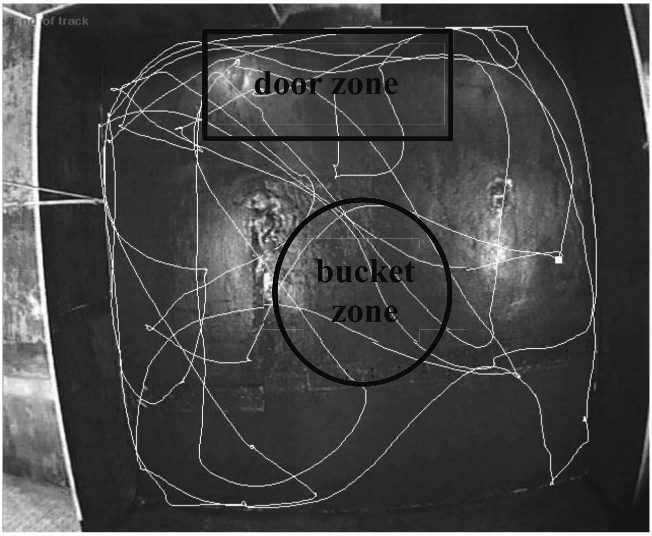

Fig. 1. Schematic view of the locomotion patterns of a pig tracked by Ethovision during the combined novel environment and novel object test. In the figure, the position of the door and bucket zone are presented.

2.3.3.2. Saliva collection and cortisol analysis

All pigs were habituated to chew on cotton buds (VWR International, Amsterdam, The Netherlands) in the week before the combined novel environment and novel object test. For the combined novel environment and novel object test, saliva samples were collected from each pig in the home pen just before (t = 0) the pig was brought to the test arena and 15, 30 and 60 min after the start of the test by allowing the pigs to chew on cotton buds until the buds were thoroughly moistened. The cotton buds were placed in test tubes (Sarstedt, Etten-Leur, The Netherlands) and the tubes were stored on ice. At the end of the day tubes were centrifuged for 10 min at 2000 g to get the saliva from the cotton buds and the saliva was stored at −20 ºC until further analysis. Cortisol concentration (ng/ml) was measured in duplicate using a radioimmunoassay kit (COAT-ACOUNT ®, Siemens Healthcare Diagnostics, Los Angeles, USA) which has been modified and validated for pigs (Ruis et al., 1997). Inter-Assay CV (n = 9 assays) and Intra-Assay CV were both 7.9%.

2.4. Statistical analyses

Statistical analyses were performed with SAS (SAS 9.2, SAS Institute Inc.). Preliminary analyses showed no differences between the results of the left and right rope latencies or percentages of time present at each rope (data not shown). Therefore the minimum latency (the latency to touch either the left or right rope) and total time present at the ropes (sum of left and right presence) were determined for each pig and those variables were used in further analyses. Three pigs were omitted from analyses of the human approach test due to health reasons. Four other pigs were omitted from analyses as well, because their touch latencies (600, 600, 600 and 569 s (overall mean ± SEM of all other pigs: 17.0 ± 0.9 s)) were outliers (based on the Grubbs' test) and significantly influenced the outcome of the results. Each recorded latency was, prior to analysis, log transformed to obtain normality of residuals. The variables of the novel rope test and human approach test were analyzed with a mixed linear model with SBV class (+SBV or –SBV), backtest classification (HR or LR), housing (barren or enriched), all two-way interactions between these effects, gender, and batch as fixed effects, and pen, nested within SBV, housing and batch, as random effect. Testing order (i.e. the order in which the pens were tested) was included as covariate in the model.

Preliminary analyses of the combined novel environment and novel object showed that pigs behaved quite differently in 5 min after the appearance of the bucket compared to the first 5 min (Table 2). The test was, therefore, separately analyzed for these two periods. To reduce the number of variables of this test and thereby making the results less complex, a principal component analysis (PCA) was conducted (e.g. Van Reenen et al., 2002) on the variables in the period before and on the variables in the period after the bucket was lowered from the ceiling. All variables measured during the test were included, except defecating and urinating, because these variables could not be normalized, and jumping because it did not occur. Bucket related behaviors (Table 1) were only included in the second PCA. The variables walking, standing alert, standing, exploring floor, exploring wall, approaching bucket, drawing back from bucket, exploring bucket, time present in door zone and time present in bucket zone were expressed as percentages of time. The variables low- and high-pitched vocalizations and visiting the door and bucket zone were expressed as absolute frequencies (Table 2). Prior to the first PCA, the frequencies of low- and high-pitched vocalizations and the percentage of time spent in the door zone were log or arcsine square root transformed, respectively, to normalize the variables. All variables were, then, entered into a PCA with orthogonal varimax rotation and principal components (PC) with an eigenvalue above one were retained. This resulted in two PC's for the period before the introduction of the bucket (Table 3). Prior to the second PCA, the frequencies of low- and high-pitched vocalizations and the latency to touch the bucket were log transformed and the percentages of time spent in the door zone, in approaching the bucket, in drawing back from the bucket and in exploring the bucket were arcsine square root transformed to normalize the variables. Here, the PCA resulted in four PC's (Table 3). The scores of each animal for each PC were subsequently analyzed with a mixed linear model with the same fixed and random effects as above (testing order is here the order in which the pigs were tested on a day) and including another random effect of test day.

Table 2
Means ± SEM of the behaviors measured during the combined novel environment and novel object test in the 5-min period before the bucket was lowered from the ceiling (before) and in the 5-min period thereafter (after)

Behaviors	Before	After	P-value
Walking (% of time)	32.0 ± 0.5	26.7 ± 0.5	< 0.001
Standing alert (% of time)	17.9 ± 0.6	31.6 ± 0.8	< 0.001
Standing (% of time)	47.0 ± 0.6	37.5 ± 0.7	< 0.001
Exploring floor (% of time)	65.0 ± 0.7	31.5 ± 0.8	< 0.001
Exploring wall (% of time)	3.7 ± 0.1	3.5 ± 0.1	0.23
Low-pitched vocalizations (freq.)	25.5 ± 1.2	39.3 ± 1.5	< 0.001
High-pitched vocalizations (freq.)	2.2 ± 0.5	5.8 ± 0.8	< 0.001
Total distance moved (m)	48.3 ± 0.9	41.7 ± 0.8	< 0.001
Time in door zone (% of time)	21.1 ± 0.7	13.9 ± 0.6	< 0.001
Frequency visiting door zone (freq.)	4.7 ± 0.1	3.5 ± 0.1	< 0.001
Time in bucket zone (% of time)	24.2 ± 0.6	24.6 ± 0.7	0.58
Frequency visiting bucket zone (freq.)	7.8 ± 0.2	8.6 ± 0.2	< 0.05
Defecating (freq.)	3.8 ± 0.1	3.2 ± 0.1	< 0.001
Urinating (% of pigs)	24.4	20.6	0.20

Table 3
Loadings[1] on the principal components (PC) with an eigenvalue above one that were extracted by principal component analysis with orthogonal varimax rotation on the recorded variables during the combined novel environment and novel object test in the 5-min period before the bucket was lowered from the ceiling (before) and in the 5-min period thereafter (after)

	Before		After			
	PC		PC			
Behaviors	1	2	1	**2**	3	4
Walking	**0.80**	0.24	0.23	**0.78**	0.24	0.22
Standing alert	-0.11	**-0.81**	-0.28	-0.16	**-0.74**	-0.00
Standing	-0.40	**0.73**	0.33	-0.11	**0.82**	-0.07
Exploring floor	-0.12	**0.92**	-0.25	0.03	**0.84**	-0.19
Exploring wall	0.22	-0.07	-0.20	0.26	0.16	0.41
Low-pitched vocalizations	0.34	-0.37	0.16	0.12	-0.15	**0.69**
High-pitched vocalizations	0.27	-0.23	0.08	0.10	-0.10	**0.72**
Total distance moved	**0.94**	0.06	0.29	**0.80**	0.10	0.36
Time in door zone	-0.31	-0.48	-0.41	0.31	-0.09	0.08
Frequency visiting door zone	**0.70**	-0.14	-0.10	**0.74**	-0.06	0.22
Time in bucket zone	0.20	0.39	**0.72**	0.09	0.06	-0.07
Frequency visiting bucket zone	**0.74**	0.12	**0.74**	**0.50**	0.09	0.17
Approaching bucket			**0.55**	**0.53**	-0.01	-0.07
Draw back from bucket			-0.02	0.40	-0.31	-0.21
Exploring bucket			**0.87**	-0.03	0.12	0.10
Latency to touch bucket			**-0.73**	-0.13	-0.04	-0.24
Eigenvalues	3.11	2.75	3.32	2.70	2.21	1.59
% of variance explained	43.34	38.32	43.49	24.02	17.55	10.07

[1] High loadings (loadings ≤ -0.50 or ≥ 0.50) are indicated in bold.

3. Results

3.1. Novel rope test

Pigs with a +SBV for the growth of their pen mates were faster to touch one of the ropes provided than pigs with a –SBV for the growth of their pen mates, but +SBV and –SBV pigs did not differ in time spent at both ropes (Table 4). Enriched housed pigs tended to touch a rope faster than barren housed pigs (140.9 ± 18.4 vs. 161.8 ± 20.1 s, $F_{1,71} = 2.92$, $P = 0.09$), but barren housed pigs were more frequently present at the ropes than enriched housed pigs (35.3 ± 2.2 vs. 20.4 ± 1.7 %, $F_{1,71}$ = 30.50, P < 0.001). Female pigs were faster than male pigs to touch one of the ropes provided (135.8 ± 13.0 vs. 166.7 ± 15.0 s, $F_{1,396}$ = 4.95, P = 0.03), but female pigs did not differ from male pigs in time spent at both ropes (27.8 ± 1.3 vs. 28.0 ± 1.3 %, $F_{1,396}$ = 0.06, P = 0.81). No other significant main or interaction effects were found on the latency to touch one of the ropes or on the percentage of time present at both ropes (data not shown).

3.2. Human approach test

SBV class did not affect the latency to approach a person, to touch a person, or the percentage of time present near the person (Table 4). Enriched housed pigs were faster than barren housed pigs to approach (6.7 ± 1.0 vs. 8.3 ± 1.1 s, $F_{1,71}$ = 4.49, P = 0.04) and touch the person (14.8 ± 1.2 vs. 19.4 ± 1.9 s, $F_{1,71}$ = 5.56, P = 0.02). Barren housed pigs, however, were more frequently present near the person than enriched housed pigs (57.8 ± 1.6 vs. 49.5 ± 1.7 %, $F_{1,71}$ = 16.58, P < 0.001). Pigs classified as HR pigs tended to be more frequently present near the person than LR classified pigs (55.2 ± 1.5 vs. 52.5 ± 1.3 %, $F_{1,387}$ = 3.31, P = 0.07). Moreover, female pigs were faster than male pigs to approach the person (6.7 ± 0.8 vs. 8.1 ± 0.9 s, $F_{1,387}$ = 5.46, P = 0.02) and tended to be faster to touch the person (15.9 ± 1.3 vs. 18.2 ± 1.4 s, $F_{1,387}$ = 3.14, P = 0.08), but the percentage of time present near the person did not differ between female and male pigs (52.9 ± 1.3 vs. 54.5 ± 1.4 %, $F_{1,387}$ = 0.72, P = 0.40). No other significant main or interaction effects were found on the latency to approach and touch the person and the presence near the person (data not shown).

3.3. Combined novel environment and novel object test

3.3.1. The period before the bucket was lowered from the ceiling

Two principal components were retained from the principal component analysis conducted on the recorded variables during the first 5 min of the novel environment test (Table 3). Original variables with loadings higher than 0.49 or lower than −0.49 on a principal component have been considered to contribute significantly to that component (Van Reenen et al., 2004; Juul-Madsen et al., 2010). The first component had high positive loadings for walking, total distance moved and the frequency of visiting the door and bucket zone and will, therefore, be referred to as the locomotion component. The second component had high positive loadings for exploring the floor of the arena and standing and had a high negative loading for standing alert and will, therefore, be referred to as the calm component.

No effect of SBV class was found on the scores of the locomotion and calm component (Table 4). Female pigs scored higher than male pigs on both the locomotion (0.16 ± 0.06 vs. −0.16 ± 0.06, $F_{1,362} = 15.39$, $P < 0.001$) and calm component (0.10 ± 0.06 vs. −0.10 ± 0.07, $F_{1,362} = 4.72$, $P = 0.03$), indicating that female pigs were both more active and more calm than male pigs. No other significant main or interaction effects were found (data not shown).

3.3.2. The period after the bucket was lowered from the ceiling

Four principal components were retained from the recorded variables during the second 5 min of the test, i.e. after a bucket was lowered from the ceiling (Table 3). The bucket behaviors loaded high on the first component which will, hence, be referred to as the bucket component. The second and third components are similar to the ‘calm’ and ‘locomotion’ component found during the first 5 min of the test and, therefore, identically labelled. Both high- and low-pitched vocalizations loaded high on the fourth component and will, therefore, be referred to as the vocalization component.

No effect of SBV class was found on the bucket, calm and vocalization component, but +SBV pigs scored lower on the locomotion component than –SBV pigs (Table 4). Pigs classified as HR scored higher than LR classified pigs on the locomotion component (0.10 ± 0.07 vs. −0.08 ± 0.05, $F_{1,362} = 3.62$, $P = 0.06$) and the vocalization component (0.16 ± 0.06 vs. −0.12 ± 0.05, $F_{1,362} = 12.33$, $P < 0.001$). Female pigs tended to have higher scores than male pigs on the bucket component (0.08 ± 0.06 vs. −0.08 ± 0.06, $F_{1,362} = 3.25$, $P = 0.07$) and they had higher scores than male pigs on the locomotion component (0.13 ± 0.06 vs. −0.13 ± 0.06, $F_{1,362} = 10.25$, $P < 0.01$). No other significant main or interaction effects were found (data not shown).

Table 4
Behavioral responses in means ± SEM to various novelty tests of pigs with a positive social breeding value (+SBV) or a negative social breeding value (–SBV) for the growth of their pen mates

	+SBV	–SBV	P-value
Novel rope test			
Latency to touch a rope (s)	130.5 ± 20.2	172.2 ± 17.9	< 0.01
Presence at both ropes (%)	29.4 ± 2.3	26.3 ± 2.4	0.25
Human approach test			
Latency to approach the person (s)	7.8 ± 1.1	7.2 ± 1.0	0.25
Latency to touch the person (s)	16.5 ± 1.4	17.7 ± 1.8	0.39
Presence near the person (%)	53.1 ± 1.9	54.2 ± 1.7	0.54
Combined novel environment and novel object test			
Before the bucket was lowered			
Principal component 1 (“locomotion”)	0.01 ± 0.07	0.00 ± 0.10	0.86
Principal component 2 (“calm”)	0.03 ± 0.07	-0.02 ± 0.08	0.54
After the bucket was lowered			
Principal component 1 (“bucket”)	0.03 ± 0.05	-0.02 ± 0.06	0.65
Principal component 2 (“locomotion”)	-0.09 ± 0.08	0.09 ± 0.06	0.04
Principal component 3 (“calm”)	-0.06 ± 0.06	0.05 ± 0.06	0.28
Principal component 4 (“vocalizations”)	0.01 ± 0.06	-0.01 ± 0.06	0.93

3.3.3. Salivary cortisol

Salivary cortisol concentrations were strongly affected by time ($F_{3,231}$ = 265.58, P < 0.001). Post hoc pairwise comparisons showed that concentrations were higher at t = 15, t = 30 and t = 60 min than at t = 0 min and higher at t = 15 and t = 30 than at t = 60 min (all, P < 0.05) (Fig. 2). Concentrations were not different between t = 15 and t = 30 min (Fig. 2). In addition, concentrations were not different between +SBV and –SBV pigs ($F_{1,72}$ = 0.55, P = 0.46) (Fig. 2A) or between HR and LR pigs ($F_{1,379}$ = 1.41, P = 0.24) (Fig. 2B). Salivary cortisol concentrations were, however, affected by housing ($F_{1,72}$ = 140.65, P < 0.001) and the interaction between housing and time ($F_{3,231}$ = 18.68, P < 0.001). Post hoc pairwise comparisons showed that concentrations were higher for barren housed pigs than for enriched housed pigs at each time point (all P < 0.001), but barren and enriched housed pigs did not differ in their response over time (Fig. 2C). Moreover, salivary cortisol concentrations were also affected by gender ($F_{1,379}$ = 8.51, P < 0.01) and the interaction between gender and time ($F_{3,1143}$ = 3.63, P < 0.05). Post hoc pairwise comparisons showed that male pigs had higher concentrations than female pigs, but only at t = 0 (P < 0.001) (Fig. 2D). No other significant interaction effects were found (data not shown).

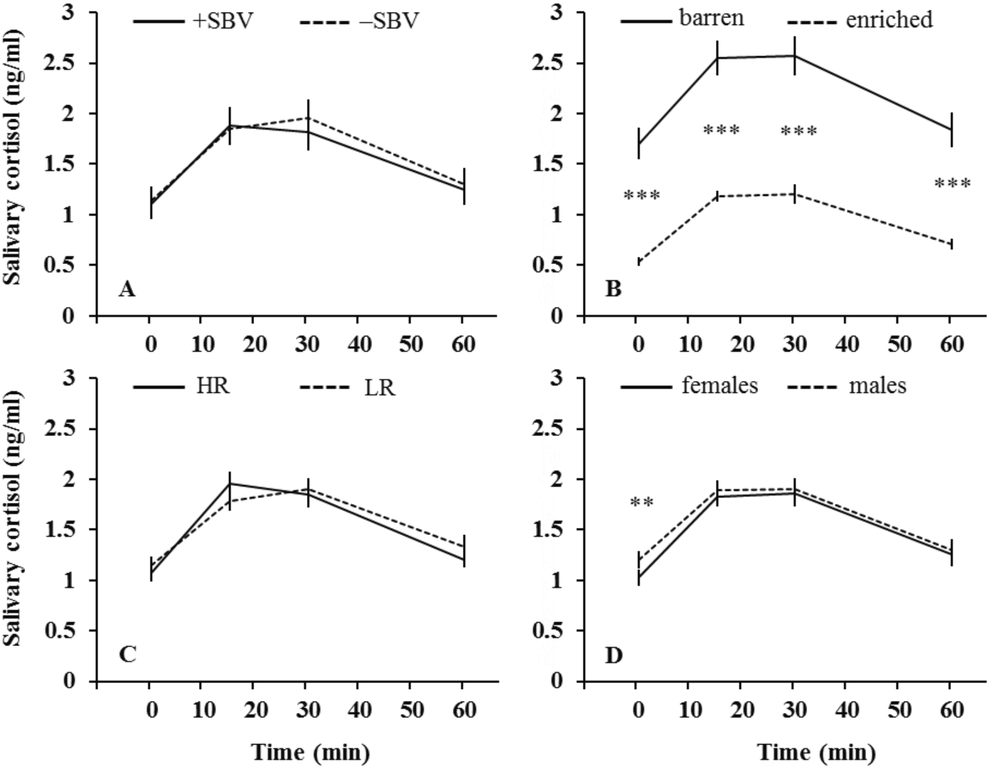

Fig. 2. Means and SEM of salivary cortisol concentrations (ng/ml) at t = 0, 15, 30 and 60 min for pigs with a positive (+) (solid line) or negative (–) social breeding value (SBV) for growth (dashed line) (A), for high-resisting (HR) (solid line) and low-resisting (LR) pigs (dashed line) (B), for barren housed (solid line) and enriched housed pigs (dashed line) (C), and for female (solid line) and male pigs (dashed line) (D). ** P < 0.01, *** P < 0.001

4. Discussion

In this study the hypothesis that pigs with a relatively positive indirect genetic effect on the growth of their pen mates (+SBV pigs) are less fearful than pigs with a relatively negative indirect genetic effect on the growth of their pen mates (–SBV pigs) was tested by subjecting the pigs to several novelty tests. Differences between +SBV and –SBV pigs were only found in the novel rope test and novel environment test. Effects of housing and backtest classification, which were also taken into account because of their known effects on pigs' responses to novelty, were more evident. There were no interactions between SBV class and backtest classification or between SBV class and housing. This suggests that the behavioral differences found between +SBV and –SBV pigs do not result from a differential response to a barren vs. enriched environment. This is in line with other studies who also found that SBV effects on behavior (Camerlink et al., 2013, submitted) and immune status (Reimert et al., submitted) were additive to those of the environment. The influence of these factors will therefore be discussed separately, as will be the gender effects.

4.1. Social breeding values for growth and novelty

In the novel rope test, +SBV pigs were faster than –SBV pigs to touch one of the ropes provided and in the novel environment test, +SBV pigs showed less locomotion after the sudden appearance of a bucket than –SBV pigs. No other differences between +SBV and –SBV pigs were found in these tests, and SBV class did not affect the pigs' responses in the human approach test. Shorter latencies to approach novel stimuli have been associated with being less fearful (e.g. Brown et al., 2009) which suggests that +SBV pigs were less fearful of the ropes than –SBV pigs. Locomotion in a novelty test is more difficult to interpret. Rutherford et al. (2012) showed that locomotion in a novel environment test could indicate that a pig is trying to escape the test as well as reflect a pig's exploration needs or a combination of both. If +SBV pigs would be less inclined than –SBV pigs to escape the arena after the sudden appearance of a bucket, it would be in line with the results of the novel rope test. In a previous study, we subjected +SBV and –SBV piglets to three novelty tests during the lactation period (Reimert et al., 2013b). In line with the present results, we found no large differences between piglets diverging in SBV for growth, except that in a novel object test at 1 week of age, +SBV piglets touched the novel object (a feeder) faster than –SBV piglets and in a human approach test at 2.5 weeks of age, +SBV piglets were found to spent more time near the person than –SBV piglets. Although few, the results from Reimert et al. (2013b) and this study do confirm our hypothesis that +SBV pigs are (somewhat) less fearful than –SBV pigs, because shorter latencies to touch and more contact time with novel stimuli have been associated with lower fearfulness (e.g. Brown et al., 2009; Dalmau et al., 2009) and less locomotion in a novel arena could indicate less intention to escape the arena (Rutherford et al., 2012).

Camerlink et al. (2013) found that +SBV pigs were less aggressive towards their familiar pen mates than –SBV pigs in the first 30 min upon reunion after a 24-hours mix test with unfamiliar pigs, indicating that +SBV pigs may cope better with stress- or fear-eliciting situations than –SBV pigs. Together these results suggests that SBV for the growth of pen mates might at least in part result from differences in fearfulness which is in line with reported effects of fear on growth (Boissy, 1995; Hemsworth, 2003) and its influence on group members (Edgar et al., 2012; Špinka, 2012; Reimert et al., 2013a) and with several laying hens studies which showed that laying hens selected with a similar breeding strategy but for a +SBV on the survival of their group members were less fearful in several stressful situations as well (Bolhuis et al., 2009; Rodenburg et al., 2009; Nordquist et al., 2011). If selection for pigs that have a positive effect on the growth of their pen mates also reduces fearfulness in pigs, this may have positive consequences for pig welfare. Caution should be made, however, because the pigs under study were selected after just one generation. Also, given that the differences in fear-related behaviors in our present and previous study (Reimert et al., 2013b) were small and subtle, other differences such as differences in (tail) biting behavior likely underlie the diverging SBV for growth as well (Camerlink et al., submitted; Reimert et al., submitted).

4.2. Housing and novelty

Straw-enriched pigs were faster than barren housed pigs to touch a rope and to approach and touch a person in the group-wise novel rope test and human approach test, respectively, but barren housed pigs spent more time at the ropes and near the person than enriched housed pigs. These results are in line with those of Stolba and Wood-Gush (1980) and Wemelsfelder et al. (2000) who found that enriched housed pigs were faster than barren housed pigs to touch a novel object in their home pen, whereas barren housed pigs spent more time near the object. Together, these results suggest that enriched housed pigs are less fearful or more curious to start exploring a novel object or a person in the home pen but, at the same time, barren housed pigs are more motivated to keep on exploring a novel object or a person in the home pen possibly due to less exploration possibilities in the home pen compared to enriched housed pigs (Wemelsfelder et al., 2000).

Contrary to the group-wise novelty tests, in the individual novel environment test no behavioral differences were found between straw-enriched and barren housed pigs in the period before as well as after the appearance of the bucket. These results are in line with Wemelsfelder et al. (2000) who also found no difference between enriched and barren housed pigs in object manipulation in a combined novel environment and novel object test. There are, however, also studies that do find differences in behavior between barren and enriched housed pigs in response to a novel environment test (e.g. Mendl et al., 1997; Beattie et al., 2000; O'Connell et al., 2004; Jansen et al., 2009). The salivary cortisol response to this test was similar for both housing types, but concentrations were higher in barren housed pigs at each time point measured. De Jong et al. (1998) also found no effect of housing on the salivary cortisol response to stressors such as relocation, isolation and restraint, whereas O'Connell et al. (2004) found that enriched housed pigs had a larger cortisol response to a novel environment test compared to barren housed pigs. In addition, Van de Weerd and Day (2009) reported, in line with our study, that salivary cortisol concentrations are higher in barren housed pigs compared to straw-enriched pigs, whereas De Jong et al. (1998, 2000) reported higher basal cortisol concentrations in enriched housed pigs compared to barren housed pigs. These behavioral and physiological contrasting results could possibly be explained by differences in enrichment chosen. In this study, barren and enriched pens were of similar size, but the enriched pens were filled with ample straw and wood shavings. In the other studies the enrichment included also more space (Beattie et al., 2000; O'Connell et al., 2004) and/or other enrichment materials such as peat or branches (Mendl et al., 1997; Beattie et al., 2000; O'Connell et al., 2004). In addition, in several studies reporting behavioral and/or physiological differences in response to novel situations between barren and enriched pigs, the contrast in housing environment was already present at time of birth (De Jong et al., 1998, 2000; Beattie et al., 2000; O'Connell et al., 2004; Jansen et al., 2009; Oostindjer et al., 2011), suggesting that rearing housing conditions and thereby early development of behavior and of (re)activity of the HPA-axis rather than current housing conditions could play a role here.

4.3. Backtest classification and novelty

Studies in a growing number of species describe two extremes in coping style or personality: proactive and reactive (Carere et al., 2010; Coppens et al., 2010; Koolhaas et al., 2010). In pigs, similar coping styles have been distinguished based on their response in a backtest. High-resisting (HR) pigs are more likely individuals with a proactive style of coping with stress (Geverink et al., 2002; Jansen et al., 2009; Reimert et al., 2013b) and they seem to act primarily on the basis of previous experience, i.e. they develop more easily routines (Bolhuis et al., 2004). Low-resisting (LR) pigs, in contrast, resemble reactive copers, as they show more passive behaviors when stressed and have a higher behavioral flexibility (Bolhuis et al., 2004, 2005b; Jansen et al., 2009). In contrast with several other studies (e.g. Hessing et al., 1993; Bolhuis et al., 2000; Ruis et al., 2000; Van Erp-van der Kooij et al., 2000; Geverink et al., 2002), we did not use the extremes of the population to characterize pigs as HR or LR pigs but simply divided our population into two groups. Yet, in the present study, behavioral differences between HR and LR pigs were still found in the human approach test and novel environment test, but not in the novel rope test. In the human approach test, HR pigs tended to spend more time near the person than LR pigs, and in the novel environment test, HR pigs tended to show more locomotion and were more vocal than LR pigs in the 5 min after the appearance of the bucket. This latter result is in line with Ruis et al. (2001) who also found that HR and LR pigs did not differ behaviorally in a novel environment test in the period before the novel object, but that HR pigs did vocalize more than LR pigs after novel object exposure. Also in other challenging situations, HR pigs have been reported to vocalize more (Geverink et al., 2002; Jansen et al., 2009).

Overall, the results of this study could suggest that differences between HR and LR pigs only emerge if a novelty test is sufficiently challenging. A person in the home pen might be more challenging than two ropes in the home pen and the sudden appearance of a bucket in an arena might be more challenging than the arena itself. This is in line with Ursinus et al. (2013) who found that pigs displayed more fear-related behaviors in a novel environment test after introduction of a novel object compared to exposure to just the arena itself. In a novel environment not combined with a novel object test at 3.5 weeks of age, a predictive effect of the backtest response was, however, found too. Piglets that struggled more in the backtest walked more in the novel environment test (Reimert et al., 2013b). This result does, however, not undermine the suggestion made, because for very young piglets, isolation from the sow and littermates could already be sufficiently challenging (Kanitz et al., 2009).

The HR pigs in this study responded with more active behaviors to the change of environment compared to the LR pigs which is in line with other studies in pigs (Hessing et al., 1994; Bolhuis and Schouten, 2002; Geverink et al., 2002; Jansen et al., 2009; Reimert et al., 2013b) and studies in other animals (Carere et al., 2010; Koolhaas et al., 2010). Our cortisol results seem to contradict several other pig studies that did find cortisol level differences between HR and LR pigs (Hessing et al., 1994; Ruis et al., 2000, 2001; Geverink et al., 2002). Koolhaas et al. (2010) demonstrated, however, that activation of the HPA-axis does not necessarily have to differ between proactive and reactive animals. We did not find any interaction effects between housing and backtest classification, whereas others (Bolhuis et al., 2003, 2004, 2005a, 2006; Melotti et al., 2011) found that the impact of housing condition (i.e. barren or enriched) was different for HR and LR pigs. All in all, at least part of the variation in behavioral responses of pigs to challenging situations seems to reflect their coping style or personality as assessed early in life by their response in a backtest.

4.4. Gender and novelty

Female pigs were faster than castrated male pigs to touch a rope in the novel rope test and to approach and touch a person in the human approach test. In the novel environment test, female pigs were more active and less alert than male pigs in the first 5 min of the test. After the appearance of the bucket, female pigs paid more attention to the bucket and remained more physically active than male pigs. These results are in line with our previous study on pre-weaning piglets in which we found that castrated male piglets responded more fearful to novel situations than female piglets (Reimert et al., 2013b). In addition, in the present study, male pigs had higher salivary cortisol concentrations than female pigs at t = 0. In Reimert et al. (2013b), we proposed that these differences could be explained by castration and its consequences, but it could also be a genuine difference between the genders. In pigs, castration is performed to avoid the presence of boar taint in meat (Prunier et al., 2006) and to reduce aggressive and mounting behaviors later in life (Von Borell et al., 2009), but to the authors' knowledge the (long-term) effects of castration on behaviors under challenging conditions have not been studied as such. During surgical castration, the testes are removed which consequently results in a loss of gonadal hormones such as testosterone (Babol et al., 2004; Prunier et al., 2006). Endogenous or exogenous testosterone has been associated with anxiolytic behavior in novelty tests in for instance sheep (Vandenheede and Bouissou, 1993a, 1996), heifers (Boissy and Bouissou, 1994), and castrated male rats (Frye and Edinger, 2004). To our knowledge, it is not known whether testosterone also makes pigs less fearful, but if so, the absence of gonadal testosterone could explain the more fearful reaction of the castrated male pigs before weaning (Reimert et al., 2013b) because normally testosterone production peaks between 2 and 4 weeks of age in male piglets (Colenbrander et al., 1978; Schwarzenberger et al., 1993). Testosterone concentrations are, however, relatively low in the period we tested the pigs after weaning (Colenbrander et al., 1978; Schwarzenberger et al., 1993), so the results cannot be solely explained by the absence of gonadal testosterone. Besides the absence of testosterone, the handling by a human during castration and the associated pain afterwards could also have made the castrated male pigs more fearful of humans (Prunier et al., 2006). As humans were present during each novelty test in this study and in the tests presented in Reimert et al. (2013b), this could also be an explanation of the results found. Even though our results (Reimert et al., 2013b; this study) show a very consistent pattern, they are not in line with other pig studies that found no differences between female and (castrated) males pigs in their response to novelty (e.g. Fraser, 1974; Taylor and Friend, 1986; Velie et al., 2009). In addition, the literature is also not consistent about differences between female and male pigs in their physiological response to novelty (e.g. Hessing et al., 1994; Ruis et al., 1997; De Jong et al., 1998). We, therefore, propose that more research into gender differences and underlying factors is needed, because it could aid in a better understanding of the behavioural and physiological needs of both sexes and thereby improve pig welfare and productivity (Vandenheede and Bouissou, 1993b).

5. Conclusions

Subtle effects of divergent selection for indirect genetic effects on growth in pigs were found on behavioral responses in novelty tests, suggesting that pigs with a relatively positive indirect genetic effect on the growth of their pen mates are less fearful, but more research is needed to confirm this.

Furthermore, the housing environment and a pig's coping style and gender affect its response in novelty tests as well.

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CHAPTER 4

Selection on social breeding values for growth, environmental enrichment and coping style affect the immune status of pigs

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submitted

Abstract

Pigs living in intensive husbandry systems may experience both acute and chronic stress through standard management procedures and limitations in their physical and social environment, which may have implications for their immune status. In this study, the effect of a new breeding method where pigs were selected on their heritable influence on their pen mates' growth, and environmental enrichment on the immune status of pigs was investigated. Hereto, 240 pigs with a relatively positive genetic effect on the growth of their pen mates (+SBV) and 240 pigs with a relatively negative genetic effect on the growth of their pen mates (–SBV) were housed in barren or straw-enriched pens from 4 to 23 weeks of age (n = 80 pens in total). A blood sample was taken from the pigs before, three days after a 24 h regrouping test, and at 22 weeks of age. In addition, effects of coping style, as assessed in a backtest, and gender were also investigated. Mainly, +SBV were found to have lower leukocyte, lymphocyte and haptoglobin concentrations than –SBV pigs. Enriched housed pigs had a lower N:L ratio and lower haptoglobin concentrations, but had higher antibody titers specific for KLH than barren housed pigs. No interactions were found between SBV class and housing. Furthermore, pigs with a proactive coping style had higher alternative complement activity and, in the enriched pens, higher antibody titers specific for KLH than pigs with a reactive coping style. Lastly, females tended to have lower leukocyte, but higher haptoglobin concentrations than castrated males. Overall, these results suggest that +SBV pigs and enriched housed pigs were less affected by stress than –SBV and barren housed pigs, respectively. Moreover, immune activation might be differently organized in individuals with different coping styles and to a lesser extent in individuals of opposite genders.

Keywords: stress, immune system, indirect genetic effects, enrichment, coping style, gender, pigs

1. Introduction

In response to stressful situations, the HPA-axis and sympathetic nervous system are activated which subsequently results in the release of glucocorticoids and catecholamines which prepare the body to fight or flight (e.g. Chrousos and Gold, 1992). However, it is now well-known that the experience of stress also has an effect on various components of the immune system (e.g. Khansari et al., 1990; Campisi and Fleshner, 2003). For instance, one of the best known effects of acute stress is a reduction in the number of several blood leukocyte types (Dhabhar, 2002). Generally, acute stress has been suggested to enhance and chronic stress to suppress immune activation (Dhabhar, 2002), although experimental results are not always that straightforward (e.g. Moynihan, 2003; Salak-Johnson and McGlone, 2007). The effect of stress on the immune system is suggested to be particularly mediated by glucocorticoids and catecholamines (Glaser, 2003; Moynihan, 2003; Nance and Sanders, 2007; Padgett and Sorrells and Sapolsky, 2007).

Pigs in intensive farming systems experience acute stress during standard management procedures such as castration, tail docking, abrupt weaning, regrouping and transport (e.g. Noonan et al., 1994; Stookey and Gonyou, 1994; Geverink et al., 1998; Von Borell, 2001; Dudink et al., 2006; Rault et al., 2011), and at the same time they have to cope with prolonged limitations in their living environment. The absence of proper substrates for oral manipulation in most intensive farming systems (Day et al., 2002; Van de Weerd and Day, 2009) prevents pigs from performing highly motivated behaviors such as rooting and chewing (Studnitz et al., 2007) and may, therefore, induce chronic stress which is reflected in changes in the HPA-axis (e.g. Beattie et al., 2000; De Jong et al., 2000), cognitive impairment (De Jong et al., 2000; Sneddon et al., 2000; Bolhuis et al., 2013) and in the expression of abnormal behaviors, such as tail biting and stereotypies (e.g. Beattie et al., 1995; Schrøder-Petersen and Simonsen, 2001; Averós et al., 2010). Chronic stress may also be caused by ongoing social stress (Turner et al., 2009, 2013). Both short-term and prolonged stressful situations have indeed been found to influence the immune status and immune reactivity of pigs (e.g. De Groot et al., 2001; Kanitz et al., 2004; Merlot et al., 2004; Salak-Johnson and McGlone, 2007), and also have major implications for pig welfare and productivity (e.g. Wiepkema and Koolhaas, 1993; Stookey and Gonyou, 1994; Ekkel et al., 1995; Hyun et al., 1998).

One solution to diminish these negative side effects of stress may be provided by genetics (Star et al., 2008; Turner, 2011; Rodenburg and Turner, 2012). For instance, social stress may be reduced by breeding pigs that perform well in group housing and do not show harmful behavior directed towards their group mates. Direct selection for pigs that perform favorable behaviors seems, however, not feasible in commercial pig breeding (Turner, 2011; Bijma, 2012), but selection on group performance is feasible and this may, indirectly, also lead to pigs with improved behavioral skills (Bijma, 2012). Early work of Griffing (1967) and later work of Muir (1996, 2005) and Bijma et al. (2007a,b) has shown that a phenotypical trait of an individual that lives in a group is not only influenced by its own genes, but also by the genes of its group members. This indirect genetic effect (Wolf et al., 1998) on another's phenotypical trait is also referred to as associative effect (Muir, 2005) or social (genetic) effect (Bergsma et al., 2008, 2013) and can relatively easy be included as a social breeding value (SBV) for production traits in commercial breeding programs (Bijma et al., 2007a; Bijma, 2012). Hence, via indirect selection on each other's performance, animals can perform better as a group. A series of selection experiments in which laying hens were selected by taking indirect genetic effects on performance of cage mates into account, not only showed that these laying hens indeed performed better as a group, but also suggested that these hens were less sensitive to stress compared to laying hens that were selected on individual performance only (reviewed in Rodenburg et al., 2010). Pigs can be selected for the genetic effect on each other's growth during the finishing phase (Chen et al., 2007; Bergsma et al., 2008, 2013) and the first results of a one generation selection experiment indicated that pigs that were selected to have a relatively positive indirect genetic effect on the growth of their pen mates (+SBV) are somewhat less fearful (Reimert et al., 2013, 2014) and less sensitive to stress (Camerlink et al., 2013) than pigs that were selected to have a relatively negative indirect genetic effect on the growth of their pen mates (–SBV). Effects of this divergent selection on indirect genetic effects for growth on immune status are so far unknown. Besides genetics, the provision of environmental enrichment is suggested to alleviate pigs from (prolonged) stress as well (reviewed by Van de Weerd and Day, 2009). Environmental enrichment has, moreover, been reported to affect certain components of the immune system (Huff et al., 2003; Marashi et al., 2003).

The aim of this study was therefore to investigate both the separate and interacting effects of this new breeding method and housing on the immune status of pigs. Furthermore, the coping style of the pigs was also taken into account, because pigs with different coping styles do not only respond differently to acute and chronic stress (Geverink et al., 2003, 2004a; Bolhuis et al., 2005a, 2006), but have also been found to differ in immune responses (Schrama et al., 1997; Bolhuis et al., 2003). To that aim, a contrast in prolonged stress was created by housing

+SBV and –SBV pigs from 4 to 23 weeks of age in either relatively barren or straw-enriched pens. Furthermore, at 9 weeks of age all pigs were subjected to a 24 h regrouping test to induce acute stress (Stookey and Gonyou, 1994). Effects of SBV class, housing and coping style on leukocyte subsets (Davis et al., 2008), haptoglobin (Cray, 2012) and innate immune components (Ayensu et al., 1995; Star et al., 2007; Oostindjer et al., 2013; Sun et al., 2013) were studied by taking three blood samples, i.e. before and after the regrouping test and at 22 weeks of age. We expected the +SBV pigs to be less affected by stress than the –SBV pigs which would be, subsequently, reflected in their immune status. In addition, we expected differences in immune status between pigs in barren and enriched housing as the latter are likely to suffer less from prolonged stress than barren housed pigs.

2. Materials and Methods

2.1. Ethics statement

The experiment described in this study was approved by the Animal Care and Use Committee of Wageningen University (Protocol Number: 2010055f). Blood sampling was carried out by trained assistants and done as quickly as possible to minimize suffering.

2.2. Animals and housing

The pigs in this study - 480 in total, equally divided over five batches - were the same pigs as described in Reimert et al. (2014). In short, pigs were born at the experimental farm of Topigs Research Center IPG in Beilen, The Netherlands and reared in conventional farrowing pens. Pigs were weaned at four weeks of age and transported to the experimental farm 'de Haar' of Wageningen University in Wageningen, The Netherlands. Here, half of the pigs were housed in barren pens (~1 m^2/ pig) with a partially slatted and partially concrete solid floor. Barren housed pigs received two hands of wood shavings each day from 6 weeks of age onwards. The other half of the pigs were housed in pens (~1 m^2/ pig) enriched with 1.5 kg of straw and 12 kg of wood shavings. All pens were cleaned daily and afterwards 3 kg of fresh wood shavings and fresh straw (250 g at the start of the experiment and then gradually increased to 1.5 kg) were added to the enriched pens. In all pens, a metal chain with a ball and, from 8 weeks of age, a jute sack were attached to the wall of the pen. The jute sack was replaced when needed.

Each group of pigs consisted of three gilts and three barrows and at least two

HR and two LR pigs (see section 2.2.1.) and groups diverged in indirect genetic effects for growth, i.e. the heritable effect on the growth of their group members. All pigs in a pen had either an estimated relatively positive indirect genetic effect (+SBV, average of 2.00 g/day) or an estimated relatively negative indirect genetic effect on the growth of their pen mates (–SBV, average of -1.62 g/day) during the finishing period (from app. 25 to 110 kg). During this period, the growth of a pig is theoretically affected by each of its pen mates which means that the total estimated indirect genetic effect on a pig's growth in this experiment was 10 g/day ((6-1) * 2.00) for the +SBV pigs and -8.1 g/day ((6-1) * -1.62) for the –SBV pigs. Pigs were obtained by mating Topigs-20 sows and Tempo boars with the most extreme positive or negative estimated indirect genetic effects for growth that were available for each batch. Direct breeding values for growth were kept as similar as possible for both SBV classes (for details about the (social) breeding value estimations see Camerlink et al., 2013). The study was approved by the Animal Care and Use Committee of Wageningen University.

2.3. Behavioral tests

2.3.1. Backtest

Pigs were subjected to the backtest at approximately two weeks of age (for details see Reimert et al., 2013) to assess their personality or coping style (Bolhuis et al., 2003). In short, a piglet was put on its back for 1 min and manually restrained. During the test, the number of struggles, the latency to struggle, the number of vocalizations, and the latency to vocalize were recorded. Piglets were classified as high-resisters (HR) if they showed two struggles and produced at least 25 vocalizations, or showed at least three struggles. Low-resisting (LR) pigs were piglets that showed 0 or 1 struggle, or 2 struggles and produced less than 25 vocalizations.

2.3.2. Regrouping test

At 9 weeks of age, pigs were exposed to a regrouping test (see Camerlink et al., 2013 for details) which is a stressful event for pigs (Stookey and Gonyou, 1994). In short, a pair of pigs was regrouped for 24 h in a new pen with two other pairs of unfamiliar pigs. Pairs of pigs were always mixed with other pairs from the same SBV class and housing condition and the new temporary group composition was balanced for gender and coping style. After the 24 h, each pair of pigs was put in its original pen and group again.

2.4. Blood collection and analyses

Blood was collected from the pigs in the week before the regrouping test at 8 weeks of age, three days after the regrouping test at 9 weeks of age, and at 22 weeks of age. Hereto, a pig was immobilized on its back in a crib (for the first and second collection) or fixated using a nose sling (for the third collection) and blood was taken by venipuncture from the jugular vein. Housing condition and SBV class were taken into account in the order of blood collection. Blood was collected in serum separating tubes (Greiner bio-one, Kremsmünster, Austria) which were stored at room temperature (RT) and in K3 EDTA tubes (Greiner bio-one, Kremsmünster, Austria) which were stored on ice after blood sampling.

In the laboratory, the serum separating tubes were incubated for one hour at 37 °C after which they were centrifuged at 5251 g for 12 min at 20 °C. Obtained sera were stored at -80 °C until further analysis (sections 2.3.1, 2.3.2 and 2.3.4). Blood from the EDTA tubes was used directly (section 2.3.3).

2.4.1. Complement activity via the classical (CPW) and alternative pathway (APW)

The hemolytic activity of both CPW and APW complement was measured using the hemolytic complement assay of Demey et al. (1993). In short, for CPW complement activity, 50 µl of serum was diluted serially in a gelatin-VBS-salt buffer and incubated with hemolysin sensitized sheep red blood cells for 90 min at 37 °C in 96-well microtiter plates. During incubation, plates were shaken every 30 min in a Titertrek (Flow Laboratories). After 90 min, the amount of light scattered by the red blood cells upon lysis was read at 655 nm in a microplate reader (BioRad model 3550). The readings were transformed using a log-log equation (Von Krogh, 1916) and the hemolytic titer was expressed as the titer that lysed 50 % of the red blood cells (CH50 U/ml). For APW complement activity, the same assay was used except that sera were diluted serially in a gelatin-VBS-EGTA buffer and incubated with rabbit red blood cells (Demey et al., 1993).

2.4.2. IgG and IgM antibody titers specific for KLH

Antibody titers of IgG and IgM specific for Keyhole Limpet Hemocyanin (KLH) (Lammers et al., 2004) were determined by a two-step enzyme-linked immunosorbent assay (ELISA) similar to Bolhuis et al. (2003) and Lammers et al. (2004). First, medium binding microtiter plates (Greiner Bio-one, Alphen a/d Rijn, The Netherlands) were coated overnight at 4 °C with 2 mg/ml KLH in coating buffer (0.05 M Na_2CO_3 x 10 H_2O, pH 9.6). After washing with tap water containing 0.05% Tween 20, serial dilutions of serum were added and incubated for one hour at RT. After washing, plates were incubated for one hour at RT with a 1:20000 diluted peroxidase (PO)-conjugated goat antibody directed to swine IgG_{FC} (Gα$Sw-IgG_{FC}$/PO, Bethyl Laboratories, Montgomery, USA) to detect binding of IgG and with 1:20000 diluted peroxidase (PO)-conjugated goat antibody directed to swine IgM_{FC} (Gα$Sw-IgM_{FC}$/PO, Bethyl Laboratories, Montgomery, USA) to detect binding of IgM, respectively. After washing, tetramethylbenzidine and 0.05% H_2O_2 were added as a substrate and incubated for 10 min at RT. The reaction was stopped with 2.5N H_2SO_4 and the absorbance was measured at 450 nm with a Multiskan (Flow, Irvine, UK). Each absorbance was expressed relatively to the absorbance of a standard positive control serum and antibody titers were determined as described in Schrama et al. (1997).

2.4.3. Leukocytes, lymphocytes and the neutrophil to lymphocyte ratio

With 150 µl of the blood in the EDTA tubes, the concentration of leukocytes (10^9/l) was determined with a Sysmex F-820 (Sysmex Corporation, Kobe, Japan) and with 10 µl, a smear was made on a microscope plate. After the smears had dried, they were fixed with a methanol solution and thereafter stained using a rapid staining kit (Hemacolor® staining kit, Merck KGaA, Darmstadt, Germany). Surplus staining solution was washed away with PBS and then smears were dried. The percentages of lymphocytes, neutrophils, monocytes and eosinophils (the last two were not used further in this study, because of low occurrence: monocytes: 8.4 ± 0.2 %, eosinophils: 2.0 ± 0.1 % (overall mean ± SEM)) were determined by microscopic examination of the smears and counting 100 leukocytes in total using an Assistant-Counter AC-8. From these counts, the neutrophil:lymphocyte ratio (N:L ratio) was determined. Furthermore, the concentration of lymphocytes (10^9/l) was determined by multiplying the percentage of lymphocytes with the leukocyte concentration.

2.4.4. Haptoglobin

Haptoglobin concentrations were determined in serum using a commercial kit based on the hemoglobin-binding capacities of haptoglobin (Phase™ Haptoglobin, Tridelta Development Limited, Maynooth, Ireland) which has been validated for pigs (GD Animal Health Service, Deventer, The Netherlands). Briefly, 100 µl of hemoglobin was added to 7.5 µl serum and solutions were gently mixed. Thereafter, 140 µl of chromogen was added and incubated for 5 min at RT. The absorbance was read immediately at 600 nm in a microplate reader. Haptoglobin concentrations (mg/ml) were calculated by using a standard linear curve with known concentrations of haptoglobin.

2.5. Statistical analyses

SAS (SAS 9.2, SAS Institute Inc.) was used for all statistical analyses. Variables could not be obtained from all 480 pigs at each sampling period, because pigs were either not healthy or had died, or the blood had clotted after sampling. Depending on the variable and week (8, 9 or 22 weeks of age), missing values ranged from 3 to 45. The variables CPW complement, haptoglobin, and N:L ratio were log transformed and the number of lymphocytes square root transformed to obtain normally distributed residuals. The effects of week, SBV class, housing, backtest classification and gender on the variables were assessed with a repeated linear mixed model with the fixed factors week, SBV class, housing, backtest classification, their interactions, gender, its interaction with week, and batch. Values in time of individual pens and pigs were taken as repeated measurements, i.e. SBV class, housing and batch effects were tested against the random effect of pen, and backtest classification and gender effects were tested against the random effect of pig. The order of collection within a sampling day was included as covariate.

To investigate the effect of SBV class, housing, backtest classification and gender on the variables after acute stress, the delta between week 9 and week 8 was calculated and subsequently analyzed with a linear mixed model with SBV class, housing, backtest classification, their two-way interactions, gender, and batch as fixed effects and pen, nested within SBV class, housing, and batch, as random effect. Prior to this analysis, CPW complement activity was log transformed to obtain normality of residuals.

For brevity, only significant interactions are reported. Significant interactions were further investigated with post hoc pairwise comparisons using the differences of the least square means. Results are presented as means ± SEM.

3. Results

3.1. Classical (CPW) and alternative (APW) complement activity

CPW complement activity was not affected by SBV class, housing or backtest classification ($P \geq 0.29$). There was, however, an effect of week on CPW complement activity ($P < 0.001$) (Fig. 1A and 1B), with higher activity in week 9, three days after regrouping, than in week 8 or 22. The increase in CPW complement activity from week 8 to 9 (i.e. the delta) was not affected by SBV class, housing or backtest classification ($P \geq 0.12$).

APW complement activity was affected by backtest classification ($P < 0.01$) and week ($P < 0.001$) (Fig. 1C and 1D). HR pigs had a higher APW complement activity than LR pigs (overall: 64.3 ± 1.1 vs. 58.9 ± 1.1 CH50 U/ml). In addition, APW complement activity was lower in week 22 compared to weeks 8 and 9. APW complement activity was not affected by SBV class or housing ($P \geq 0.54$). Although APW complement activity did, overall, not differ between weeks 8 and 9, the delta between weeks 8 and 9 was affected by backtest classification ($P < 0.05$). From week 8 to 9, after regrouping, APW complement activity decreased slightly for HR pigs, but increased for LR pigs (-1.0 ± 1.7 vs. 3.4 ± 1.6 CH50 U/ml). The delta in APW complement activity was not affected by SBV class or housing ($P \geq 0.93$).

3.2. IgG and IgM titers specific for KLH

KLH-IgG titers were affected by housing ($P < 0.001$) and week ($P < 0.001$) and by the interaction between housing and backtest classification ($P < 0.05$) (Fig. 1E and 1F). Post hoc analysis showed that enriched housed HR pigs (4.0 ± 0.1) had a higher titer than enriched housed LR pigs (3.8 ± 0.1) and that both had a higher titer than the barren housed HR and LR pigs (both: 3.5 ± 0.1). Furthermore, KLH-IgG titers increased from week 8 to 9 to 22. KLH-IgG titers were not affected by SBV class ($P = 0.43$). The increase of the KLH-IgG titer from week 8 to 9 tended to be smaller for +SBV pigs than for –SBV pigs (0.37 ± 0.06 vs. 0.47 ± 0.06, $P < 0.1$) and was larger for enriched housed pigs than for barren housed pigs (0.59 ± 0.07 vs. 0.32 ± 0.08, $P < 0.05$), but was not affected by backtest classification ($P = 0.15$).

KLH-IgM titers were affected by week ($P < 0.001$) and the interaction between housing, backtest classification and week ($P < 0.001$) (Fig. 1G and 1H). Post hoc analysis showed that KLH-IgM titers, similar to the KLH-IgG titers, increased from week 8 to 9 to 22. Housing and backtest classification did not affect the KLH-IgM titer in weeks 8 and 9, but HR enriched housed pigs had a higher KLH-IgM titer than the other pigs in week 22. KLH-IgM titers were not affected by SBV class ($P = 0.19$). The increase of the KLH-IgM titer from week 8 to 9 was not affected by SBV class, housing or backtest classification ($P \geq 0.16$).

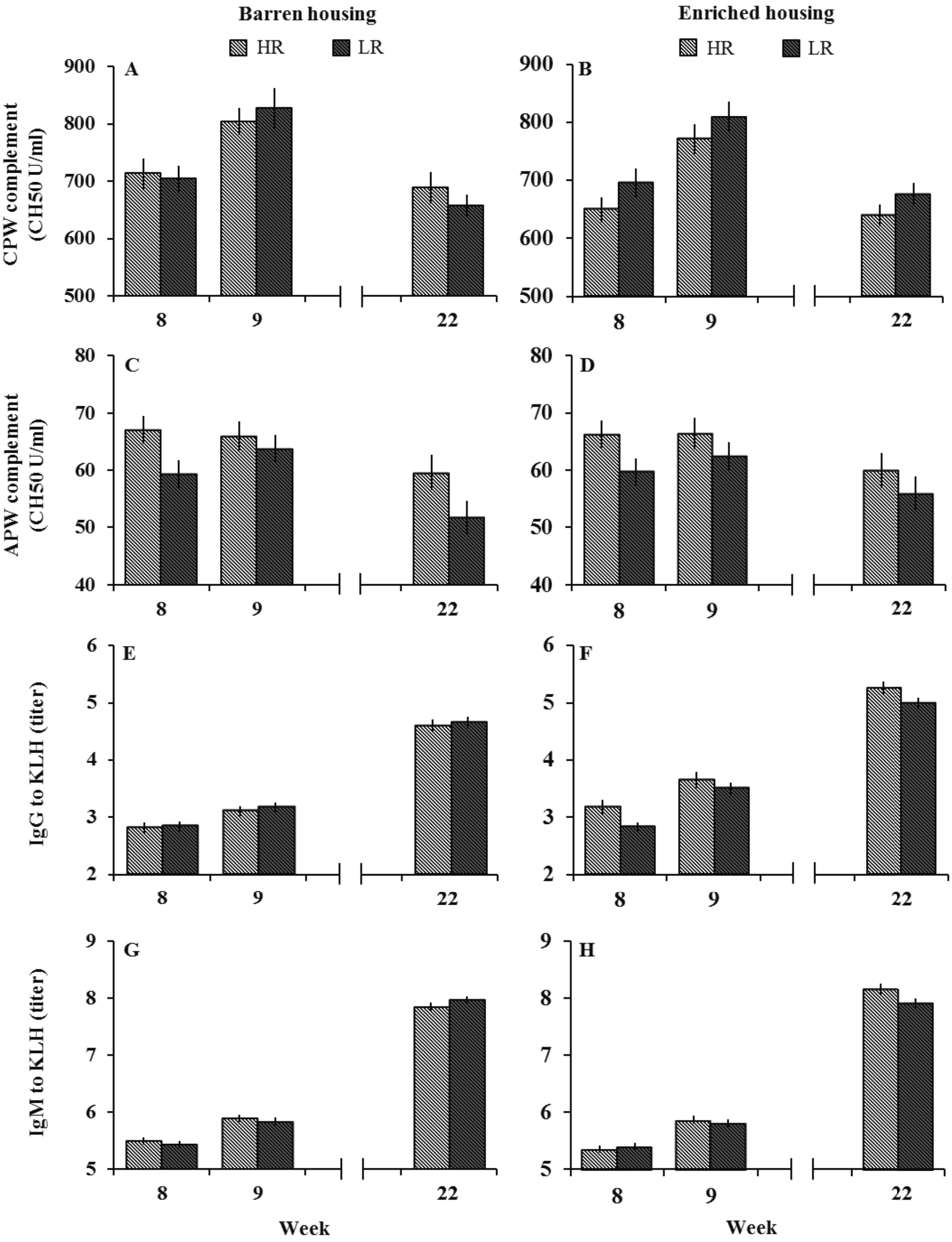

Fig. 1. Means and SEM of complement activity and antibody titers. Compliment activity via the classical (CPW) (panels A and B) and alternative pathway (APW) (panels C and D), and IgG (panels E and F) and IgM titers (panels G and H) to Keyhole Limpet Hemocyanin (KLH) of pigs with a high-resisting (HR) and low-resisting (LR) backtest classification in barren and enriched housing measured before a 24 h regrouping test at 8 weeks of age, after the regrouping test at 9 weeks of age and at 22 weeks of age. Significance of effects of housing and backtest classification is given in the text.

3.3. Leukocytes, lymphocytes and the ratio of neutrophils to lymphocytes (N:L ratio)

The concentration of leukocytes was affected by SBV class ($P < 0.05$). Pigs with a +SBV had, overall, lower concentrations than –SBV pigs (17.8 ± 0.2 vs. 18.6 ± 0.3 10^9/l). The concentration of leukocytes was also affected by week ($P < 0.001$) and by the interaction between housing and week ($P < 0.01$) (Fig. 2A and 2B). Post hoc analysis revealed that leukocyte concentrations of enriched housed pigs were lower in weeks 9 and 22 compared to week 8, whereas leukocyte concentrations of barren housed pigs were lower in week 9 compared to weeks 8 and 22. In addition, leukocyte concentrations did not differ between enriched and barren housed pigs in weeks 8 and 9, but enriched housed pigs had lower leukocyte concentrations than barren housed pigs in week 22 (Fig. 2A and 2B). Leukocyte concentrations were not affected by backtest classification ($P = 0.82$). The decrease in the concentration of leukocytes from week 8 to 9 was larger for +SBV pigs than for –SBV pigs (-2.5 ± 0.4 vs. -1.6 ± 0.4 10^9/l, $P < 0.05$), but was not affected by housing or backtest classification ($P \geq 0.13$).

The concentration of lymphocytes was also affected by SBV class ($P < 0.05$) and week ($P < 0.01$) (Fig. 2C and 2D). Pigs with a +SBV had lower lymphocyte concentrations than –SBV pigs (8.6 ± 0.2 vs. 9.2 ± 0.2 10^9/l). Furthermore, lymphocyte concentrations were lower in week 22 compared to weeks 8 and 9. Lymphocyte concentrations were not affected by housing or backtest classification ($P \geq 0.22$). The delta in the lymphocyte concentration between weeks 8 and 9 was also not affected by SBV class, housing or backtest classification ($P \geq 0.20$).

The N:L ratio was affected by housing ($P < 0.05$) and week ($P < 0.001$) (Fig. 2E and 2F). Enriched housed pigs had a lower N:L ratio than barren housed pigs (0.90 ± 0.03 vs. 0.96 ± 0.04). Moreover, the N:L ratio was higher in week 8 compared to weeks 9 and 22. The delta in N:L ratio between weeks 8 and 9 was not affected by housing ($P = 0.57$), but was affected by the interaction between SBV class and backtest classification ($P < 0.05$). Post hoc analysis showed, however, no differences between the groups.

3.4. Haptoglobin

Haptoglobin concentrations tended to be affected by SBV class ($P < 0.1$) (Fig. 2G and 2H). Concentrations tended to be lower for +SBV pigs than –SBV pigs (0.58 ± 0.03 vs. 0.65 ± 0.03 mg/ml). Moreover, haptoglobin concentrations were affected by housing ($P < 0.01$) and week ($P < 0.001$) (Fig. 2G and 2H).

Enriched housed pigs had lower haptoglobin concentrations than barren housed pigs (0.57 ± 0.03 vs. 0.66 ± 0.03 mg/ml) and haptoglobin concentrations were higher in week 9 compared to weeks 8 and 22. Haptoglobin concentrations were not affected by backtest classification ($P = 0.83$). The increase in haptoglobin from week 8 to 9 was not affected by SBV class, housing or backtest classification ($P \geq 0.32$).

Gender did not affect any of the immune variables, except that over the three sampling points, gilts tended to have lower leukocyte concentrations than barrows (17.9 ± 0.2 vs. 18.4 ± 0.2 10^9/l, $P < 0.1$) and higher haptoglobin concentrations (0.63 ± 0.02 vs. 0.60 ± 0.02 mg/ml, $P < 0.1$).

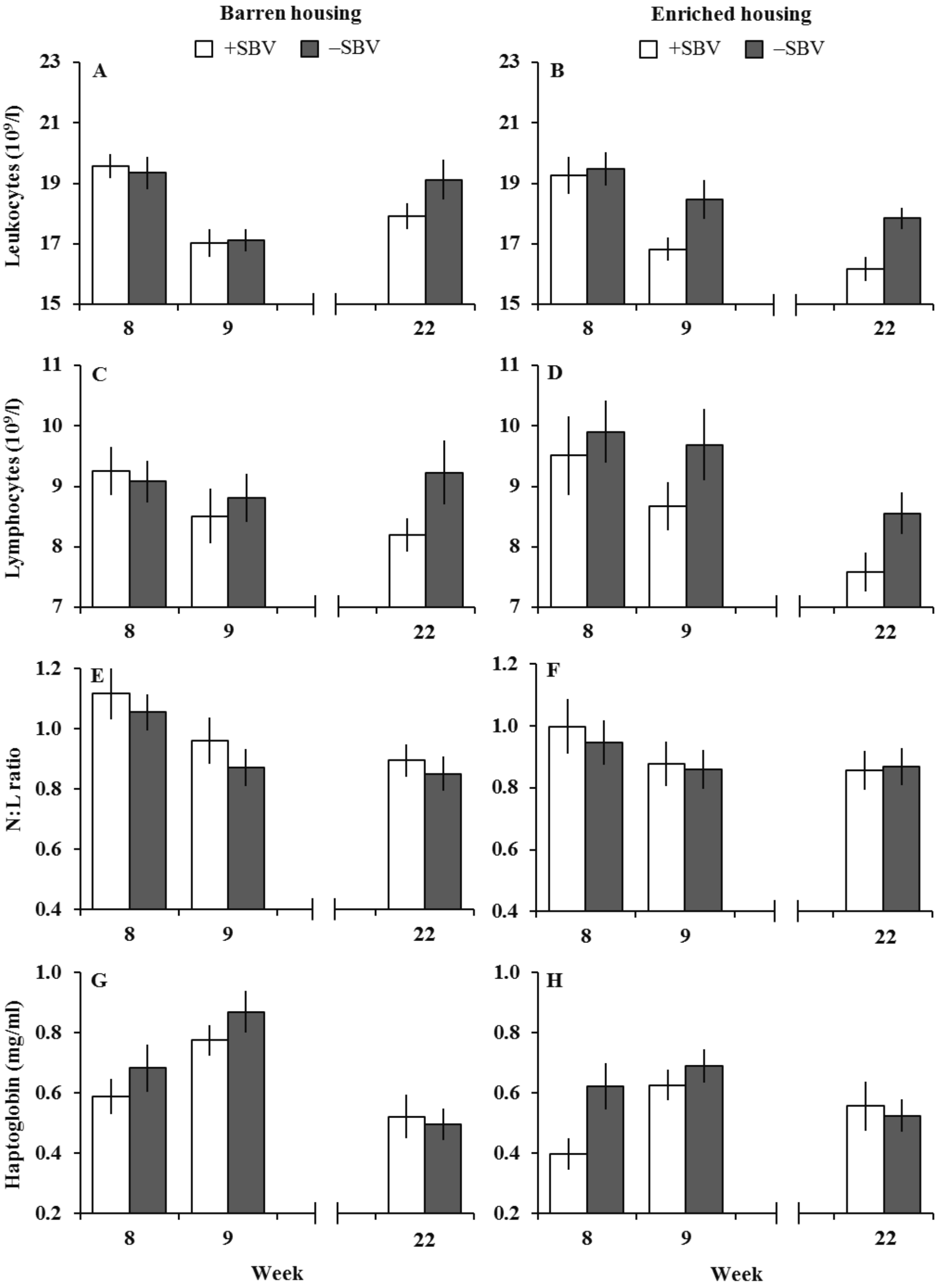

Fig. 2. Means and SEM of leukocytes, lymphocytes, N:L ratio and haptoglobin. The concentrations of leukocytes (panels A and B), lymphocytes (panels C and D), the neutrophil to lymphocyte ratio (N:L ratio) (panels E and F), and haptoglobin concentrations (panels G and H) of pigs that have an estimated relative positive genetic effect (+SBV) or negative genetic effect (–SBV) on the growth of their pen mates in barren and enriched housing measured before a 24 h regrouping test at 8 weeks of age, after the regrouping test at 9 weeks of age and at 22 weeks of age. Significance of effects of housing and SBV is given in the text.

4. Discussion

In this study, we investigated the effects of divergent selection for indirect genetic effects on growth (+SBV pigs vs. –SBV pigs) and environmental enrichment on the immune status of pigs.

In response to acute stress or inflammation, the acute phase response is activated which results, amongst others, in an increase of acute phase proteins, such as haptoglobin, and in complement activation (Ayensu et al., 1995; Campisi and Fleshner, 2003; Cray et al., 2009). The increased CPW complement activity and haptoglobin concentrations found three days after the regrouping test could, thus, indicate that the pigs experienced the test as stressful, but may also have resulted from skin inflammations caused by vigorous fighting during regrouping (Camerlink et al., 2013). Moreover, pigs also had lower leukocyte concentrations and a lower N:L ratio after regrouping, whereas lymphocyte concentrations did not differ between before and after regrouping. These results are not in line with the generally reported effects of acute stress on different leukocyte types, which could be due to differences in the timing of blood sampling (see Davis et al., 2008), i.e. the leukocyte levels three days after regrouping in our study may have partly reflected recovery from acute stress than the effect of regrouping stress per se.

The other effects of week on the variables measured could point to an effect of age. For instance, the increase in KLH-IgG and KLH-IgM natural antibody titers from week 8 to 9 to 22 is in line with other studies reporting rising natural antibody titers with age (e.g. Parmentier et al., 2004; Star et al., 2007). On the other hand, APW complement activity, the concentration of lymphocytes and the N:L ratio decreased over weeks in our study. A similar result was found by Blount et al. (2005) for the concentration of lymphocytes, but other studies showed a different pattern with age for these three variables (Star et al., 2007; Juul-Madsen et al., 2010). These inconsistencies might be due to individual variation as distinct individual variation in age-related immune changes has been reported (Lutgendorf and Costanzo, 2003).

Even though the pigs in this study were all relatively healthy and were not deliberately immunologically challenged, SBV class, housing and coping style did have clear effects on the immune variables measured. Housing affected all immune variables, except the CPW and APW complement activity and lymphocyte concentrations, and SBV class mainly affected the leukocyte, lymphocyte and haptoglobin concentrations. Moreover, effects of coping style were mainly found on the innate immune variables APW complement activity and KLH-IgG and

KLG-IgM natural antibody titers. Effects of gender were also found, but these effects were rather subtle. The interpretation of these results with respect to health and (chronic) stress is, for clarity reasons, discussed in the separate sections below.

4.1. Housing

Effects of housing were found on all variables measured except for complement activity and lymphocyte concentrations. Enriched housed pigs had, partly in line with other studies, overall a higher KLH-IgG titer (Kelly et al., 2000; Ernst et al., 2006), a lower N:L ratio (Merlot et al., 2012) and lower haptoglobin concentrations (Scott et al., 2006; Scollo et al., 2013) than barren housed pigs. Enriched housed pigs also had lower leukocyte concentrations than barren housed pigs, in line with Manciocco et al. (2011), but only at 22 weeks of age. On the other hand, Merlot et al. (2012) found no effect of conventional or enriched housing on haptoglobin concentrations in a conventional pig breed and Manciocco et al. (2011) actually found a higher N:L ratio and higher CPW complement activity in enriched housed pigs. This could be explained by differences in type of enrichment (e.g. straw, extra outdoor area, or toys), duration of enrichment provided, and age of the animals tested. The lower N:L ratio and lower haptoglobin concentrations in enriched housed pigs could indicate that enriched housed pigs were less stressed (Piñeiro et al., 2007a; Davis et al., 2008; Salamano et al., 2008) which confirms our expectation and is in accordance with the well-established benefits of straw on behavior and welfare (Van de Weerd and Day, 2009). Straw bedding has been described as unfavorable for pathogen presence and hygiene (Tuyttens, 2005) which could explain the higher circulating KLH-IgG in enriched housed pigs, but more research is needed to confirm this. All in all, the results of this study show that housing (i.e. relatively barren vs. deep straw bedding) has a substantial impact on variables related to both stress physiology and (innate) immunity which could, therefore, have consequences for both pig health and welfare.

4.2. Social breeding values for growth

Most notably, +SBV pigs had overall lower concentrations of leukocytes and lymphocytes and tended to have overall lower haptoglobin concentrations than –SBV pigs. In addition, the decrease in the concentration of leukocytes from week 8 to 9 was larger for +SBV pigs than for –SBV pigs, suggesting that they respond differently to the 24 h regrouping test at 9 weeks of age.

Previously, it was found that these +SBV pigs behaved somewhat less fearful than the –SBV pigs in several novelty tests (Reimert et al., 2013, 2014) and were less stressed than the –SBV pigs upon reunion after the 24 h regrouping test (Camerlink et al., 2013). In addition, injurious biting behavior directed at pen mates (e.g. tail and ear biting) has been reported to occur less in the +SBV pens than in the –SBV pens (Camerlink et al., submitted). As all pigs within a pen either had a +SBV or –SBV, these behavioral results could indicate that +SBV pigs create a less stressful social environment for themselves than the –SBV pigs. The found lower leukocyte, lymphocyte and haptoglobin concentrations in the +SBV pigs support this indication and confirms our expectation, because higher leukocyte and lymphocyte levels have been associated with more stress (Ots et al., 1998; Boscarino and Chang, 1999; Dhabhar, 2002; Lewis et al., 2008) and a higher level of haptoglobin with chronic or repeated stress (Piñeiro et al., 2007a; Salamano et al., 2008). Some caution should be made, however, because lower leukocyte and lymphocyte levels have also been associated with more stress (reviewed in Davis et al., 2008). The observed behavioral and immunological differences between the +SBV and –SBV pigs are likely related, but in what way is, at present, not clear. A higher concentration of leukocytes, lymphocytes and haptoglobin in the –SBV pigs could indicate that the –SBV pigs have a more active immune system which, in turn, could have led to an increased need of specific amino acids (e.g. for synthesis of acute phase proteins such as haptoglobin) and, thus, a reduced availability for other systems such as growth (Van de Kampman-Hoek et al., 2013). This may have stimulated these –SBV pigs to search for food and thereby have led to more stress and biting behavior (Taylor et al., 2010). On the other hand, as all pigs in one pen were either +SBV or –SBV pigs, more biting behavior in the –SBV pens also meant receiving more bites which, likely, resulted in more inflammations and that could have led to a more active immune system in the –SBV pigs. It should be noted, though, that significantly less biting behavior was observed in the enriched pens (Camerlink et al., submitted; Ursinus et al., in press), whereas the immunological differences between the +SBV and –SBV pigs were independent of housing condition (see below), suggesting that the higher leukocyte, lymphocyte and haptoglobin levels of the –SBV pigs are not the sole cause or consequence of the injurious biting behaviors. Whether the found immunological differences between the +SBV and –SBV pigs have different implications for their health is difficult to say, because both higher and lower leukocyte concentrations have been associated with better health (reviewed in Davis et al., 2008) and the found haptoglobin concentrations are much lower compared to haptoglobin concentrations of pigs with health problems (Petersen et al., 2002). It is, however, worthwhile to further investigate the health of pigs from this selection method compared with conventional selection in which selection is based on individual performance only, because one of the consequences of conventional selection has been suggested to be a heightened susceptibility to disease (Rauw et al., 1998; Prunier et al., 2010).

Interestingly, the differences between the +SBV and –SBV pigs are comparable with the differences found between enriched and barren housing: pigs housed in a better physical environment (i.e. enriched pens) may experience less stress and pigs housed in a better social environment (i.e. +SBV pens) also seem to experience less stress. In addition, no interactions were found between SBV class and housing condition which is in line with behavioral results of these pigs (Camerlink et al., 2013; Reimert et al., 2014). This suggests that effects of this selection method on pig behavior and physiology are independent from those of housing. This is important as it may show that only a combined effort of optimizing both the breeding program and the housing environment will yield optimal results in terms of pig welfare.

4.3. Coping style

In many different animal species two extremes in coping style or personality have been described: proactive and reactive (Carere et al., 2010; Coppens et al., 2010; Koolhaas et al., 2010). Generally, proactive animals are aggressive, active, bold, prone to take risks, and they hardly pay attention to environmental cues, whereas reactive animals are less aggressive, more cautious, avoid taking risks, but are very attentive to cues from the environment (Sih et al., 2004; Coppens et al., 2010; Koolhaas et al., 2010). In pigs, similar coping styles have been distinguished based on their response in a backtest at a young age. Pigs that struggle and vocalize relatively much in this test are classified as high-resisting (HR) pigs and they resemble proactive copers, and pigs that hardly struggle and vocalize in the backtest are classified as low-resisting (LR) pigs and they resemble reactive copers (Hessing et al., 1994; Ruis et al., 2000, 2002; Geverink et al., 2002; Bolhuis and Schouten, 2002; Bolhuis et al., 2004, 2005a,b, 2006; Jansen et al., 2009).

In this study we also found immunological differences between HR and LR pigs, even though we simply divided the population of pigs into HR or LR and did not use the extremes of the population as was done in earlier pig studies (e.g. Hessing et al., 1993; Bolhuis et al., 2000; Geverink et al., 2002; Ruis et al., 2000; Van Erp-van der Kooij et al., 2000). HR pigs had an overall higher APW complement activity, although the regrouping test had a more substantial effect on

APW complement activity in the LR pigs. Furthermore, HR pigs also had a higher KLH-IgG titer, but only in enriched housing. At 22 weeks of age, the same result was found for the KLH-IgM titer. Together these results could indicate that on the long term HR pigs have a more active innate immune system, but that acute stress due to regrouping has a larger impact on antibody titers of LR pigs. As LR or reactive pigs are more attentive to environmental cues (Bolhuis et al., 2004; Koolhaas et al., 2010), a change of environment (i.e. regrouping) could indeed have more impact on them than on HR or proactive pigs. The more chronic higher innate immune activity in the HR pigs could be related to their behavior. They explore a new environment faster than LR pigs. This could mean that HR pigs are earlier exposed to pathogens than LR pigs and, perhaps, also to different pathogens. To defend themselves against these pathogens, having an active innate immune system which is the first line of defense may fit with this suggestion (Barber and Dingemanse, 2010; Kortet et al., 2010). In addition, several other pig studies investigated whether HR and LR pigs differed in specific immune responses and their results indicated that HR pigs had a stronger cell-mediated immune response, while LR pigs had a more pronounced humoral immune response (Hessing et al., 1995; Schrama et al., 1997; Bolhuis et al., 2003). Hessing et al. (1995) proposed that a difference in balance between T-helper 1 (Th1) and T-helper 2 (Th2) cells was underlying this difference with a shift towards Th1 in HR pigs and Th2 in LR pigs, because activation of Th1 cells leads to an inflammatory response and activation of Th2 to the production of antibodies (Hessing et al., 1995; Sanders, 2006). In support of this, results of an unpublished pig study by Bolhuis et al. indeed found more pro-inflammatory cytokine production in HR pigs. Furthermore, as complement activation is also associated with the activation of pro-inflammatory cytokines (Ricklin et al., 2010), the results of our study seem to be in line with these results. Together, these results suggest that HR and LR pigs may have different strategies to deal with immune challenges. However, studies that investigated differences in immune responses in proactive and reactive animals are scarce and the results of these studies do not always agree with each other (Geverink et al., 2004b; Koolhaas, 2008; Sild et al., 2011; Niemelä et al., 2013). In addition, in this study housing had a modulating effect on the natural antibody titers of the HR pigs, whereas Bolhuis et al. (2003) found that housing had a modulating effect on specific antibody titers of the LR pigs. We propose, therefore, that more research is needed not only to be able to draw more definite conclusions about differences in immune function between proactive and reactive animals (Barber and Dingemanse, 2010), but also because of the relevance of personality in actual disease susceptibility (e.g. Friedman, 2008; Mehta and

Gosling, 2008).

4.4. Gender

Gilts were found to have lower concentrations of leukocytes, but higher haptoglobin concentrations than barrows. The barrows were surgically castrated at 3 days of age which could explain their higher concentrations of leukocytes, because Prunier et al. (2006) described that surgical castration might have long-term negative effects on the health of the pigs. The higher concentrations of haptoglobin in the gilts have also been found by others (Clapperton et al., 2005; Piñeiro et al., 2007b, 2009) and could indicate a fundamental difference between female and male pigs. It has been suggested that females have both a more active humoral and a more active cell mediated immune system which could be reflected in a higher susceptibility to parasites and infections in males, and females being more prone to autoimmune diseases (Baum and Grunberg, 1991; Kurtz et al., 2000; Stefanski and Grüner, 2006; Darnall and Suarez, 2009). These differences have been attributed to differences in sex steroids, but also to differences in how females and males deal with stress on both a physiological and psychological level (Baum and Grunberg, 1991; Kurtz et al., 2000; Stefanski and Grüner, 2006; Darnall and Suarez, 2009). As the male pigs in this study were castrated, we will refrain from speculating whether sex steroids could underlie the found immunological differences in the gilts and barrows, but differences in dealing with stress is a likely possibility, because these same gilts and barrows have been found to behave very differently in various novelty tests (Reimert et al., 2013, 2014) and in the regrouping test (Camerlink et al., 2013).

5. Conclusions

Environmental enrichment is known to alleviate stress in animals. In this study, enriched housed pigs were found to have a lower N:L ratio and lower haptoglobin concentrations than the barren housed pigs which indeed suggests that enrichment has stress-reducing effects. Stress-reducing effects were also seen in pigs selected for a relatively positive genetic effect on the growth of their pen mates (i.e. +SBV pigs), because these pigs had lower leukocyte, lymphocyte and haptoglobin concentrations compared to pigs that were selected for a relatively negative genetic effect on the growth of their pen mates. Together these results indicate that both genetics and environmental enrichment can be used to improve the welfare of pigs and that the use of both together likely yields the best results.

Two effects of gender were found, but these effects were rather subtle. On the other hand, clear differences were found between pigs with a proactive or reactive coping style. Pigs with a proactive coping style seemed to have a more active innate immune status compared to pigs with a reactive coping style, pointing to a difference in dealing with immune challenges. The implications of the results found for health merit further research.

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CHAPTER 5

Indicators of positive and negative emotions and emotional contagion in pigs

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Abstract

For the welfare of group-housed animals, such as pigs, the emotional state of an individual pig is relevant, but also the extent to which pen mates are affected by the distress or pleasure of other individuals, i.e. emotional contagion, a simple form of empathy. Therefore, indicators of positive and negative emotions were investigated in pigs during anticipation and experience of a rewarding (access in pairs to a compartment with straw, peat and chocolate raisins) or aversive (social isolation combined with negative, unpredictable interventions) event. Thereafter the same indicators were investigated in naive pigs during anticipation and experience of a rewarding or aversive event by their trained pen mates. Positive emotions could be indicated by play, barks and tail movements, while negative emotions could be indicated by freezing, defecating, urinating, escape attempts, high-pitched vocalizations (screams, squeals or grunt-squeals), tail low, ears back and ear movements. Salivary cortisol measurements supported these behavioral observations. During anticipation of the aversive event, naive pigs tended to show more tail low. During the aversive event, naive pigs tended to defecate more, while they played more during the rewarding event. These results suggest that pigs might be sensitive to emotional contagion, which could have implications for the welfare of group-housed pigs. Pig emotions and the process of emotional contagion merit, therefore, further research.

Keywords: positive emotions, emotional contagion, empathy, pigs, play behavior

1. Introduction

Emotions such as fear and anxiety could have large impacts on behavior, health and welfare of animals (Held et al., 2009). Therefore, the study of emotions has become a focus of attention in animal welfare research (Fraser and Duncan, 1998; Désiré et al., 2002; Mendl and Paul, 2004; Boissy et al., 2007). Emotions are composed of behavioral, (neuro)physiological, cognitive and conscious components (Désiré et al., 2002; Mendl and Paul, 2004; Paul et al., 2005). In animals, emotions have mainly been measured using the behavioral or physiological component (Paul et al., 2005), despite several limitations (described in Mendl and Paul, 2004; Paul et al., 2005; Mendl et al., 2009). The interpretation of physiological or behavioral measures in terms of emotions is often difficult. An increased heart rate, for instance, can indicate a positive as well as a negative emotional state (Paul et al., 2005; Mendl et al., 2009). Moreover, it is unclear whether an animal that stops swimming in the forced swim test is in a state of depression or coping with the situation (Paul et al., 2005; Mendl et al., 2009). Another limitation is that at present there are few measures of positive emotions despite their increasing importance in animal welfare research (Désiré et al., 2002; Mendl and Paul, 2004; Paul et al., 2005; Boissy et al., 2007; Mendl et al., 2009). Such limitations may be overcome by using measures that make use of the cognitive component (Mendl and Paul, 2004; Paul et al., 2005; Mendl et al., 2009). In humans, cognitive processes are thought to underlie the emotion that arises during the experience of an event via a process called 'appraisal'. During this process, the event is evaluated according to so called checks, for example, suddenness, familiarity, predictability or pleasantness (Paul et al., 2005; Mendl et al., 2009). The outcome of this process determines which emotions will arise. In this process, complex cognitive abilities such as the recollection of memories from previous events can be involved, but the process can also occur rapidly and automatically. There are several appraisal theories (Mendl and Paul, 2004; Paul et al., 2005; Mendl et al., 2009) of which the theory of Rolls (2000) may be applicable in animals (Mendl and Paul, 2004; Paul et al., 2005). Rolls proposes that events are mainly appraised according to whether they are rewarding or aversive. A rewarding event may elicit emotions such as happiness, while an aversive event may elicit emotions such as fear (Rolls, 2000). Animals can be trained to anticipate these rewarding or aversive events via Pavlovian conditioning (Spruijt et al., 2001; Hansen and Jeppesen, 2004; Moe et al., 2006; Zimmerman et al., 2011). During anticipation, i.e. the period during which an animal expects the event, animals can express how they feel about this event by showing anticipatory behavior (Spruijt et al., 2001). As the cognitive component is also involved in anticipation (Mendl and Paul, 2004; Paul et al., 2005), the responses observed during both measures, appraisal and anticipation, could be used as indicators of putative animal emotions. We decided to use both measures, because the experience of an event involves a different behavioral system, both in time and function, than expecting that event (Spruijt et al., 2001).

For the welfare of group-housed animals, such as pigs, it is not only relevant what an individual pig feels but also the extent to which its pen mates are affected by its distress or pleasure. This process is called emotional contagion, a simple form of empathy (Hatfield et al., 1993; Langford et al., 2006; De Waal, 2008; Edgar et al., 2011). It has been suggested that animals may become distressed by receiving signals from conspecifics which are frightened or in pain during situations such as routine handling procedures, transport or slaughter (Edgar et al., 2011). If so, the welfare on a group level is diminished. On the other hand, individuals may become positively excited by receiving signals from conspecifics that are feeling ‘happy’. This could occur during situations such as play, because play seems to induce a state of pleasure (Held and Špinka, 2011) and as it has been suggested that play is contagious, the accompanying emotional state might also be contagious (Held and Špinka, 2011). If so, the welfare on a group level might then be increased. Signals through which distress or pleasure could be transferred to other pigs could be auditory (Kiley, 1972; Schrader and Todt, 1998; Manteuffel et al., 2004), olfactory (Amory and Pearce, 2000; Marchant et al., 2001) or gustatory (Held et al., 2009). Whether pigs also use visual signals is difficult to say, because vision is thought to be not well developed in pigs (Kiley, 1972; Lomas et al., 1998; Zonderland et al., 2008), although pigs are able to discriminate other pigs based on vision alone (Ewbank et al., 1974; McLeman et al., 2008). Whether and to what extent pigs are affected by the emotions of their pen mates is unknown.

The first aim of this study was to investigate indicators of positive and negative emotions in pigs during anticipation and experience of a rewarding (access in pairs to a compartment with straw, peat and chocolate raisins) or aversive (social isolation combined with negative, unpredictable interventions) event. We hypothesized that pigs would show more play behavior (Fraser and Duncan, 1998; Špinka et al., 2001; Boissy et al., 2007; Held and Špinka, 2011) during anticipation and the experience of a rewarding event and would show more freezing (Paul et al., 2005), high-pitched vocalizations (Kiley, 1972; Fraser, 1974; Schrader and Todt, 1998; Manteuffel et al., 2004), escape attempts (Mendl and Paul, 2004) and high levels of defecating (Fraser, 1974; Mendl et al., 1997; Mendl and Paul 2004) and urinating (Mendl et al., 1997) during anticipation and the experience of an aversive event. Recently, ear and tail postures have been postulated as useful indicators of positive and negative emotions (Reefman et al., 2009a,b; Forkman et al., 2007; Boissy et al., 2011; Jones and Boissy, 2011). In pigs only little research has been performed on tail postures (e.g. Kiley-Worthington, 1976), and, as far as we know, not on ear postures. We, therefore, investigated whether ear and tail postures could be indicators of positive and negative emotions in pigs. In addition, we determined salivary cortisol of pigs which may respond to both positive (Paul et al., 2005; Jones and Boissy, 2011; Koolhaas et al., 2011) and negative (Parrott et al., 1989; Hemsworth et al., 1996; De Jong et al., 1998; Geverink et al., 1998; Mormède et al., 2007; Merlot et al., 2010) situations.

The second aim was to investigate whether pigs show signs of emotional contagion. To that aim, the same behaviors as above and the salivary cortisol response of naive pigs were investigated during anticipation and experience of a rewarding or aversive event by their trained pen mates.

2. Materials and methods

2.1. Animals and housing

For this study, 24 Tempo × Cambourough pigs were used, which were 12 weeks of age at the start of the study. The pigs had previously, before weaning, participated in an experiment in which they were brought to a test room (Oostindjer et al., 2011), so they were already familiar with the presence of humans and going in and out of their pens. Pigs were weaned at 4 weeks of age and from 5 weeks of age housed in six groups of six unrelated pigs in 8.3 m^2 pens. Each group was composed of four gilts and two boars and composition was balanced for backtest classification (see Bolhuis et al., 2003). Pigs could be identified by an ear tag and a number sprayed on their backs. Food (a standard commercial diet for growing pigs) and water were available ad libitum. Lights were on between 7 am and 7 pm. The floors of the pens were covered with wood shavings (34 l) and fresh straw (around 1.8 kg) was provided daily after cleaning the pens. All pigs were habituated to chewing on cotton buds for saliva sampling before the training procedure (section 2.3.) of the test pigs started.

The study was approved by the Animal Care and Use Committee of Wageningen University.

2.2. Experimental set up

The two boars of each pen, the test pigs, were trained in ten days to associate one combination of cues with a rewarding event and another combination of cues with an aversive event. Thereafter they were exposed to the cues and events in the presence of two naive, non-trained pen mates (two gilts).

Anticipatory behavior in the test pigs was induced using Pavlovian conditioning in which an initially neutral stimulus (conditioned stimulus, CS) was repeatedly followed by a supposedly rewarding event or a supposedly aversive event (unconditioned stimulus, US). The supposedly rewarding event consisted of 5 min access in pairs to a compartment (10.6 m^2) containing straw (around 10 kg), peat (200 l) and approximately 20 chocolate raisins hidden in the substrate. The supposedly aversive event consisted of 5 min social isolation in a barren compartment (3.3 m^2) accompanied by negative unpredictable interventions (section 2.3.). As conditioned stimuli, two combinations of auditory and visual cues were used: the sound of a bicycle bell combined with blue light from a rope light and the sound of a siren combined with white light from a rope light. For half of the pens, the bicycle bell combined with blue light announced the supposedly rewarding event and the siren combined with white light announced the supposedly aversive event; for the other pens this was the other way around. The rope lights signaling the visual cues were wrapped around the top of the doors of both the aversive and rewarding compartments. An experimenter was sitting in the test room to manually operate the cues when the pigs were in the start box. The pigs could not see or hear this experimenter.

The experimental pen (Fig. 1) was located in a test room and consisted of four compartments: a start box, a rewarding compartment and two aversive compartments. Pigs entered the start box from a hall way via a sliding door (will be referred to as start box door in the remaining of the article). From the start box, pigs could go to the rewarding or aversive compartment after an experimenter had opened the corresponding door. Microphones and cameras were suspended from the ceiling of the test room in order to make video and audio recordings that could be analyzed later.

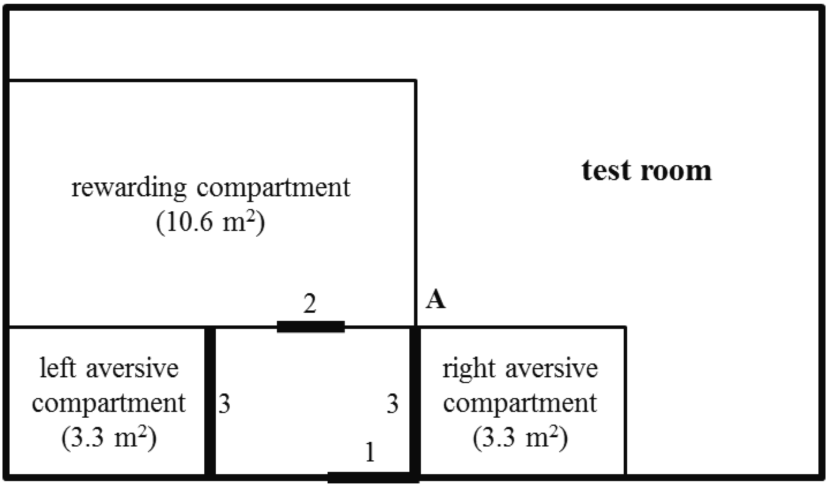

Fig. 1. A schematic drawing of the test room. All compartments were 1 m high. The letter A indicated the place where an experimenter operated the cues. The thickened lines indicated the position of the doors. Door 1 is the start box door through which pigs entered the start box from the hall way. Doors 2 and 3 are the rewarding door and aversive doors through which pigs entered the rewarding compartment and aversive compartments respectively.

2.3. Training procedure of test pigs

The day before the training procedure started, the test pigs were brought in pairs to the rewarding compartment and were allowed to explore it for 5 min to avoid novelty-stress during the first training trial.

The training procedure lasted ten days. Each pair of test pigs was exposed to two training trials, one in the morning and one in the afternoon, on days 1, 3, 4, 5, 6, 9 and 10, in one of which they were exposed to the cues followed by the supposedly rewarding event, and in the other to the cues followed by the supposedly aversive event. There were at least two hours between the two daily trials for each pen. The order in which the test pigs were trained, and the order of rewarding and aversive events on a day was randomized for pen and day throughout the entire training period.

During each trial, the two test pigs of each pen were brought to the start box where the combination of auditory and visual cues was provided. The length of the cues, i.e. the anticipation period, was gradually increased from 5 s on days 1 and 3, to 10 s on day 4, to 15 s on days 5 and 6 and to 20 s on days 9 and 10. When the cues signaled the supposedly rewarding event, an experimenter entered the start box directly after the cues had stopped and guided the pair of test pigs to the rewarding compartment after opening the corresponding door. The door was closed as soon as the pair of test pigs had entered the rewarding compartment. After 5 min in this compartment, the test pigs were brought back to their home pens. When the cues signaled the supposedly aversive event, an experimenter entered, directly after the cues had stopped, the start box with a wooden board to separate the test pigs and guided them individually into one of the aversive compartments after opening the corresponding door. The door was closed as soon as the pig had entered the aversive compartment. After 5 min in this compartment, the test pigs were brought back to their home pens. Negative, unpredictable interventions were carried out during the supposedly aversive event: on days 1, 9 and 10, a person (not the experimenter) stepped first into the left aversive compartment after 1, 2 and 1 min, respectively, to restrain the pig there with a nose sling for 30 s after which he went to the right aversive compartment to restrain the pig there with a nose sling for 30 s. On days 3 and 5, a person stepped first into the right aversive compartment after 3 and 0 min, respectively, to restrain the pig there with a nose sling for 30 s after which he went to the left aversive compartment to restrain the pig there with a nose sling for 30 s. On days 4 and 6, pigs were not exposed to the nose sling, but switched compartments.

2.4. Habituation of naive pigs

Before the testing procedure (section 2.5.), each pair of naive pigs was brought to the start box four times: twice, once in the morning and once in the afternoon, on days 2 and 8. In the start box, they were exposed to the combinations of visual and auditory cues for 20 s. After the 20 s exposure to the cues, the naive pigs were brought back to their home pens. They were not allowed to go to the rewarding or aversive compartments and thus were not trained to associate the cues with these compartments. There were at least one and a half hours between the two daily trials for each pen. The order in which the pairs of naive pigs were tested and which cues were given in the morning and which in the afternoon was randomized for each pen on these two days.

To ensure that the test pigs were not disturbed by the company of the naive pigs and vice versa in the testing procedure, the four (test and naive) pigs of each pen were brought to the start box together once on day 7. After 20 s they were brought back to their home pens. No cues were given during these 20 s.

2.5. Testing procedure

In the morning of days 11 and 12, the test pigs and their naive pen mates were brought to the start box where the cues were given for 20 s, after which the test pigs were exposed to the corresponding rewarding or aversive event for 3 min. No interventions were carried out during the aversive event. The naive pigs remained in the start box during these 3 min. After the 3 min all four pigs were brought back to their home pens. Half of the pens were exposed to the supposedly rewarding event on day 11 and the supposedly aversive event on day 12 and for the other pens this was the other way around.

2.6. Behavioral analyses

Video recordings were analyzed using focal sampling and continuous recording with The Observer 5.0 and The Observer 9.0 XT (Noldus Information Technology, Wageningen, The Netherlands). The ethogram in Table 1 was used for scoring the behaviors during the rewarding and aversive events on days 1, 5, 10, 11 and 12. On days 11 and 12, the behaviors nosing start box door, nosing rewarding door and nosing aversive doors were additionally scored for the naive pigs only. The same ethogram was used for scoring the behaviors during the display of cues in the start box on all training and testing days. Preliminary investigation of the video recordings showed that escape attempts, play, urinating and vocalizations did not occur during the display of cues and were therefore not scored during the display of cues. Vocalizations were scored as a total of two pigs in the training procedure and as a total of four pigs in the testing procedure, because it was not possible to identify them per individual pig.

Table 1
Ethogram used to score the behaviors of the test pigs during the rewarding and aversive events on day 1, 5, 10, 11 and 12 and of the naive pigs in the start box on day 11 and 12 during the experience of the aversive or rewarding event by the test pigs. This ethogram is also used to score the behaviors of the test pigs during the display of cues in the start box on day 1, 3, 4, 5, 6, 9, 10, 11 and 12 and of the naive pigs during the display of cues in the start box on day 11 and 12. Behaviors were scored as states unless indicated otherwise.

Behavior	Description
Behavior	
Freezing	Standing motionless with whole body and head fixed
Escape attempts *(event)*[1]	Moving away from the person with the nose sling
Play *(event)*[1]	Running, gamboling, pivoting or playing with straw by shaking head
Defecating *(event)*	Defecating
Urinating *(event)*[1]	Urinating
Nosing start box door[2]	Sniffing or touching the start box door of the start box with the snout
Nosing rewarding door[2]	Sniffing or touching the door of the rewarding compartment with the snout
Nosing aversive doors[2]	Sniffing or touching either door of the aversive compartments with the snout
Ears, tail and head	
Ears back	One or both ears directed backwards (Tate et al., 2006, page 2159)
Tail in curl	Tail coiled up in a curl on top of the body
Tail wagging	Tail swinging in any direction, but mostly from side to side
Tail low	Tail hanging down against the body
Head to start box door[2]	Head oriented to the start box door of the start box
Head to rewarding compartment[2]	Head oriented to either one of the aversive compartments
Head to aversive compartment[2]	Head oriented to the rewarding compartment
Vocalizations[1]	
Low-pitched vocs. *(event)*	Screams, squeals or grunt-squeals
High-pitched vocs. *(event)*	Short or long grunts
Barks *(event)*	A low tone that sounds like “woof”

[1] These behaviors were only scored during the events.

[2] These behaviors were only scored during the display of cues, except on day 11 and 12. On these days the behaviors nosing start box door, nosing rewarding door and nosing aversive doors were additionally scored for the naive pigs only. The other behaviors were scored during the events as well as during the display of cues.

2.7. Saliva collection and cortisol analysis

Saliva samples were collected in the home pen from both the two test and two naive pigs just before (t = 0) and 20 min after (t = 20) both trials on days 1 and 10 of the training procedure and in the testing procedure by allowing the pigs to chew on cotton buds (VWR International, Amsterdam, The Netherlands) until the buds were thoroughly moistened. The cotton buds were placed in test tubes (Sarstedt, Etten-Leur, The Netherlands) and were centrifuged for 10 min at 2000 x g to get the saliva from the buds. The saliva was stored at −20 ºC until further analysis. Cortisol concentration was measured in duplicate using a radioimmunoassay kit (COAT-A-COUNT®, Siemens Healthcare Diagnostics, Los Angeles, USA) which has been modified and validated for pig salivary cortisol (Ruis et al., 1997). Inter-Assay CV was 3.7 % (based on two assays) and Intra-Assay CV was 5.3 %.

2.8. Statistical analyses

SAS (SAS 9.2, SAS Institute Inc.) was used for statistical analyses. Variables that were scored as events were expressed as number of occurrences per min and all other variables were expressed as percentages of time. Correcting the variables for the time the test pigs were in the nose sling during the aversive events did not change the results, so percentages of time spent on various behaviors are reported without this correction. Two new variables were made which were ear posture changes per min and tail posture changes per min. All variables were averaged per pen, for the test and naive pigs separately, prior to analysis. If needed to obtain normality of residuals, arcsine square root, square root and log transformations were applied to skewed distributions of proportions, frequencies and concentrations, respectively. For a few residuals a normal distribution could not be realized. These variables were, therefore, analyzed with the Fisher's Exact Test. Effects of treatment (rewarding or aversive) and the interaction between treatment and day on the behaviors of the test pigs during the display of cues and during the events in the training procedure were assessed with a mixed linear model in which treatment, days (1, 3, 4, 5, 6, 9 and 10 for the display of cues and 1, 5 and 10 for the events), their interaction and order (first the rewarding event and then the aversive event or vice versa) were taken as fixed factors and pen nested in treatment as random factor. In addition, effects of treatment on the behaviors of the test pigs during the display of cues in the training procedure were assessed for each day separately using a mixed linear model in which treatment and order were taken as fixed factors and pen nested within order as random factor. Effects of treatment on the behaviors of the test and naive pigs during the display of cues and the events in the testing procedure were assessed with a mixed linear model in which treatment, days (11 and 12) and order were taken as fixed factors and pen nested in order as random factor.

Behaviors that did not occur during either the aversive or the rewarding events were transformed into a binomial distribution on pen level. For instance, if one or both of the pigs of a pen played during the rewarding treatment on day 1, play behavior was scored as 1 for that pen. All these behaviors were then analyzed with a Fisher's Exact Test for treatment. These results were confirmed with a Wilcoxon matched-pairs signed-ranks test. To test whether salivary cortisol concentrations changed from $t = 0$ to $t = 20$, effects of time and order were assessed per day (day 1 of the training procedure, day 10 of the training procedure and the testing procedure) and treatment, using a mixed linear model in which time (0 and 20) and order were taken as fixed factors and pen nested within order as random factor. In addition, the difference between salivary cortisol concentrations at $t = 20$ and $t = 0$ was calculated and fixed effects of treatment and order on this difference were assessed with a mixed linear model that also contained pen nested within order as random factor.

Data analyzed with a mixed linear model are presented as untransformed means ± SEM. Data analyzed with the Fisher's Exact Test are presented as percentages of total occurrences.

3. Results

3.1. Test pigs

3.1.1. Display of cues in the training procedure

During the display of cues preceding the aversive event, test pigs oriented, over the whole training period, their heads more towards the aversive compartment ($F_{1,10} = 10.2$, $P < 0.01$) (Fig. 2, upper left panel). No other overall treatment or treatment x day effects on the behaviors during the display of cues were found. Analyses per day revealed, however, that on days 6 and 10 test pigs showed less nosing of the rewarding door (both $P < 0.05$) (Fig. 2, upper right panel) and oriented their heads less towards the start box door (day 6: $P < 0.05$, day 10: $P < 0.01$) (Fig. 2, lower left panel) during the aversive cues than during the rewarding cues. In addition, they tended to show more ears back on these training days (days 6 and 10: $P < 0.1$) (Fig. 2, lower right panel) during the aversive cues.

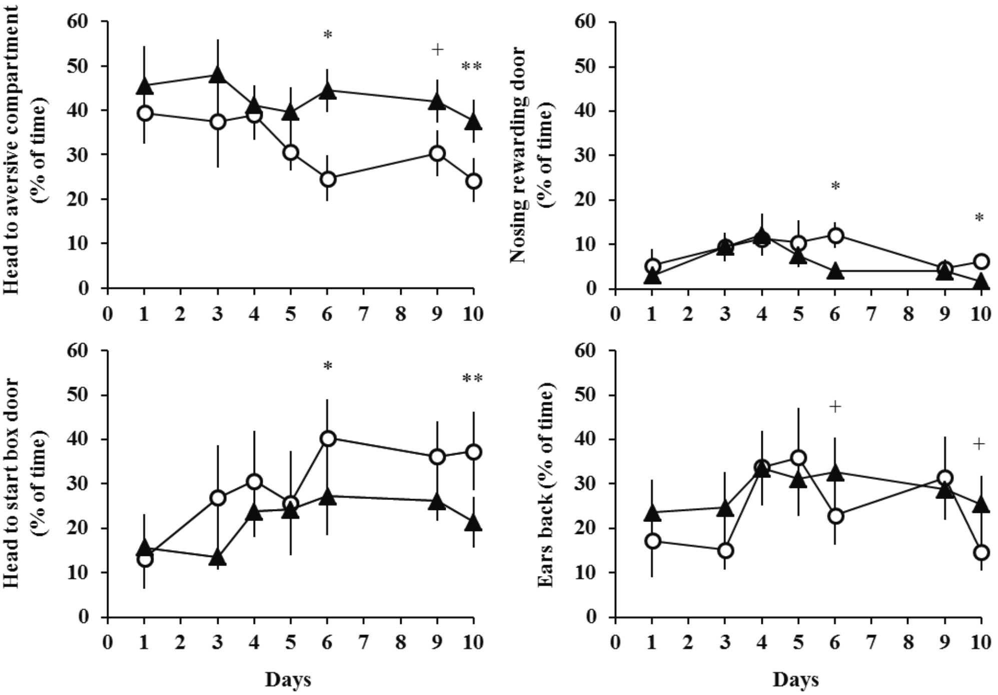

Fig. 2. Orientation of the test pigs' heads towards the aversive compartment (upper left), nosing the rewarding door (upper right), orientation of the test pigs' heads towards the start box door (lower left) and position of test pigs' ears backwards (lower right) on each day of the training procedure during the display of rewarding (○) and aversive cues (▲).
** $P < 0.01$, * $P < 0.05$, $^{+}P < 0.1$

3.1.2. Display of cues in the testing procedure

The behaviors of the test pigs during the display of cues in the testing procedure are shown in Table 2. During the aversive cues, test pigs showed less nosing of the start box door ($P < 0.05$) than during the rewarding cues. Similar to the training procedure, they oriented their head less towards the start box door ($P < 0.01$) and tended to orient their heads more towards the aversive compartment ($P < 0.01$) during the aversive cues. Furthermore, they showed more ears back ($P < 0.05$) and more ear posture changes ($P < 0.01$) during the aversive cues. No other treatment effects were found.

Table 2
Behavior of test pigs during the display of cues in the testing procedure

Behavior	Rewarding cues	Aversive cues	P-value
Behavior			
Freezing	24.1 ± 6.0	23.6 ± 2.3	NS
Nosing start box door	27.6 ± 9.0	5.4 ± 2.8	*
Nosing rewarding door	5.3 ± 4.2	5.4 ± 2.1	NS
Nosing aversive doors	0.4 ± 0.4	3.9 ± 2.5	NS
Defecating[1]	3.2 ± 1.1	1.6 ± 0.5	NS
Head, ears and tail			
Head to start box door	54.1 ± 5.9	27.4 ± 6.6	**
Head to rewarding compartment	22.4 ± 7.1	35.1 ± 8.3	NS
Head to aversive compartment	22.2 ± 3.8	35.2 ± 4.1	+
Ears back	28.3 ± 7.2	37.6 ± 8.4	*
Ear posture changes[1]	11.4 ± 1.8	17.2 ± 2.4	**
Tail in curl	99.1 ± 0.7	96.0 ± 2.0	NS
Tail wagging	0.2 ± 0.2	0.7 ± 0.7	NS
Tail low	0.7 ± 0.7	3.3 ± 2.1	NS
Tail posture changes[1]	1.3 ± 0.9	1.1 ± 0.5	NS

[1] These behaviors are presented as number of occurrences per min. All other behaviors are presented as percentages of time.

** $P < 0.01$, * $P < 0.05$, $^{+}$ $P < 0.1$, NS $P \geq 0.1$

3.1.3. Rewarding and aversive events in the training procedure

Table 3 shows the behaviors of the test pigs during the rewarding and aversive events in the training procedure. During the aversive event on days 1, 5 and 10 test pigs showed more freezing (P < 0.001), more defecating (P < 0.001) and more urinating (P < 0.001) than during the rewarding event. In addition, escape attempts (P < 0.001) occurred only during the aversive event, while play (P < 0.001) occurred only during the rewarding event. During the rewarding event, test pigs also showed more tail wagging (P < 0.001) and more tail posture changes (P < 0.001), whereas they showed more ears back (P < 0.001), ear posture changes (P < 0.01), tail in curl (P < 0.05) and tail low (P < 0.05) during the aversive event. Low-pitched vocalizations (P < 0.001) were heard more during the aversive event. In addition, high-pitched vocalizations (P < 0.001) were heard only during the aversive event, while barks (P < 0.05) were heard only during the rewarding event.

Table 3
Behavior of test pigs during the rewarding and aversive events in the training procedure

Behavior	Rewarding event	Aversive event	P-value
Behavior			
Freezing	3.4 ± 1.0	21.8 ± 2.2	***
Escape attempts[1]	0	77.8	***
Play[1]	88.9	0	***
Defecating[2]	0.3 ± 0.1	1.0 ± 0.1	***
Urinating[1]	0	83.3	***
Ears and tail			
Ears back	4.6 ± 1.0	27.2 ± 2.3	***
Ear posture changes[2]	3.6 ± 0.6	7.4 ± 1.2	**
Tail in curl	93.1 ± 1.4	97.0 ± 1.0	*
Tail wagging	6.3 ± 1.3	0.2 ± 0.1	***
Tail low	0.6 ± 0.4	2.8 ± 1.0	*
Tail posture changes[2]	1.8 ± 0.3	0.4 ± 0.1	***
Vocalizations			
Low-pitched vocalizations[2]	0.2 ± 0.2	19.1 ± 4.0	***
High-pitched vocalizations[1]	0	100	***
Barks[1]	27.8	0	*

[1] These behaviors are presented as percentages of occurrences.

[2] These behaviors are presented as number of occurrences per min. All other behaviors are presented as percentages of time.

*** P < 0.001, ** P < 0.01, * P < 0.05

3.1.4. Rewarding and aversive events in the testing procedure

The behaviors of the test pigs during the rewarding and aversive events in the testing procedure are shown in Table 4. During the aversive event test pigs showed more defecating ($P < 0.05$) and urinating ($P < 0.01$) than during the rewarding event. No treatment effect was found for freezing ($P = 0.14$). Escape attempts were not observed in the testing procedure. Similar to the training procedure, play ($P < 0.01$) occurred only during the rewarding event. In addition, test pigs also showed more tail wagging ($P < 0.01$) and more tail posture changes ($P < 0.01$) during the rewarding event, whereas they showed more ears back ($P < 0.1$) and tail in curl ($P < 0.01$) during the aversive event. No treatment effects were found on ear posture changes ($P = 0.27$) and tail low ($P = 1.00$).

Table 4
Behavior of test pigs during the rewarding and aversive event in the testing procedure

Behavior	Rewarding event	Aversive event	P-value
Behavior			
Freezing	16.9 ± 8.6	24.1 ± 4.5	NS
Escape attempts	-	-	-
Play[1]	100	0	**
Defecating[2]	0.4 ± 0.1	1.2 ± 0.1	*
Urinating[1]	0	83.3	**
Ears and tail			
Ears back	6.8 ± 2.8	16.4 ± 2.6	+
Ear posture changes[2]	4.5 ± 1.3	6.3 ± 1.4	NS
Tail in curl	86.9 ± 6.1	99.9 ± 0.1	**
Tail wagging	12.8 ± 6.1	0.1 ± 0.1	**
Tail low	0.2 ± 0.2	0.0 ± 0.0	NS
Tail posture changes[2]	2.1 ± 0.6	0.1 ± 0.1	**

[1] This behavior is presented as percentage of occurrences.

[2] These behaviors are presented as number of occurrences per min. All other behaviors are presented as percentages of time.

** $P < 0.01$, * $P < 0.05$, $^{+}$ $P < 0.1$, NS $P \geq 0.1$

3.1.5. Salivary cortisol

During the aversive event, salivary cortisol concentrations increased from t = 0 to t = 20 on days 1 (P = 0.05) and 10 (P< 0.01) of the training procedure and tended to increase in the testing procedure (P = 0.10) (Fig. 3). During the rewarding event, salivary cortisol concentrations increased from t = 0 to t = 20 on day 10 of the training procedure (P < 0.001), but did not increase on day 1 of the training procedure (P = 0.77) and in the testing procedure (P = 0.23) (Fig. 3). The increase in salivary cortisol was higher for the aversive event than for the rewarding event on day 1 of the training procedure (aversive: 1.8 ± 0.7 vs. rewarding: 0.2 ± 0.6, P < 0.05). On day 10 of the training procedure (2.3 ± 0.8 vs. 1.0 ± 0.1, P = 0.13) and in the testing procedure (1.2 ± 0.7 vs. 0.5 ± 0.4, P = 0.43), the increase in salivary cortisol did not significantly differ between treatments, although results were in the same direction as on day 1.

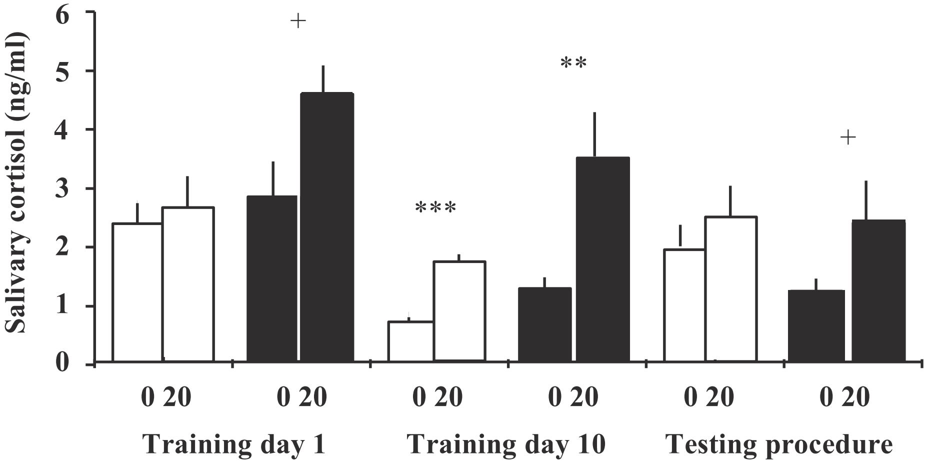

Fig. 3. Salivary cortisol (ng/ml) concentrations of the test pigs before (0) and 20 min after (20) a rewarding (open bars) or aversive (black bars) event on days 1 and 10 of the training procedure and in the testing procedure.
*** P < 0.001, ** P < 0.01, + P ≤ 0.1

3.2. Naive pigs (emotional contagion)

3.2.1. Display of cues in the testing procedure

During the habituation of the naive pigs on days 2 and 8, naive pigs did not show any play during the display of cues and there was no difference in defecating between the display of the aversive and rewarding cues (data not shown).

During the 20 s display of the rewarding cues in the testing procedure, naive pigs tended to show more tail in curl (rewarding: 90.3 ± 7.5 vs. aversive: 86.7 ± 7.5 % of time, $P < 0.1$) and less tail low (7.8 ± 5.6 vs. 12.6 ± 7.6 % of time, $P < 0.1$) than during the aversive cues. No treatment effects were found for freezing (rewarding: 15.1 ± 2.0 vs. aversive: 18.4 ± 4.8 % of time, $P = 0.50$), defecating (0.7 ± 0.3 vs. 0.5 ± 0.3 occurrences/min, $P = 0.64$), ears back (11.0 ± 3.6 vs. 17.5 ± 6.6 % of time, $P = 0.16$), ear posture changes (10.3 ± 3.0 vs. 11.3 ± 2.5 occurrences/min, $P = 0.48$), tail wagging (1.9 ± 1.9 vs. 0.7 ± 0.5% of time, $P = 0.91$) and tail posture changes (2.7 ± 1.3 vs. 2.2 ± 0.9 occurrences/min, $P = 0.93$).

3.2.2. Rewarding and aversive events in the testing procedure

During the rewarding event of the test pigs, naive pigs spent more time nosing the rewarding door (5.3 ± 2.0 vs. 1.1 ± 0.4 % of time, $P < 0.01$) than during the aversive event. In addition, play (83.0 vs. 0 % of occurrence, $P < 0.05$) occurred only during the rewarding event. During the aversive event of the test pigs, naive pigs spent more time nosing the aversive doors (16.7 ± 3.8 vs. 2.0 ± 0.7 % of time, $P < 0.01$) and they tended to show more defecating (0.9 ± 0.2 vs. 0.6 ± 0.3 occurrences/min, $P < 0.1$) than during the rewarding event. Escape attempts and urinating were not observed in the testing procedure. No treatment effects were found for freezing (rewarding: 11.4 ± 4.0 vs. aversive: 6.1 ± 1.3 % of time, $P = 0.24$), ears back (11.0 ± 2.3 vs. 11.9 ± 4.3 % of time, $P = 1.00$), ear posture changes (5.2 ± 0.9 vs. 6.0 ± 1.4 occurrences/min, $P = 0.59$), tail wagging (0.6 ± 0.3 vs. 0.2 ± 0.1 % of time, $P = 0.44$), tail low (5.9 ± 5.9 vs. 7.1 ± 6.0 % of time, $P = 0.36$) and tail posture changes (0.7 ± 0.3 vs. 0.5 ± 0.2 occurrences/min, $P = 0.81$).

3.2.3. Salivary cortisol

During the aversive event of the test pigs, salivary cortisol concentrations of the naive pigs tended to increase from $t = 0$ to $t = 20$ on day 10 of the training procedure ($P < 0.1$), but did not increase on day 1 of the training procedure ($P = 0.92$) or in the testing procedure ($P = 0.14$) (Fig. 4). During the rewarding event of the test pigs, salivary cortisol concentrations of the naive pigs did not increase from t = 0 to t = 20 on days 1 (P = 0.46) and 10 (P = 0.19) of the training procedure, but they did increase in the testing procedure (P < 0.05) (Fig. 4). The increase in salivary cortisol was not significantly different for the rewarding or aversive event on days 1 (aversive: 0.1 ± 1.1 vs. rewarding: 0.4 ± 0.5, P = 0.65) and 10 (2.6 ± 1.6 vs. 0.5 ± 0.4, P = 0.24) of the training procedure or in the testing procedure (1.2 ± 0.8 vs. 0.5 ± 0.2, P = 0.48).

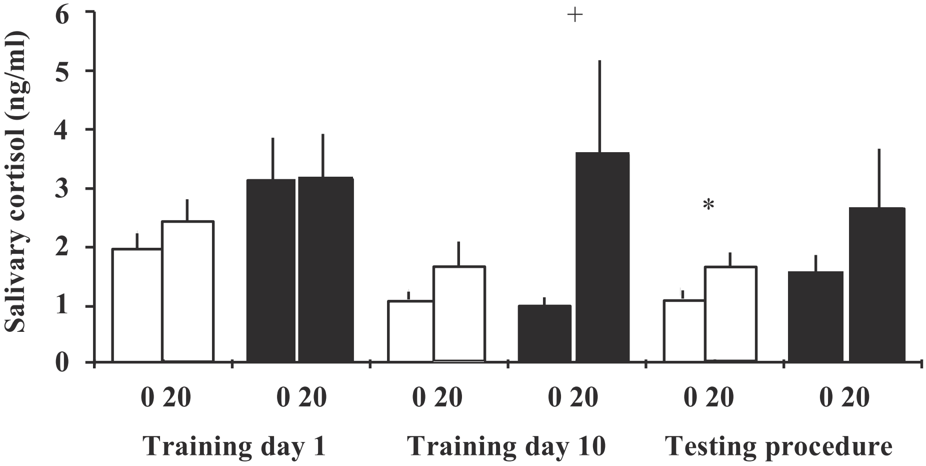

Fig. 4. Salivary cortisol (ng/ml) concentrations of the naive pigs before (0) and 20 min after (20) the rewarding (open bars) or aversive (black bars) event experienced by the test pigs on days 1 and 10 of the training procedure and in the testing procedure. * P < 0.05, + P < 0.1

3.3. Vocalizations during the rewarding and aversive events in the testing procedure

Vocalizations were scored as a total of four pigs. Barks were heard only during the rewarding event, but this was not significantly different from the aversive event (50 vs. 0 % of occurrence, P = 0.18). High-pitched vocalizations were heard only during the aversive event, but this was not significantly different from the rewarding event (50 vs. 0 % of occurrence, P = 0.18). Low-pitched vocalizations were also heard only during the aversive event and this was significantly different from the rewarding event (100 vs. 0 % of occurrence, P < 0.01).

4. Discussion

4.1. Indicators of positive and negative emotions in pigs

The first aim of this study was to investigate indicators of positive and negative emotions in pigs by assessing their response during anticipation and experience of a rewarding or aversive event.

As rewarding event we chose for pairwise access to a compartment filled with straw and peat, materials that seem to satisfy the behavioral need of pigs to explore (Studnitz et al., 2007), in which highly favored (Gieling et al., 2011; personal observation) chocolate raisins were hidden. Play occurred often during the rewarding event, both during training and testing, and never during the aversive event. As play has been shown to be a reliable indicator of positive emotions (Fraser and Duncan, 1998; Špinka et al., 2001; Boissy et al., 2007; Held and Špinka, 2011), this confirms that the rewarding event was valued as rewarding by the test pigs in this study. Also the bark, which has been associated with positive events such as play (Newberry et al., 1988; Chan and Newberry, 2011), occurred more during the rewarding event. Barks can, however, also be heard during alarming situations (Kiley, 1972; Newberry et al., 1988; Chan et al., 2011), indicating that there might be, at least, two types of bark: a ‘play’ bark and an ‘alarm’ bark (Chan et al., 2011) which differ in acoustic morphology and, moreover, elicit different behavioral responses in pigs (Chan and Newberry, 2011). As the barks in this study were heard only during the rewarding event, these were likely of the ‘play’ bark type and could therefore be indicative of positive emotions. Tail wagging and tail postures changes also occurred more often during the rewarding than during the aversive event. Other studies also reported tail wagging in pigs to be related with positive situations such as social greeting (Kiley-Worthington, 1976; Terlouw and Porcher, 2005), play (Newberry et al., 1988) and eating (Kleinbeck and McGlone, 1993). Tail wagging can, however, also be found in less positive situations such as after tail docking (Noonan et al., 1994), after castration (Hay et al., 2003) or following tail damage due to tail biting (Zonderland et al., 2009), but in these circumstances tail wagging is likely related to skin irritation (Kiley-Worthington, 1976) rather than emotional expression. Tail posture changes during which the tail is moving, as with tail wagging, occurred also more during the rewarding event, whereas the more static tail postures ‘tail in curl’ and ‘tail low’ occurred less during the rewarding event. These results suggest that tail movements in general could be a new potential behavioral indicator of positive emotions in pigs.

Social isolation and unpredictable, negative interventions, both stressful ((Schrader and Ladewig, 1999; Herskin and Jensen, 2000; Ruis et al., 2001) and (Weiss, 1970; Harding et al., 2004), respectively), were combined during the aversive event. Freezing, high-pitched vocalizations, escape attempts, defecating and urinating occurred more during this aversive event, which is in line with other studies associating these behaviors with aversive situations (Kiley, 1972; Fraser, 1974; Boissy, 1995; Mendl et al., 1997; Schrader and Todt, 1998; Herskin and Jensen, 2000; Marchant et al., 2001; Manteuffel et al., 2004; Mendl and Paul, 2004; Forkman et al., 2007; Manteuffel et al., 2007; Düpjan et al., 2008; Von Borell et al., 2009; Jones and Boissy, 2011) and they could, therefore, be indicators of negative emotions. Low pitched vocalizations, short and long grunts, also occurred more during the aversive event. Grunts are thought to be social contact calls (Marchant et al., 2001; Held et al., 2009). Therefore, test pigs probably grunted more during the aversive event, as they were, unlike during the rewarding event, deprived of visual and tactile social contact. In addition, test pigs showed more ears back, more ear posture changes and more tail low during the aversive event. In other species, ears back have also been associated with negative situations (sheep: (Tate et al., 2006; Veissier et al., 2009; Boissy et al., 2011), horses: (Waring, 2003), dogs: (Kiley-Worthington, 1976; Haupt, 2011)). While tail movements could be indicative of positive emotions, ear movements (i.e. changes between the ear postures 'front' and 'back') could be indicative of negative emotions in pigs, which has also been found in sheep (Reefman et al., 2009a,b). Tail low has been associated with negative situations in pigs (Kiley-Worthington, 1976; Noonan et al., 1994), horses (Waring, 2003) and dogs (Kiley-Worthington, 1976; Haupt, 2011). From these studies, it seems that ears back, ear movements and tail low are indeed indicators of negative emotions. If so, this study seems to have revealed two new behavioral indicators, ears back and ear movements, of negative emotions in pigs. It has been hypothesized that the posture of the ears in sheep (Boissy et al., 2011) could relay information to conspecifics about the environment. Whether pigs also use their ears to communicate (environmental) information to conspecifics is not known. The higher levels of tail in curl during the aversive event were probably the result of lower levels of tail wagging compared to the rewarding event as the three tail postures were mutually exclusive. Hence, we suggest that tail in curl is probably not related to emotional state, but can be considered as a neutral default posture from which other tail postures can occur during positive or negative emotions.

Behaviors of the test pigs during the events in the training procedure (without naive pen mates present) and during the events in the testing procedure (with naive pen mates present) were to a large extent similar. Test pigs still seemed to value the aversive event as aversive (results of defecating, urinating, ears back and vocalizations) and the rewarding event as rewarding (results of play, tail wagging and tail posture changes). There was, however, no longer a treatment effect on freezing as levels of freezing were relatively high during the rewarding event in the testing procedure as compared to the training procedure, which could indicate that test pigs were alerted by the presence of the naive pigs in the start box. In addition, treatment did not affect ear posture changes and tail low in the testing procedure, and escape attempts were not observed. Pigs may have valued the aversive event in the testing procedure as less negative compared to the reported aversive events in the training procedure for two reasons. First, we chose not to restrain the test pigs with a nose sling during the aversive event in the testing procedure, because a person entering the test room could have disturbed the naive pigs. Second, the presence of the naive pen mates in the start box during the aversive event could have reduced stress levels of the test pigs, i.e. social support.

Salivary cortisol was increased after the aversive event on days 1 and 10 of the training procedure and in the testing procedure. In addition, the increase in salivary cortisol from t = 0 to t = 20 was higher for the aversive event than for the rewarding event on day 1 of the training procedure. On day 10 of the training procedure, salivary cortisol was also increased after the rewarding event. As found by other studies (Paul et al., 2005; Jones and Boissy, 2011; Koolhaas et al., 2011), the results of this study confirm that cortisol rises in response to both positive and negative situations, indicating that the test pigs were aroused by the events. The behaviors shown by the test pigs in both events may, therefore, indeed reflect the pigs' emotional state.

During the anticipation of the events, when the cues were displayed, test pigs did not show as much behavioral indicators of positive and negative emotions as was hypothesized. In two other studies (Düpjan et al., 2008; Imfeld-Mueller et al., 2011), pigs did vocalize with high-pitched vocalizations (i.e. screams) during anticipation of an aversive event. In those studies, however, the pigs were, unlike the pigs in our study, alone during the anticipation period which could explain this difference (Mason and Mendl, 1997; Van den Bos et al., 2003). It could be that for the other behaviors the anticipation period was too short to have elicited them. Test pigs did show, however, more ears back and ear posture changes during the display of aversive cues towards the end of the training procedure and in the testing procedure. As these behaviors are associated with negative emotions, showing these behaviors during the aversive cues could indicate that pigs knew what was going to happen after the cues. This is also supported by the fact that test pigs oriented their heads more towards the aversive compartment during the display of cues preceding the aversive event in the training procedure and also tended to do so during the testing procedure. In addition, they nosed the rewarding door more and oriented their heads more towards the start box door during the rewarding cues at the end of the training procedure. During the display of cues preceding the rewarding event in the testing procedure, test pigs nosed the start box door more and they also oriented their heads more towards the start box door, possibly looking forward to see the experimenter to come through that door to bring them to the rewarding compartment. The orientation of the test pigs' heads towards the aversive compartment during the aversive cues could have been some sort of vigilance behavior, because vigilant animals may direct their attention towards the relevant stimulus (Welp et al., 2004), i.e. the aversive compartment.

4.2. Indicators of emotional contagion in pigs

The second aim was to investigate whether pigs show signs of emotional contagion. To that aim, the behavior and salivary cortisol response of naive pigs was measured during anticipation and experience of a rewarding or aversive event by their trained pen mates.

During the display of cues preceding the aversive event, naive pigs tended to show more tail low, at the expense of tail in curl which they tended to show more during the display of cues preceding the rewarding event. During the aversive event of the test pigs, naive pigs showed more nosing of the aversive door and tended to show more defecating. Naive pigs showed more nosing of the rewarding door and play occurred only and often during the rewarding event. In addition, salivary cortisol tended to increase in the naive pigs after the aversive event on day 10 of the training procedure and increased after the rewarding event in the testing procedure. Recently, Edgar et al. have argued that many results found by previous emotional contagion studies (Anil et al., 1997; Langford et al., 2006; De Waal, 2008; Edgar et al., 2011) may just indicate arousal rather than emotional contagion (Edgar et al., 2012). In this study, nosing of the doors could indicate that the naive pigs wanted to investigate what was going on behind the doors and, thus, indicate that the naive pigs were aroused by the test situation. Furthermore, the cortisol results of the naive pigs could also be explained in terms of arousal, for instance on day 10 of the training procedure due to screams of the test pigs during the aversive event, or following social interaction (Knapska et al., 2010) with the test pigs upon their return to the home pen. During the rewarding event in the testing procedure, naive pigs could have been aroused, because they heard the test pigs play and bark.

These results, however, do not provide any evidence that the naive pigs became incited with the emotions of the test pigs. The differences in play, defecating and tail low of the naive pigs during the rewarding event, the aversive event and the display of cues preceding the aversive event, respectively, are, however, hard to explain by differences in arousal alone. Naive pigs were habituated to the start box, so these behaviors were probably not elicited by exposure to the test room. In addition, play did not occur at all during habituation to the start box or during the aversive event and defecating occurred less during the rewarding event. Moreover, test pigs did not show more tail low during the display of cues preceding the aversive event and during the events, naive pigs could not see the test pigs indicating that the naive pigs were not just copying these behaviors from the test pigs. Additionally, these behaviors have, in contrast to investigatory (i.e. nosing) behaviors and cortisol concentrations, been associated with only positive or only negative situations as described in section 4.1, suggesting that the naive pigs were not only aroused, but were actually in a positive or negative emotional state which provides evidence for emotional contagion. Further research is needed, however, to support this. Signals by which emotional contagion could have occurred during the display of cues could have been visual and/or olfactory (Vieuille-Thomas and Signoret, 1992; Amory and Pearce, 2000), but probably not auditory, because the test pigs did not make any sounds during the display of cues except for breathing and sniffing sounds. During the events, signals could have been olfactory and/or auditory, but not visual, because the naive pigs could not see the test pigs. Further research into emotional contagion is warranted, not only in pigs but also in other animals, because the existence of emotional contagion could have profound implications for the welfare (Špinka, 2012), health (Vieuille-Thomas and Signoret, 1992; Amory and Pearce, 2000; Hemsworth, 2003) and performance (Hemsworth, 2003) of animals that are kept in large numbers within one room. Routine handling procedures with a negative connotation for the animals, such as, for instance, mutilations or medical treatments could lead to stress. The vocalizations, alarm pheromones (Vieuille-Thomas and Signoret, 1992; Amory and Pearce, 2000), and behaviors associated with a negative emotional state displayed by the stressed animals could in turn induce stress in the other animals within the room which were not subjected to the negative procedures themselves. Düpjan et al. have described the anecdote that stress responses could indeed spread through a group: when a pig starts screaming in a slaughterhouse, others pigs also start to scream (Düpjan et al., 2011). If animals are negatively affected by not only the stressful events they are exposed to, but also by those – unpredictably – imposed on their conspecifics within the room, they could potentially suffer from repeated or even chronic stress with negative consequences for health (Vieuille-Thomas and Signoret, 1992; Amory and Pearce, 2000; Hemsworth, 2003) and performance (reviewed in Hemsworth, 2003). On the other hand, emotional contagion of a positive emotional state, signaled by, for instance, play behavior, could be a powerful tool to improve the welfare, health and performance of animals. Furthermore, by investigating emotional contagion we could learn more about which signals animals use to communicate to their conspecifics (Düpjan et al., 2011).

In conclusion, behavioral indicators of emotions which have been described in other studies were also found in the current study. In addition, the results indicated three new potential behavioral indicators of emotions in pigs: tail movements, ears back and ear movements. Furthermore, the indications of emotional contagion in pigs suggest that it is worthwhile to continue research in this area.

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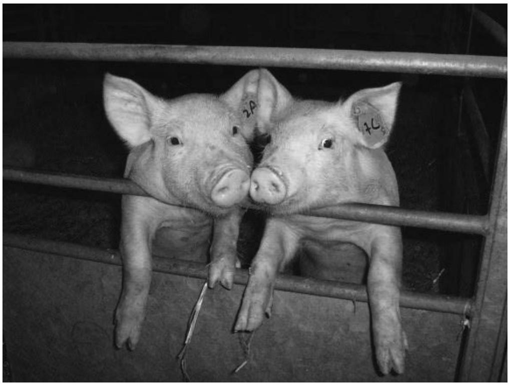
CHAPTER 6

Emotions on the loose: emotional contagion and the role of oxytocin in pigs



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Abstract

In this study, the process of emotional contagion, a simple form of empathy, and the role of oxytocin in this process were studied in pigs. To that aim, two pigs, the test pigs, per pen (n = 16 pens) were subjected to a positive (i.e. pairwise access to a large compartment filled with peat, straw and some chocolate raisins) and a negative treatment (i.e. social isolation in a small empty compartment) in a test room. Thereafter, two pen mates, the naive pigs, joined the test pigs to the test room, but they were not given access to the treatments. This allowed testing for emotional contagion. Subsequently, this procedure was repeated but 30 min prior to the emotional contagion test the naive pigs were given an intranasal administration of 24 IU of oxytocin or a placebo. The behavioral differences found between the positive and negative treatment (e.g. play and tail wagging vs. standing alert, urinating, defecating, and ears backwards) show that the treatments induced a positive and negative emotional state in the test pigs, respectively. Comparisons of the behaviors of the test pigs with and without naive pigs present and of the naive pigs with and without test pigs present indicated that emotional contagion did occur between the test and naive pigs, especially during the negative treatment. Oxytocin had no effect on the behavior of the treated naive pigs, but did affect the behavior of the test pigs which had not received oxytocin. This suggests a role for oxytocin in pig communication, which merits further research.

Keywords: behavior, emotions, emotional contagion, empathy, oxytocin, pigs

1. Introduction

Empathy is recognized as a multilayered phenomenon (De Waal, 2008; Preston and De Waal, 2002) which can be defined as “the capacity to be affected by and share the emotional state of another, assess the reasons for the other’s state and identify with the other, adopting his or her perspective” (De Waal, 2008). At the most simple level of empathy, emotional contagion, only the emotional state of the other is shared, but no cognitive perspective taking takes place (De Waal, 2008; Preston and De Waal, 2002). Emotional contagion is perhaps best illustrated by the situation in which the cry of an infant induces other infants to start crying too (Geangu et al., 2010; Simner, 1971), because it shows that the other infants share the distress of the first infant, but they do not understand why the first infant started to cry. As sharing another’s emotional state is thought to be essential for group bonding and communication (Spoor and Kelly, 2004), emotional contagion is considered to be the phylogenetically oldest level of empathy (De Waal, 2008; Preston and De Waal, 2002). Hence, it is likely that emotional contagion is not a process confined to humans, but exists in many different animal species (De Waal, 2008; Špinka, 2012). Indeed, emotional contagion has been described to occur in, for instance, primates, birds, rats and mice (reviewed in De Waal, 2008; Edgar et al., 2012a; Panksepp and Lahvis, 2011).

The peptide oxytocin is traditionally implicated in parturition and lactation (Uvnäs-Moberg, 1998). At present, however, it is also known that oxytocin plays a role in various social processes such as bond formation, social support and trust (Bartz and Hollander, 2006; Lim and Young, 2006). Moreover, it has been suggested that oxytocin plays a role in processing emotional information (Graustella and MacLeod, 2012) and in emotional contagion (De Dreu, 2012; Shamay-Tsoory, 2011). For instance, Hurlemann et al. (2010) found that human male subjects that were given an intranasal administration of oxytocin were emotionally more affected by photos of other humans expressing a range of emotions, positive and negative, than subjects that received a placebo. That oxytocin could play a role in emotional contagion is very plausible, because oxytocin has been shown to exert effects on brain regions such as the amygdala, anterior insula, anterior cingulate cortex, inferior frontal gyrus and inferior parietal lobe (De Dreu, 2012; Sofroniew, 1983; Zink and Meyer-Lindenberg, 2012), all brain regions that have been found to be involved in emotional contagion (Bastiaansen et al., 2009; Preston and De Waal, 2002; Shamay-Tsoory, 2011; Singer, 2006).

Pigs and other farm animals in intensive husbandry systems are usually kept at high stocking densities in a confined space (Spoolder et al., 2000; van de Weerd and Day, 2009). Moreover, they are also commonly subjected to quite a few standard management procedures such as mutilations (e.g. tail docking), abrupt weaning, regrouping and transport from which they cannot escape and which lead to distress (e.g. Dudink et al., 2006; Geverink et al., 1998; Noonan et al., 1994; Stookey and Gonyou, 1994). Under such housing conditions and management procedures, it is likely that farm animals can be affected by the distress of their group members. Apart from this, farm animals may also be affected by positive emotional states of their group members during, for instance, times of play (Held and Špinka, 2011; Špinka, 2012). The extent to which they are affected depends on their capacity for empathy or emotional contagion (Edgar et al., 2011). Emotional contagion has to the authors' knowledge, however, only very sparsely been studied in farm animals (sheep (Anil et al., 1996; Colditz et al., 2012; Edgar et al., 2012a), chickens (Edgar et al., 2011; Edgar et al., 2012b) or, more specifically, in pigs (Anil et al., 1997; Düpjan et al., 2011; Reimert et al., 2013)). Both Anil et al. (1997) and Düpjan et al. (2011) found no evidence for emotional contagion in pigs, but that could have been due to their experimental design. For example, the study of Anil et al. (1997) lacked a control treatment and the pigs in that study were not habituated to the test procedures (Edgar et al., 2012a). In addition, both Anil et al. (1997) and Düpjan et al. (2011) studied emotional contagion of negative emotional states only. In Reimert et al. (2013), a completely different design was used to study emotional contagion of negative as well as positive emotional states during anticipation (Moe et al., 2011; Spruijt et al., 2001) and during a positive and negative treatment (Rolls, 2000). With this design some evidence of emotional contagion was found, but results were still rather subtle.

The first aim of the present study was, therefore, to establish emotional contagion more clearly in pigs. To that aim, the same experimental design as in Reimert et al. (2013) was used, but some changes were made to this design with the intention to get clearer results. The second aim was to investigate whether oxytocin could play a role in emotional contagion in pigs. Based on our previous study, we hypothesized that emotional contagion does indeed occur in pigs (i.e. that the emotional state of pigs as reflected in their behavior would be affected by the emotional state of their group members) and, based on literature, that oxytocin makes emotional contagion stronger both in a positive and negative way (De Dreu, 2012; Hurlemann et al., 2010).

2. Materials and methods

2.1. Animals and housing

For this study, 96 Pietrain x (Great Yorkshire x Dutch Landrace) gilts, equally divided over two rounds, were used. Gilts were born at the organic farm of the Pig Research Centre of Wageningen Livestock Research, Raalte, The Netherlands. At nine weeks of age, 48 healthy gilts per round were transported to the experimental farm 'Carus' of Wageningen University, Wageningen, The Netherlands, where they were housed in eight groups of six unrelated pigs in 5.1 m^2 pens. The floors of the pens were covered with wood shavings (68 l) and straw (around 1.5 kg). Pens were cleaned every day after which fresh straw and wood shavings (together about 500 g) were added. Food (a standard commercial diet for growing pigs) and water were available ad libitum. Lights were on between 7 am and 7 pm. Pigs could be individually recognized by an ear tag and a number sprayed on their backs. The study was approved by the Animal Care and Use Committee of Wageningen University.

2.2. Experimental set up

Two pigs of each pen, the training pigs, were trained over a period of about three weeks to anticipate and experience a positive or negative treatment. Thereafter, the training pigs were joined by two non-trained pen mates during anticipation and experience of the treatments to test for emotional contagion. Subsequently, the effect of oxytocin, administered to the non-trained pen mates, on emotional contagion was studied.

Anticipatory behavior in the training pigs was induced using Pavlovian conditioning in which an initially neutral stimulus (conditioned stimulus, CS) was repeatedly followed by a supposedly positive or negative treatment (unconditioned stimulus, US). The supposedly positive treatment consisted of four min access in pairs to a compartment (15.5 m^2) containing about five kg of straw, 350 l of peat and eight chocolate raisins hidden in the substrate. The supposedly negative treatment consisted of four min social isolation in a much smaller and empty compartment (2.3 m^2) combined with other negative, unpredictable handlings (see next section). As conditioned stimuli, two auditory cues were used: a repetition of 12 s of piano music from Bach and a repetition of 11 s of a military march (both pieces of music are part of the auditory files of Microsoft PowerPoint 2010). For half of the pens, the piano piece announced the supposedly positive treatment and the military march the supposedly negative treatment. This was the other way around for the other half of the pens. The auditory cue started when both pigs were present in the anticipation compartment with the door closed and ended at the end of the four min treatment period. The cue was played during the treatment as well to increase the likelihood of associating a particular cue with a particular treatment.

The experimental setup (Fig. 1) was located in a test room and consisted of five compartments: an anticipation compartment, a positive compartment, two negative compartments and a compartment where the non-trained pen mates stood during the test for emotional contagion (from here on referred to as the neutral compartment). From the anticipation compartment, the training pigs could go to the positive or negative compartments via the neutral compartment after an experimenter had opened the corresponding doors. Cameras were fixed onto the setup to make video recordings that were analyzed later.

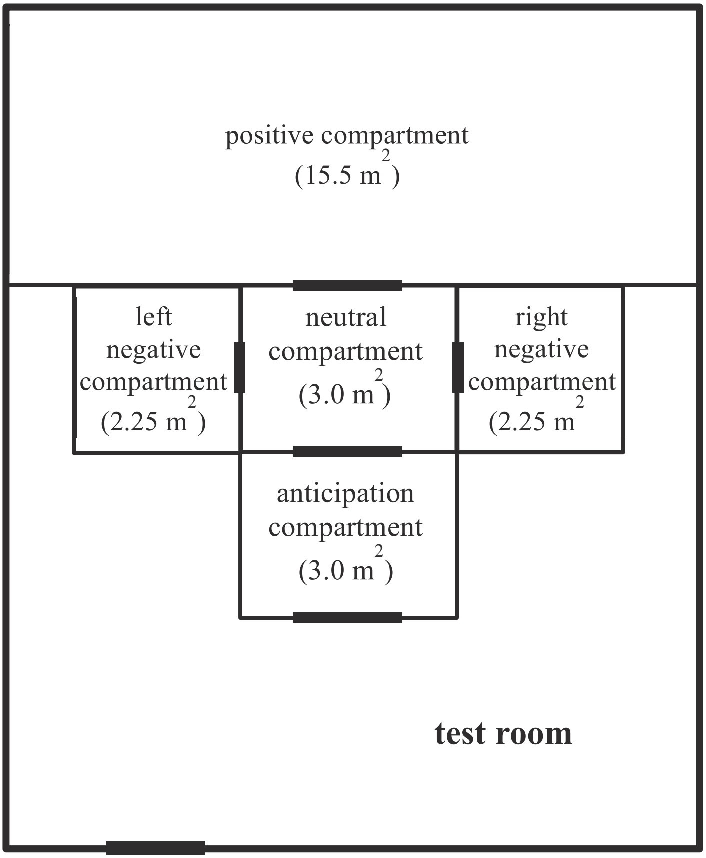

Fig. 1. A layout of the test room. The thickened lines indicate the position of the doors. The doors are named according to which compartment they gave entrance to. The route from entering the test room to entering the anticipation compartment was separated from the rest of the test room with wooden partitions. Compartment walls were at least 1 m high.

2.3. Training procedure of training pigs

The training procedure lasted about three weeks. During these three weeks, each pair of training pigs was subjected to two trials per day, one in the morning and one in the afternoon, in one of which they were exposed to the cue followed by the supposedly positive treatment, and in the other to the cue followed by the supposedly negative treatment, except for four training days. On these days, the same treatment was given both in the morning and in the afternoon, in a balanced way. We did this, so that the training pigs could not learn which treatment would be given in the afternoon based on the treatment given in the morning and, thus, already started to show anticipatory behavior in the home pen. There were at least three hours between the two daily trials for each pen. The order in which the training pigs were trained, and the order of positive and negative treatments on a day was randomized for pen and day throughout the entire training period, but in such a way that all pens experienced the positive and negative treatment the same number of times.

During each trial, the two training pigs of each pen were brought in the anticipation compartment after which the cue started. The length of the anticipation period was gradually increased every two days from 5 s on the first test day to a maximum of 35 s. When the cue signaled the supposedly positive treatment, one experimenter entered the anticipation compartment directly after the end of the anticipation period and guided the pair to the positive compartment while another experimenter opened the corresponding doors. The door of the positive compartment was closed as soon as the pair had entered it. After four min in this compartment, the pair was brought back to its home pen. When the cue signaled the supposedly negative treatment, an experimenter entered the anticipation compartment directly after the anticipation period had ended and guided each pig into one of the negative compartments while another experimenter opened the corresponding doors. The door was closed as soon as a pig had entered the negative compartment. After four min in this compartment, the training pigs were brought back to their home pen. In addition to social isolation, other negative handlings were carried out in an unpredictable way during the negative treatment. On test day 2, 4, 9, 12, 16, 19, 22 and 25 a person (not one of the experimenters) entered one of the negative compartments either directly or two min after the start of the treatment and restrained the pig there with a nose sling for 15 s. Thereafter, the same handling was done to the pig in the other negative compartment. On test day 3, 5, 8, 11, 17 and 18, a person also entered one of the negative compartments either directly or one or two min after the start of the treatment but now only threatened to restrain the pig in that compartment. Thereafter, again, the same handling was done to the pig in the other negative compartment. On test day 6 and 10, air from a noisy vacuum cleaner was blown for 15 s into both negative compartments at floor level at one min after the start of the treatment and on test day 7 and 15, two balloons, one at the level of each compartment, were simultaneously punctured with a needle at one min after the start of the treatment. On test day 1, 23, 24 and 26 no additional handlings were carried out. Assignment of compartments (left or right) and (start of the) negative handlings were all balanced for the different test days and over the total training period. Days 13, 14, 20 and 21 were two weekends during which pigs were not trained.

The behaviors of the training pigs during anticipation and during the experience of a positive and negative treatment on test day 23 and 24 were considered as ‘normal’ behaviors expressed during a particular training trial and were used to compare with their behavior on test day 26, when two non-trained pen mates were also present during anticipation and the treatments which allowed testing for emotional contagion (see *test for emotional contagion*).

2.4. Habituation procedure of the pen mates

Before testing for emotional contagion on test day 26, the four pen mates of each pair of training pigs were habituated to the test room and to the cues, but were not given access to the treatments. Two of these pigs will later on join the training pigs to the test room to test for emotional contagion (see *test for emotional contagion*). These two are from here on referred to as naive pigs or naive pen mates as they are, with regard to the training pigs, naive to the treatments. The other two will later on be used to test whether oxytocin has an effect on behavior in itself, irrespective of the treatment of the training pigs (see *test for the effect of oxytocin on emotional contagion*). These two are from here on referred to as control pigs.

Habituation started on test day 19 by bringing these four pigs to the anticipation compartment and after the door was closed, one of the cues started. After 35 s, pigs were guided by an experimenter to the neutral compartment, while another experimenter opened the door for them. Subsequently, the four pigs spent two min in the neutral compartment after which the cue ended and they were brought back to their home pen. Thereafter, it was the turn of the next four pigs. Similarly to the training pigs, these four pigs also had a morning and afternoon trial, one with the Bach cue and one with the military march cue, matching the positive and negative cue of their trained pen mates. The four pigs were habituated in this way for two test days. In the following two test days, a similar procedure was carried out, but now with only two of the four pigs, i.e. the naive pigs, and the duration in the neutral compartment was set to four min. The order in which these pigs were habituated and which cue was given in the morning and which in the afternoon trial was randomized, but balanced for pen and day on these four test days. There were at least three hours between the two daily trials for each pen.

The behaviors of the two naive pigs in the anticipation and neutral compartment on the last two habituation days, test day 23 and 24, were used to compare with their behavior in the same situation on test day 26, i.e. the emotional contagion test day.

To reduce any disturbance of the company of these two naive pen mates with the training pigs and vice versa on test day 26, these four (training and naive) pigs of each pen were brought to the anticipation compartment once on test day 25. After 35 s they were brought back to their home pen. No cue was given during these 35 s.

2.5. Test for emotional contagion

In the morning and afternoon of test day 26, the training pigs and their two naive pen mates were brought to the anticipation compartment where a cue was given for 35 s, after which the training pigs were exposed to the corresponding positive or negative treatment for four min. The naive pen mates stayed in the neutral compartment during these four min. After the four min, all four pigs were brought back to their home pen. Half of the pens was exposed to the supposedly positive treatment in the morning and to the supposedly negative treatment in the afternoon and for the other pens this was the other way around.

2.6. Test for the effect of oxytocin on emotional contagion

In the week after test day 26 (a Friday), the test for emotional contagion was repeated but this time the naive pen mates received a dose of oxytocin 30 min before they were brought to the test room together with the training pigs. To avoid that the naive pen mates started to anticipate themselves, we kept the number of test trials to a minimum by continuing with only one cue (i.e. the Bach cue) for all pens. So from here on, half of the training pigs only experienced the positive treatment and the other half only the negative treatment.

In the morning of the first three days of this week, the training pigs of each pen went through a regular training trial to keep the association between the Bach cue and the subsequent treatment. After the training trials on the first day of this week, the four pen mates of the training pigs were reminded of the test room in the same way as described above, but also with just the Bach cue. After the training trials on the second and third day (i.e. test day 30 and 31) of this week, the effect of oxytocin on behavior in itself was studied with the control pigs. On the first of these two days, therefore, half of the pairs received a single dose of 24 IU of oxytocin (VWR International, Amsterdam, The Netherlands) 30 min before they were brought to the test room and the other half received a placebo. For the oxytocin, this was done by diluting 50 µg of oxytocin in 0.5 ml of 0.9 % saline and administering 0.25 ml in each nostril of each pig using a Mucosal Atomizer Device (MAD 300, Vandeputte Medical Nederland BV, Nieuwegein, The Netherlands) connected to a 1 ml syringe (Rault et al., 2013). The placebo consisted of 0.5 ml of 0.9 % saline which was administered in the same way as the oxytocin solution. Subsequently, the pair of control pigs was brought to the test room 30 min later and the same procedure as described above was carried out, meaning that control pigs were brought to the test room without training pigs. On the second day, the pairs that received oxytocin the day before were now given the placebo and vice versa.

On the last two test days of this week (test day 32 and 33), the effect of oxytocin on emotional contagion was tested. Hereto, the same procedure for the oxytocin and placebo administration was used as described above, except that here the pen mates, i.e. the naive pigs, were used that were also used on test day 26. Further, the same procedure as described for test day 26 was followed, meaning that both naive and training pigs were brought to the test room.

2.7. Behavioral analyses

Video recordings were analyzed using focal sampling and continuous recording with the Observer XT 10 software of Noldus Information Technology B.V., Wageningen, The Netherlands. The ethogram in Table 1 was used for scoring behaviors displayed in the anticipation compartment on test days 9, 12, 17, 18, 23, 24, 26, 30-33 by all pigs and during the positive and negative treatments by the training pigs and in the neutral compartment by the naive pen mates on test days 23, 24, 26 and 30-33. Not all behaviors were scored for each pig in each situation, because that was either not possible (e.g. exploring anticipation door for the training pigs during the treatment) or because behaviors were regarded to be relevant for one situation only (e.g. head postures were only scored in the anticipation compartment, because the number of transitions between both head postures could be indicative for hyperactive behavior which has been associated with anticipation of positive stimuli (Moe et al., 2011; Spruijt et al., 2001)). The vocalizations were scored as a total of two pigs on test days 9 – 24, 30 and 31 and as a total of four pigs on test days 26, 32 and 33, because it was not possible to identify them per individual pig.

Table 1
Ethogram used to score the behaviors of both the training pigs and naive pen mates in the anticipation compartment on test days 9, 12, 17, 18, 23, 24, 26 and 30-33 and during the treatments (in the positive and negative compartment for the training pigs and in the neutral compartment for the naive pen mates) on test days 23, 24, 26 and 30-33. Behaviors were scored as states unless indicated otherwise.

Behavior	Description
Behavior	
Standing alert	Standing motionless with whole body and head fixed
Escape attempts	Jumping in air or against the wall or door of the compartment
Play	Running, gamboling, pivoting or playing with straw by shaking head
Urinating *(event)*	Urinating
Defecating *(event)*	Defecating
Exploring anticipation door[1]	Sniffing, nosing or rooting the door of the anticipation compartment
Exploring neutral door[2]	Sniffing, nosing or rooting the door of the neutral compartment
Exploring positive door[3]	Sniffing, nosing or rooting the door of the positive treatment compartment
Exploring negative doors[3]	Sniffing, nosing or rooting the door of the negative treatment compartments
Ears postures	
Ears front	Both ears directed to the front
Ears back	One or both ears directed backwards
Tail postures	
Tail in curl	Tail coiled up in a curl on top of the body
Tail wagging	Tail swinging in any direction, but mostly from side to side
Tail low	Tail hanging down against the body
Head postures[1]	
Head up	Head directed forward or actively up
Head down	Head directed downwards or to the floor of the compartment
Head orientation[1]	
Head to anticipation door	Head oriented to the door of the anticipation compartment
Head to neutral door	Head oriented to the door of the neutral compartment
Vocalizations (events)	
Low-pitched vocalizations	Short or long grunts
High-pitched vocalizations	Screams, squeals or grunt-squeals
Barks	A low tone that sounds like “woof”

[1] These behaviors were only scored when the pigs were in the anticipation compartment.
[2] This behavior was not scored for the training pigs during the treatments.
[3] These behaviors were not scored when the pigs were in the anticipation compartment and exploring the negative doors and exploring the positive door were not scored for the training pigs when they were in the positive and negative treatment, respectively.

2.8. Statistical analyses

SAS (SAS 9.2, SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. Preliminary analyses showed that the behavior of the training pigs on test day 23, 24 and 26 differed substantially between the positive and negative treatment. However, their behavior on these and earlier test days in the anticipation compartment showed only subtle differences between anticipation for positive and negative stimuli (data not shown). In addition, the behavior of the naive pen mates on test day 26 seemed to indicate that they were (emotionally) affected by the training pigs during the two treatments, but not during anticipation. Therefore, we decided to omit the results of the training and naive pigs in the anticipation compartment and thus only present the results of the training and naive pigs during the positive and negative treatments.

2.8.1. Emotional contagion without a possible effect of intranasal oxytocin

Before analyses, the behaviors of the training pigs in the different treatments (i.e. positive or negative) were averaged per pen. Subsequently, the behaviors of the pairs during the treatments were also averaged over test day 23 and 24 to have one representative value of the behaviors of a pair of pigs during the positive and negative treatments. Preliminary analyses showed no effect of cue (i.e. Bach or military march) on the behaviors of the training pigs during the treatments. This factor was, therefore, not included in the final models. Behaviors were analyzed with three separate analyses: 1) differences between the treatments were investigated in the situation without naive pen mates present (i.e. using the pen averages of the behaviors expressed in the treatments over test day 23 and 24), 2) differences between treatments were investigated in the situation with naive pen mates present (i.e. using the pen averages of the behaviors expressed in the treatments on test day 26), and 3) differences between treatments were investigated by using a model that included both situations. For the first and second analysis, a general linear model (GLM) was used with treatment (i.e. positive or negative) and round (i.e. round 1 and 2) as fixed effects and for the third analysis a GLM was used with treatment, situation (without or with naive pen mates present), their interaction and round as fixed effects. The behaviors that were far from normally distributed (e.g. pigs generally urinated once or not at all) were transformed into a 0-1 variable on pen level and were analyzed with a generalized linear model with a logit link and binary distribution and with the same fixed effects as used in the GLM. When those behaviors also did not occur during either the negative or the positive treatment (e.g. no play behavior was observed in the negative treatment), they were analyzed with a Fisher's Exact Test for treatment for the first and second situation separately and over both situations and with a Fisher's Exact Test for situation within each treatment and over the two treatments.

For the behaviors of the naive pen mates similar (statistical) procedures were followed. For the first analysis (i.e. the situation without training pigs present in the test room), the fixed effect treatment was, however, changed into cue (i.e. Bach or military march) as naive pigs were exposed to two different cues, but not to the actual treatments. Cue did not affect any of the behaviors (see Table 2). Therefore, differences in behavior in the situation with training pigs present in the positive or negative treatment were analyzed using a GLM with treatment (i.e. average of both cues, positive or negative) and round as fixed effects.

Table 2
Behavior of the naive pen mates in the neutral compartment of the test room without training pigs present but with two different cues

	without training pigs present		
Behaviors	Bach	military march	C[1]
Behavior			
Standing alert (% of time)	5.6 ± 1.4	3.6 ± 1.0	NS
Escape attempts (freq.)	0.9 ± 0.3	0.5 ± 0.2	NS
Urinating (% of pens)	75.0	68.8	NS
Defecating (freq.)	4.2 ± 0.3	3.8 ± 0.3	NS
Exploring neutral door (% of time)	7.0 ± 1.0	6.0 ± 1.2	NS
Exploring positive door (% of time)	2.6 ± 0.9	1.3 ± 0.3	NS
Exploring negative door (% of time)	2.2 ± 0.3	2.3 ± 0.3	NS
Ear posture			
Ears back (% of time)	3.8 ± 0.8	3.7 ± 1.0	NS
Vocalizations (voc.)			
Low-pitched voc. (freq.)	10.2 ± 2.5	11.0 ± 2.3	NS
High-pitched voc. (freq.)	6.2 ± 3.2	5.9 ± 2.4	NS
Barks (% of pens)	0	6.3	NS

[1] Significance of effect of cue (C) is indicated: NS $P \geq 0.10$.

2.8.2. Emotional contagion with a possible effect of intranasal oxytocin

Preliminary analyses showed no effect of oxytocin on the behavior of the control pigs in the test room on test day 30 and 31 (see Table 3). Moreover, order (i.e. receiving oxytocin first and then the placebo or vice versa) also did not affect the behavior of pigs on these test days and test day 32 and 33. Order was, therefore, not included in the final models.

The behaviors of the training and naive pigs on test day 32 and 33 were analyzed on pen level with a mixed linear model with treatment (i.e. positive or negative), intranasal administration (i.e. oxytocin or a placebo), their interaction, and round as fixed effects and pen nested within treatment and round as a random effect. Similarly to before, behaviors that were far more normally distributed were transformed into a 0-1 variable on pen level and analyzed with a generalized linear model with a logit link and binary distribution and with the same fixed effects as used in the mixed linear model or with a Fisher's Exact Test for treatment, intranasal administration and treatment within each administration when the behavior also did not occur during either the negative or the positive treatment.

Table 3
Behavior of the control pigs in the neutral compartment of the test room 30 min after receiving an intranasal administration of oxytocin or a placebo

Behaviors	oxytocin	placebo	A[1]
Behavior			
Standing alert (% of time)	13.6 ± 2.0	12.8 ± 1.5	NS
Escape attempts (% of pens)	6.3	6.3	NS
Urinating (% of pens)	50	68.8	NS
Defecating (freq.)	4.3 ± 0.3	3.9 ± 0.4	NS
Exploring neutral door (% of time)	7.8 ± 2.0	8.0 ± 1.2	NS
Exploring positive door (% of time)	4.2 ± 0.9	5.5 ± 1.3	NS
Exploring negative door (% of time)	1.8 ± 0.4	1.5 ± 0.3	NS
Ear posture			
Ears back (% of time)	4.8 ± 1.3	4.5 ± 1.3	NS
Tail postures			
Tail in curl (% of time)	98.5 ± 1.0	97.9 ± 1.2	NS
Tail wagging (% of time)	0.6 ± 0.5	1.1 ± 0.9	NS
Tail low (% of time)	0.9 ± 0.8	1.0 ± 0.6	NS
Vocalizations (voc.)			
Low-pitched voc. (freq.)	4.5 ± 1.3	2.5 ± 1.2	NS
High-pitched voc. (freq.)	0.2 ± 0.1	0.3 ± 0.2	NS
Barks (% of pens)	0	0	NS

[1] Significance of effect of intranasal administration (A) is indicated: NS $P \geq 0.10$.

As the two ear postures are complementary to each other, only the percentage of time ears back are presented as this posture has been associated with a negative emotional state (Reimert et al., 2013). For the naive pen mates, play behavior and tail postures were not statistically analyzed, because play did not occur and the tail was almost 100 % of the time in a curl (tail in curl: 99.7 ± 0.1 % of time; tail wagging: 0.1 ± 0.1 % of time; tail low: 0.1 ± 0.1 % of time). The behaviors analyzed with the GLM or mixed linear model were expressed as percentage of time or as absolute frequencies and the behaviors analyzed with the Fisher's Exact Test or generalized linear model as percentage of pens that showed this behavior. For the GLM and mixed models skewed residuals were normalized if needed using arcsine square root and square root transformations for proportions and frequencies, respectively, and significant interactions were further explored with post hoc pairwise comparisons using the differences of the least square means.

3. Results

3.1. Behavior of training and naive pigs without intranasal oxytocin administration

3.1.1. Training pigs

In the situation without the presence of the naive pen mates, treatment affected all behaviors of the trainings pigs except the tail posture tail low which did not differ between the positive and negative treatment (Table 4). Play behavior occurred and barks were heard during the positive treatment only. In addition, training pigs wagged their tail far more during the positive treatment than during the negative treatment. Escape attempts occurred and high-pitched vocalizations were heard during the negative treatment only. Also, training pigs showed more standing alert behavior, were more likely to urinate and defecate, showed more exploring of the compartment door, had their ears more in a backwards posture and their tail more in a curl posture, and produced more low-pitched vocalizations during the negative treatment than during the positive treatment. In the situation with two naive pen mates present in the neutral compartment, treatment affected the behaviors of the trainings pigs similar to the situation where training pigs were tested alone (Table 4).

When comparing both situations, training pigs generally tended to explore the door of the treatment compartment less when their naive pen mates were present in the neutral compartment than without their presence (Table 4). In addition, interaction effects were found for standing alert, ears back, and the tail postures tail in curl and tail wagging (Table 4). Post hoc pairwise comparisons revealed that training pigs spent more time standing alert but had their ears less backwards and were less likely to urinate during the negative treatment with two naive pen mates present than during the same treatment without their presence (Table 4). During the positive treatment training pigs had their tails in a curl more frequently and wagged their tails less when their two naive pen mates were present than during the same treatment without their presence (Table 4). Training pigs also tried to escape less from the negative treatment compartment when their naive pen mates were present in the neutral compartment than without their presence, but that was not significant (Table 4).

Table 4
Behavior of the training pigs during a positive and negative treatment in two situations: without the presence of two naive pen mates and in the presence of two naive pen mates in the test room

	without naive pigs present			with naive pigs present		effects^1		
Behaviors	positive	negative	T^2	positive	negative	T^2	S	TS
Behavior								
Standing alert (% of time)	0.3 ± 0.1^a	32.9 ± 3.1^b	***	2.8 ± 1.2^a	49.0 ± 4.6^c	***	**	*
Escape attempts (% of pens)^3	0	62.5	***	0	31.3	*	NS	-
Play (% of pens)^3	100	0	***	93.8	0	***	NS	-
Urinating (% of pens)^3	6.3	93.8^g	***	0	62.5^h	***	NS	-
Defecating (freq.)	0.7 ± 0.2	4.7 ± 0.5	***	0.7 ± 0.3	4.5 ± 0.4	***	NS	NS
Exploring treatment door (% of time)	0.5 ± 0.1	3.4 ± 0.7	***	0.4 ± 0.2	2.3 ± 0.6	***	+	NS
Ear posture								
Ears back (% of time)	1.9 ± 0.7^a	17.3 ± 4.7^b	***	1.3 ± 0.5^a	7.3 ± 2.0^c	**	*	+
Tail postures								
Tail in curl (% of time)	87.3 ± 3.5^a	99.8 ± 0.2^b	***	93.1 ± 2.4^c	99.2 ± 0.7^b	**	NS	+
Tail wagging (% of time)	12.3 ± 3.4^a	0.1 ± 0.0^b	***	6.7 ± 2.3^c	0.2 ± 0.1^b	***	+	+
Tail low (% of time)	0.4 ± 0.2	0.1 ± 0.1	NS	0.3 ± 0.2	0.6 ± 0.5	NS	NS	NS
Vocalizations (voc.)								
Low-pitched voc. (freq.)	0.2 ± 0.2	24.8 ± 2.9	***					
High-pitched voc. (% of pens)	0	50.0	**					
Barks (% of pens)	87.5	0	***					

Means with different superscript letters differ significantly (a/b/c: $P < 0.05$, g/h: $P < 0.1$).

[1] Significance of effects of treatment (T), situation (S) and their interaction (TS) is indicated:
*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, + $P < 0.10$, NS $P \geq 0.10$, - no statistical analysis performed

[2] These treatment effects belong to the first and second situation, respectively. Treatment effects over both situations were equal to the situation without naive pigs present.

[3] The effect of situation within treatment was significant for urinating within the negative treatment, but not within the positive treatment nor for escape attempts and play.

3.1.2. Naive pen mates

The behavior of the naive pen mates in the neutral compartment of the test room was not affected by hearing Bach music or a military march (Table 2). There were differences, however, between the situation where naive pigs were tested alone versus the situation with training pigs present in either the positive or negative treatment compartment for standing alert, exploring of the compartment doors and ears back (Table 5). Post hoc analysis showed that the naive pen mates spent more time standing alert when the training pigs were in the negative treatment than when the training pigs were in the positive treatment or in the situation without training pigs present in the test room (Table 5). Furthermore, naive pen mates spent more time exploring the door of the compartment that held the training pigs during both treatments, but they spent more time exploring the door of the neutral compartment in the situation without the training pigs present than in the two situations with the training pigs present in the test room (Table 5). Moreover, naive pen mates had their ears more backwards when the training pigs were in the negative treatment than when the training pigs were in the positive treatment or in the situation without training pigs present in the test room (Table 5).

Table 5
Behavior of the naive pen mates in the neutral compartment of the test room in three situations: without training pigs present and with training pigs present in the positive or negative treatment compartments

Behaviors	without training pigs present	with training pigs present		T[1]
		positive	negative	
Behavior				
Standing alert (% of time)	4.6 ± 1.2^{a}	3.8 ± 0.8^{a}	10.7 ± 1.6^{b}	***
Escape attempts (freq.)	0.7 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	NS
Urinating (% of pens)	87.5	62.5	43.8	NS
Defecating (freq.)	4.0 ± 0.3	3.8 ± 0.3	3.9 ± 0.3	NS
Exploring neutral door (% of time)	6.5 ± 1.0^{ay}	3.7 ± 0.7^{bz}	4.3 ± 0.8^{z}	+
Exploring positive door (% of time)	2.0 ± 0.5^{a}	6.4 ± 1.9^{b}	2.6 ± 0.7^{a}	*
Exploring negative door (% of time)	2.2 ± 0.2^{a}	2.1 ± 0.4^{a}	3.6 ± 0.6^{b}	*
Ear posture				
Ears back (% of time)	3.8 ± 0.9^{ay}	4.5 ± 1.0^{y}	7.2 ± 1.4^{bz}	+

Means with different superscript letters differ significantly (a/b: $P < 0.05$, y/z: $P < 0.10$).

[1] Significance of effect of treatment (T) is indicated:
*** $P < 0.001$, * $P < 0.05$, + $P < 0.10$, NS $P \geq 0.10$

3.1.3. Vocalizations in the situation with four pigs in the test room

Vocalizations were not compared between both situations, because vocalizations were scored as a total of two pigs in one and as a total of four pigs in the other situation. In the situation with the training pigs in one of the treatment compartments and their naive pen mates in the neutral compartment, more low- and high-pitched vocalizations were recorded with the training pigs in the negative treatment compartment than in the positive treatment compartment (low-pitched vocalizations: 33.6 ± 3.6 vs. 7.9 ± 2.5, $P < 0.001$ and high-pitched vocalizations: 10.7 ± 3.5 vs. 2.9 ± 1.4, $P < 0.001$). In contrast, more barks were heard with the training pigs in the positive treatment compartment than in the negative treatment compartment (56.3 vs. 6.3 % of pens, $P < 0.01$).

3.2. Behavior of training and naive pigs with intranasal oxytocin administration

3.2.1. Training pigs

Irrespective of whether their naive pen mates received an intranasal administration of oxytocin or a placebo, training pigs still only played during the positive treatment and wagged their tails more during this treatment than during the negative treatment (Table 6). During the negative treatment, training pigs still spent more time standing alert, were more likely to urinate, and had their tails more in a curl than during the positive treatment. No main effect of treatment was found for escape attempts, exploring of the treatment door, and ears backwards (Table 6).

Defecating and tail low were both affected by treatment, intranasal administration and their interaction (Table 6). Post hoc analysis showed that training pigs were less likely to defecate during the positive treatment than during the negative treatment, but were also less likely to defecate during the negative treatment when their naive pen mates had received oxytocin compared to a placebo (Table 6). Furthermore, the tail of the training pigs was most frequently low during the positive treatment and with a placebo given to their naive pen mates compared to the other situations (Table 6). Exploring the treatment door was also affected by the interaction between treatment and administration. Post hoc analysis showed that training pigs spent less time exploring the door of the treatment compartment during the positive treatment when their naive pen mates had received a placebo than during the positive treatment when their naive pen mates had received oxytocin. In addition, they tended to spend less time exploring the door of the treatment compartment during the positive treatment when their naive pen mates had received a placebo than during the negative treatment when their naive pen mates had received a placebo (Table 6).

Table 6
Behavior of the training pigs during a positive and negative treatment in the presence of their naive pen mates who received an administration of oxytocin or a placebo 30 min before they went to the test room with the training pigs

	positive treatment		negative treatment		effects[1]		
Behaviors	oxytocin	placebo	oxytocin	placebo	T	A	TA
Behavior							
Standing alert (% of time)	3.5 ± 2.1	4.3 ± 3.0	33.9 ± 3.2	34.3 ± 4.0	***	NS	NS
Escape attempts (% of pens)[2]	0	12.5	0	0	NS	NS	-
Play (% of pens)[2]	100^{g}	100^{g}	0^{h}	0^{h}	***	NS	-
Urinating (% of pens)[2]	0^{g}	0^{g}	75.0^{h}	87.5^{h}	***	NS	-
Defecating (freq.)	0.3 ± 0.2^{a}	0.3 ± 0.1^{a}	4.1 ± 0.4^{b}	5.1 ± 0.3^{c}	***	*	*
Exploring treatment door (% of time)	3.2 ± 1.2^{a}	1.1 ± 0.6^{b}	2.3 ± 0.5^{ab}	3.2 ± 0.4^{b}	NS	NS	*
Ear posture							
Ears back (% of time)	10.3 ± 4.0	13.5 ± 2.7	6.1 ± 1.6	6.8 ± 1.3	NS	NS	NS
Tail postures							
Tail in curl (% of time)	85.0 ± 5.9	82.0 ± 7.0	99.9 ± 0.1	100 ± 0.0	**	NS	NS
Tail wagging (% of time)	11.6 ± 4.8	9.2 ± 3.9	0	0.0 ± 0.0	**	NS	NS
Tail low (% of time)	3.4 ± 2.2^{a}	8.8 ± 4.2^{b}	0.1 ± 0.1^{a}	0^{a}	+	+	*

Means with different superscript letters differ significantly (g/h: $P < 0.01$; a/b/c: $P < 0.05$).

[1] Significance of effects of treatment (T), intranasal administration (A) and their interaction (TA) is indicated: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, + $P < 0.10$, NS $P \geq 0.10$, - no statistical analysis performed

[2] The effect of treatment within the oxytocin or placebo administration was not significant for escape attempts, but was significant for play ($P < 0.001$) and for urinating ($P < 0.01$).

3.2.2. Naive pen mates

Irrespective of whether the naive pen mates received oxytocin or a placebo, they spent more time standing alert when the training pigs were in the negative treatment than when the training pigs were in the positive treatment (Table 7). In addition, naive pen mates also tended to have their ears more backwards when the training pigs were in the negative treatment than when the training pigs were in the positive treatment (Table 7). On the other hand, naive pen mates spent more time exploring the door of the positive treatment compartment when the training pigs were in the positive treatment than when training pigs were in the negative treatment (Table 7). A significant interaction effect between treatment and intranasal administration was found for exploring the neutral door, which was during the negative treatment performed more by the placebo treated pigs and during the positive treatment more by the oxytocin treated pigs, although post hoc analysis revealed no differences between the treatment groups (Table 7).

Table 7
Behavior of the naive pen mates in the neutral compartment of the test room 30 min after receiving an intranasal administration of oxytocin or a placebo and during a positive or negative treatment experienced by the training pigs

	positive treatment		negative treatment		effects[1]		
Behaviors	oxytocin	placebo	oxytocin	placebo	T	A	TA
Behavior							
Standing alert (% of time)	5.5 ± 2.11	9.0 ± 2.61	13.8 ± 2.0	14.4 ± 2.8	*	NS	NS
Escape attempts (% of pens)[2]	0	0	25	0	NS	NS	-
Urinating (% of pens)	37.5	75.0	62.5	75.0	NS	NS	NS
Defecating (freq.)	3.0 ± 0.5	2.9 ± 0.6	3.9 ± 0.6	3.6 ± 0.4	NS	NS	NS
Exploring neutral door (% of time)	6.1 ± 3.0	3.2 ± 1.4	5.1 ± 1.2	7.2 ± 2.2	NS	NS	*
Exploring positive door (% of time)	35.0 ± 10.4	32.3 ± 11.1	5.4 ± 1.4	4.2 ± 1.4	*	NS	NS
Exploring negative door (% of time)	0.8 ± 0.4	0.9 ± 0.2	1.0 ± 0.3	1.2 ± 0.3	NS	NS	NS
Ear posture							
Ears back (% of time)	5.8 ± 1.6	6.1 ± 4.2	10.0 ± 1.9	9.9 ± 1.7	+	NS	NS

[1] Significance of effects of treatment (T), intranasal administration (A) and their interaction (TA) is indicated:
* $P < 0.05$, + $P < 0.10$, NS $P \geq 0.10$, - no statistical analysis performed.

[2] The effect of treatment within the oxytocin or placebo administration was also not significant.

3.2.3. Vocalizations

The four pigs together produced more low-pitched vocalizations and tended to produce more high-pitched vocalizations during the negative treatment than during the positive treatment of the training pigs (low-pitched vocalizations: 25.5 ± 4.1 vs. 3.6 ± 1.2, $P < 0.01$ and high-pitched vocalizations: 11.8 ± 4.8 vs. 0.2 ± 0.1, $P < 0.10$). In contrast, barks were only heard during the positive treatment of the training pigs (62.5 vs. 0 % of pens, $P < 0.001$). Moreover, more low-pitched vocalizations were produced when the naive pen mates were given oxytocin than a placebo (16.4 ± 4.5 vs 12.7 ± 3.7, $P < 0.05$). No other significant (interaction) effects were found.

4. Discussion

The aim of this study was to test whether emotional contagion occurs in pigs during a positive and negative treatment, and whether oxytocin augments emotional contagion. The results of this study show indeed, although subtle, that pigs can be affected by the emotional state of their pen mates. Furthermore, no effect of oxytocin was found on the behavior of the treated naive pigs, but surprisingly the training pigs did behave differently in the treatments when their naive pen mates were given oxytocin or a placebo.

4.1. Emotional contagion without a possible effect of intranasal oxytocin

During the treatments, training pigs showed many behavioral differences. The design of the positive and negative treatment was (partly) based on other studies (see Reimert et al., 2013) and the behaviors displayed in both treatments indeed showed that the positive treatment elicited a positive emotional state in the training pigs and the negative treatment a negative emotional state (e.g. Boissy et al., 2007; Manteuffel et al., 2007; Mendl and Paul, 2004; Newberry et al., 1988 and see also Reimert et al., 2013). This was not only true for the situation without, but also for the situation with two of their pen mates present in the neutral compartment of the test room. However, training pigs stood alert more, but had their ears back less frequently, were less likely to urinate and seemed to try to escape the compartment less during the negative treatment with two of their naive pen mates present than without their presence. These differences suggest that the training pigs were overall less negatively affected by the negative treatment when their pen mates were present in the neutral compartment, which could indicate that the training pigs took the presence of their pen mates as social support (Reimert et al., 2013, 2014). On the other hand, training pigs wagged their tails less during the positive treatment with two of their naive pen mates present than without their presence (see Reimert et al., 2013 for a discussion on the tail posture tail in curl). The presence of their pen mates might have made the training pigs more vigilant during the positive treatment (percentage of time standing alert was also increased in this situation, although not significantly so) which resulted therefore in less tail wagging. The experiment was set up for naive pen mates to become affected by the emotional state of the training pigs, but training pigs may just as well respond to their naive pen mates. Whether it was an actual emotional state of the pen mates (see below) or just their presence that caused these changes in the behavior of the training pigs can, however, not be elucidated from these results.

The naive pen mates of the training pigs did not behave differently when hearing either Bach or a military march which indicates that these cues in themselves did not have an effect on the behavior of the naive pen mates. In addition, it also indicates that any differences seen in their behavior in the situation when training pigs were present in either the positive or negative treatment compartment is likely due to the (emotional state of) the training pigs. During both treatments, naive pigs spent more time exploring the door of the compartment that held the training pigs. This probably indicated that the naive pen mates realized other pigs were present behind the door and wanted to investigate that, but these behaviors do not necessarily indicate that the naive pigs were emotionally affected by the training pigs. The naive pen mates also spent time more standing alert and had their ears more backwards during the negative treatment of the training pigs than during the positive treatment of the training pigs or without training pigs present in the test room. As standing alert behavior and ears back have been associated with a negative emotional state (Boissy, 1995; Boissy et al., 2011; Paul et al., 2005; Tate et al., 2006), the naive pen mates were thus, just as the training pigs, likely in a negative emotional state during the negative treatment of the training pigs and that suggests that emotional contagion had occurred in this negative situation. Some caution is warranted, however, because the other behaviors expressed by the naive pen mates do not indicate that emotional contagion had occurred and something other than the (emotional state of) the training pigs could also have caused the differences in standing alert behavior and ears backwards (Edgar et al., 2012a). In our previous study, the naive pen mates played during the positive treatment of their trained pen mates, but not during the negative treatment experienced by the training pigs. That the naive pigs did not play in the neutral compartment in the present study could have been due to their somewhat negative emotional state during testing. Their frequencies of urinating and defecating, for instance, are in all three situations comparable to the frequencies of the training pigs during the negative treatment and these behaviors have been associated with a negative emotional state (Mendl et al., 1997; Mendl and Paul, 2004). The naive pigs already displayed this negative emotional state on the first day of habituation. Thus, they evaluated the test room as unpleasant already on the first day and persisted in that evaluation until the end of the experiment. In our previous study, naive pigs were also habituated to the test room, but only for 20 s (i.e. length of the anticipation period) at a time which was perhaps too short to evaluate the test room as negative or positive for that matter. It is not clear, however, why the naive pigs of the present study experienced the first and subsequent habituation trials as negative. Nevertheless, the results of this study, although subtle, do provide evidence for emotional contagion in pigs.

4.2. Oxytocin and emotional contagion

Similar to the situation without intranasal oxytocin administration, the naive pen mates also spent more time standing alert and had their ears backwards more frequently during the negative treatment of the training pigs than during the positive treatment of the training pigs. As the behavior of the training pigs indicates that they were still in a negative emotional state during the negative treatment (see below), these results suggest that emotional contagion had occurred. No effect of oxytocin was found on the behavior of the control pigs, suggesting that any effect of oxytocin on the behavior of the naive pigs is likely due to the (emotional state of the) training pigs. However, oxytocin did not seem to have an effect on the behaviors of the naive pigs and subsequently also not on their emotional state. This was not as expected. At present, we do not have an explanation for this. The dose used and the time period between administration and testing have been used frequently by other studies, including a pig study, where clear effects of oxytocin on (emotional) behavior were found (Churchland and Winkielman, 2012; MacDonald and MacDonald, 2010; Rault et al., 2013; Zink and Meyer-Lindenberg, 2012). Moreover, comparing the behaviors of the control pigs with the behaviors of the naive pigs suggests that control and naive pigs were in a similar emotional state. A lack of an effect of oxytocin on the behaviors of the naive pigs during the positive and negative treatment of the training pigs seems therefore also not due to the emotional state of the naive pigs themselves. In this study, female pigs were used because it has been suggested that females respond more strongly to oxytocin (Kubzansky et al., 2009; Rault et al., 2013) and because females are proposed to be more empathetic (e.g. Baron-Cohen and Wheelwright, 2004; Rueckert and Naybar, 2008; Schulte-Rüther et al., 2008). Whether our results would have been as expected if male pigs were used cannot be concluded from the present study, but is interesting for future research, especially because effects of oxytocin on human social behavior have been studied mostly in male subjects (MacDonald and MacDonald, 2010; Zink and Meyer-Lindenberg, 2012).

Training pigs were now only subjected to either the positive or negative treatment, but their behavior remained fairly consistent with before. Training pigs still only played during the positive treatment and wagged their tails more during the positive treatment than during the negative treatment, whereas training pigs spent more time standing alert and were more likely to urinate and defecate during the negative treatment than during the positive treatment. These differences indicate that training pigs continued to value the positive treatment as positive and the negative treatment as negative. Surprisingly, effects of oxytocin given to the naive pigs were found on the behavior of the training pigs which had not received oxytocin or a placebo themselves. Trainings pigs were namely less likely to defecate during the negative treatment when their naive pen mates had received oxytocin than during the same treatment when their naive pen mates had received a placebo. Moreover, training pigs explored the door of the treatment compartment more during the positive treatment when their naive pen mates had received oxytocin than during the same treatment when their naive pen mates had received a placebo. However, training pigs also explored the door more during the negative treatment when their naive pen mates had received a placebo. Moreover, training pigs had their tails less frequently in the low posture during the positive treatment when their naive pen mates had received oxytocin as compared with a placebo, although this did not differ from the percentage of time tail low during the negative treatment with or without oxytocin given to the naive pen mates. These effects of oxytocin on the behavior of the training pigs could be explained if oxytocin has had an effect on the naive pigs which subsequently influenced the training pigs. At present, we can only speculate what this effect was, because we apparently were not able to measure it. Naive and training pigs could not see each other, so perhaps the administration of exogenous oxytocin stimulated the release of endogenous oxytocin (Churchland and Winkielman, 2012; Uvnäs-Moberg and Petersson, 2005) which subsequently affected the naive pigs' vocalizations (Seltzer et al., 2010) or pheromone production (Ågren and Lundeberg, 2002; Sanchez-Andrade and Kendrick, 2009). In the present study, vocalizations were scored, but not per individual pig and thus we cannot say whether oxytocin had an effect on the vocalizations of the naive pigs during the positive or negative treatment of the training pigs. However, oxytocin was found to increase the number of low-pitched vocalizations in the emotional contagion test situation which does suggest that vocalizations could underlie the effect of oxytocin on the behavior of the training pigs. Apart from some exceptions (see Ågren and Lundeberg, 2002), effects of oxytocin are to the authors' knowledge only studied in the individuals that were also treated with oxytocin. Inter-individual effects of oxytocin merit, therefore, further research.

5. Conclusions

In contrast to two earlier pig studies, the results of this study do provide evidence for emotional contagion in pigs, especially during a negative situation. Surprisingly, oxytocin had no effect on the behavior of the pigs which were given an intranasal administration of oxytocin, but did have some effects on the behavior of other pigs which were not treated with oxytocin. This suggests a role for oxytocin in auditory or olfactory communication between pigs as the oxytocin treated pigs and the other pigs could not see each other.

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CHAPTER 7

Social support in pigs with different coping styles

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Abstract

The presence of a conspecific during a stressful situation, i.e. social support, can considerably lower the stress response of an individual compared to experiencing the stressful situation alone. Pigs also benefit from social support, but it is not known whether the extent to which they benefit is dependent on their personality or coping style. In this study, therefore, the effect of social support on behavioral and physiological (i.e. salivary cortisol, heart rate and the heart rate variability parameters standard deviation (SDNN) and root mean square of successive differences (RMSSD)) stress responses was studied in pigs with different coping styles. Based on the backtest, piglets were classified as high-resisting (HR) or low-resisting (LR). At 11 weeks of age, 12 gilts of each coping style were subjected to a 15 min restraint test in a weighing cage without a pen mate present (i.e. no support treatment) and 12 other gilts of each coping style were subjected to this test with a pen mate, a boar with the same coping style, present (i.e. support treatment). With the pen mate present, LR gilts showed less standing alert behavior and they had their ears back less often than without the pen mate present. On the other hand, HR gilts seemed to spend less time on escaping the cage and more HR gilts seemed to urinate in the situation when the pen mate was present than without the pen mate present, but this was not significant. Independently of the test situation, HR gilts grunted more than LR gilts and they were more likely to urinate than LR gilts. Salivary cortisol concentrations were not affected by treatment or coping style, but were increased at 30 min after the start of the test after which concentrations decreased again to starting levels. The heart rate and heart rate variability parameters RMSSD and RMSSD/SDNN ratio were not affected by treatment or coping style, but the SDNN was lower in the LR pigs during the first and last 5 min of the test when a pen mate was present than without the pen mate present. In addition, heart rate and heart rate variability were increased during the test compared to before and after the test. No differences in behavior and salivary cortisol concentrations were found between the HR and LR accompanying boars. Although effects of social support were not found on all variables measured, the results do indicate that pigs may benefit from social support during a stressful situation and that pigs with a low-resisting coping style will likely benefit more than pigs with a high-resisting coping style.

Key words: behavior, coping style, cortisol, heart rate variability, pigs, social support

1. Introduction

The main advantage of living in a group is probably the protection against predators and other environmental threats (Kikusui et al., 2006; Estevez et al., 2007), but there are many other potential advantages as well (described in Estevez et al., 2007). One of these other advantages is social support. Social support or social buffering is defined as the ability of one (or more) social partner(s) to lower the stress response of an individual that is subjected to a stressful experience (Kikusui et al., 2006; Hennessy et al., 2009). To date, the positive effects of social support on (neuro)physiological, behavioral and immunological responses to stress are evident (reviewed by Uchino et al., 1996; DeVries et al., 2003; Kikusui et al., 2006; Hennessy et al., 2009; Rault et al., 2012). Squirrel monkeys, for instance, vocalize less and are less agitated when confronted with a snake in pairs compared to being confronted with a snake alone (Coe et al., 1982). Furthermore, the cortisol response of adult female guinea pigs to a novel environment test was lowered in the presence of a cage mate (Hennessy et al., 2008). Social support has been described in a wide range of species including humans, non-human primates, rodents, dogs, farm animals and even zebra finches (reviewed by Kikusui et al., 2006; Hennessy et al., 2009; Rault et al., 2012). The mechanism through which social support can lower stress responses is part of ongoing research, but a major role for oxytocin has been proposed as well as an involvement of opioids (Kikusui et al., 2006; Hennessy et al., 2009; Rault et al., 2012). Factors such as familiarity, age and the emotional state of the giver of social support have been found to mediate its effectiveness (Kikusui et al., 2006; Hennessy et al., 2009; Rault et al., 2012). Another factor that might influence the effectiveness of social support is the personality or coping style of both the giver and receiver. In a growing number of species, two extreme types of personalities or coping styles have been found: proactive and reactive (reviewed by Carere et al., 2010; Coppens et al., 2010; Koolhaas et al., 2010). Proactive rats are, for instance, rats that in response to brief contact with an electric probe actively bury the probe with bedding material, whereas reactive rats respond by avoiding the probe and remaining immobile (Koolhaas et al., 2010). Research has shown that the differences between proactive and reactive animals could stem from a difference in cue dependency: proactive animals act primarily on the basis of previous experience and more easily develop routines, whereas reactive animals act primarily to actual environmental information (Koolhaas et al., 2010).

In pigs, similar coping styles have been distinguished based on their response in a backtest at young age (e.g. Hessing et al., 1994; Ruis et al., 2001a; Geverink et al., 2002; Bolhuis et al., 2003; Jansen et al., 2009). During the backtest, a piglet is put on its back and manually restrained for one min. Piglets that struggle and vocalize relatively much in this test have been classified as high-resisting (HR) or proactive piglets and piglets that hardly struggle and vocalize have been classified as low-resisting (LR) or reactive piglets. In line with findings on coping styles in other species, HR pigs also seem to act primarily on the basis of previous experience and form routines, whereas LR pigs more readily respond to a change in the environment (Bolhuis et al., 2004, 2005). Pigs have also been suggested to benefit from social support during stressful experiences (Fraser, 1974; Arnone and Dantzer, 1980; Geverink et al., 1998; Ruis et al., 2001b; Hameister et al., 2012) and not only when the support was given by a conspecific, but by humans as well (Bolhuis et al., 2006). In these studies and in other social support studies, the coping style was, to our knowledge, not taken into account, while this could be relevant because of the difference in use of environmental cues between both coping styles.

The aim of this study was, therefore, to investigate whether HR (proactive) and LR (reactive) pigs differ in social support. We hypothesized that reactive pigs might benefit more from social support than proactive pigs, because reactive animals are more attentive to cues (such as the presence of a conspecific) from the environment and, hence, could use this cue in order to feel less stressed. Reactive pigs might also benefit more from social support, because it has been found that within a cichlid colony reactive cichlids maintain more affiliative relationships than proactive cichlids (Schürch et al., 2010). To test this hypothesis, pigs were subjected to a backtest at a young age and later subjected to a 15 min restraint test in a weighing cage (Bolhuis et al., 2006) with and without a pen mate present. During this test, behavioral indicators of stress such as escape and standing alert behavior (Boissy, 1995; Ramos et al., 1998; Mendl and Paul, 2004; Paul et al., 2005), high-pitched vocalizations (e.g. Manteuffel et al., 2007; Düpjan et al., 2008), defecations (Mendl et al., 1997; Ramos et al., 1998; Mendl and Paul, 2004) and a backwards ear posture (Boissy et al., 2011; Reimert et al., 2013a) were recorded and salivary cortisol was measured at various time points to have an indication of the pigs' state of arousal (Paul et al., 2005). Furthermore, the restrained pig was equipped with a heart rate belt to measure its heart rate and heart rate variability (Von Borell et al., 2007).

2. Materials and methods

2.1. Animals and housing

For this study a total of 72 Tempo x Camborough pigs, equally divided over two rounds, were used. These pigs were selected from a larger pool of pigs which had previously, before weaning, participated in another experiment (for details see Oostindjer et al., 2011). Selection was based on health, weight, sex and backtest classification (see section 2.2.). Pigs were weaned at four weeks of age and from five weeks of age housed in 12 groups of six unrelated pigs in 8.3 m^2 pens. Each group was composed of two HR and two LR gilts and one HR and one LR boar. Pigs could be identified by an ear tag and a number sprayed on their backs. Food (a standard commercial diet for growing pigs) and water were available ad libitum. Lights were on from 7 am to 7 pm. The floors of the pens were covered with wood shavings and straw and this was cleaned daily. The study was approved by the Animal Care and Use Committee of Wageningen University.

2.2. Backtest

At the age of 10 days, piglets were subjected to a backtest to assess their coping style (Bolhuis et al., 2003). In short, a piglet was put on its back and manually restrained for 60 s. During the test, the number of struggles, the latency to struggle, the number of vocalizations and the latency to vocalize were recorded. Piglets were selected and classified as high-resister (HR) when they struggled at least three times and classified as low-resister (LR) when they did not struggle or struggled once. Five piglets that struggled two times had to be selected as well to complete the experimental design. These piglets were classified as either HR or LR based on the other variables recorded (i.e. piglets with latencies under 40 s were classified as HR and with latencies above 40 s as LR) (Melotti et al., 2011).

2.3. Habituation period

In the five weeks before the social support test was carried out, pigs were familiarized with the experimenters and with chewing on cotton buds for saliva sampling (section 2.6.). Furthermore, the gilts of each pen were habituated to wearing a heart rate belt (section 2.7.) and being separated from their pen mates by a wire partition (1 m high and 2.7 m long, 5 x 5 cm openings interspersed with 7 mm wire) placed in their home pen, allowing visual, auditory and snout contact.

In order to avoid novelty stress of the test room (Fig. 1) during the actual social support test (section 2.4.), all pigs were also brought to the test room twice for a period of 5 and 7.5 min, respectively. The weighing cage (section 2.4.) was not placed in the test room during habituation.

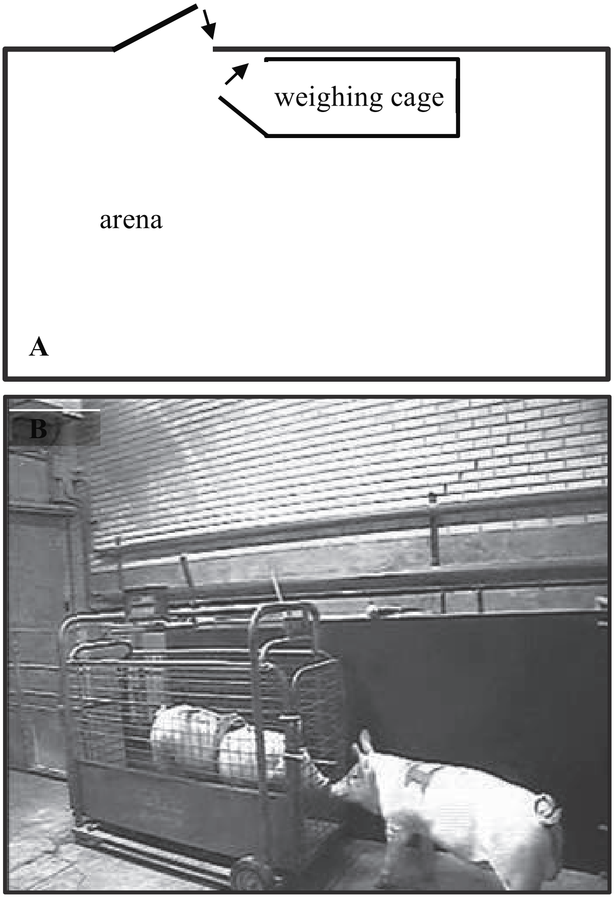

Fig. 1. A schematic drawing of the test arena and the position of the weighing cage (A) and a photo of the 15 min restraint test with a pig in the weighing cage and a pen mate present in the arena (B).

2.4. Social support test

At 11 weeks of age, the social support test was carried out. In this test a gilt was placed in a weighing cage for a first time and subjected to 15 min restraint in this cage which is a stressful event for pigs (Bolhuis et al., 2006). The weighing cage (inside: 1.4 x 0.5 x 1 m (l x w x h)) was placed in an arena (4.4 x 2.4 x 1 m (l x w x h)) (Fig. 1A) located in a test room at least 10 m from where the pigs were housed.

The social support test was performed four times for each pen: one of the gilts of each backtest classification was subjected to the restraint test without a pen mate present (from here on referred to as the no support treatment) and the other gilt was accompanied by a pen mate (i.e. a boar) with the same backtest classification (from here on referred to as the support treatment). The social support tests were carried out over four consecutive days with six tests, one test per pen, per day and in an order balanced for backtest classification and support condition. A test day started at app. 9 am and ended at app. 1.30 pm.

For a test, one or both pigs of a pen, depending on the test situation (i.e. support or no support treatment), was or were brought to the arena and the gilt was placed in the weighing cage which was then closed tightly (Fig. 1B). In case the accompanying boar was also brought along, he was allowed to move freely in the arena (Fig. 1B). Thereafter, the door of the arena was closed and the experimenters left the test room after which the test started. After 15 min, the pig was or the pigs were brought back to the home pen. Microphones and cameras were placed in the test room in such a way that video and audio recordings of both pigs could be made for subsequent analyses.

2.5. Behavioral analyses

The ethogram in Table 1 was used to score the behaviors and vocalizations of the restrained and accompanying pigs. The behaviors in the ethogram were scored from the video recordings in three mutually exclusive classes using focal sampling and continuous recording with the Observer XT 10.1 software (Noldus Information Technology B.V., Wageningen, The Netherlands) and the vocalizations were scored from the audio recordings using the Observer XT 5.0 software (Noldus Information Technology B.V., Wageningen, The Netherlands). The scoring of the behaviors was performed separately for the restrained pig and the accompanying pig. In the support treatment, the vocalizations were scored as a total of the two pigs, because it was not possible to distinguish which pig was vocalizing.

Table 1
Ethogram used to score the behaviors of the restrained and accompanying pigs during the social support test. Behaviors were scored as states, except the behaviors defecating, urinating and vocalizations which were scored as events.

Behavior	Description
Postures and locomotion	
Standing	Standing with all four paws on the floor
Standing alert	Standing motionless with head fixed (up or down) and ears upright
Lying/sitting	Sitting, kneeling or lying (on side or belly) on the floor with eyes open or closed
Walking	Moving in a forward or backward direction
Escaping	Jumping against the cage or trying to get out by showing digging-like behavior
Behaviors	
Exploring cage floor[1]	Sniffing, nosing or rooting the floor of the weighing cage
Exploring cage walls	Sniffing, nosing or rooting the wall (in- or outside) of the weighing cage
Exploring arena[2]	Sniffing, nosing or rooting the floor or walls of the arena
Exploring arena door[3]	Sniffing, nosing or rooting the exit door of the arena
Social contact[3]	Touching or sniffing any part of the other pig
Defecating	Defecating
Urinating	Urinating
Ears and tail postures	
Ears back	One or both ears directed backwards
Tail in curl	Tail coiled up in a curl on top of the body
Tail wagging	Tail swinging in any direction, but mostly from side to side
Tail low	Tail hanging down against the body
Vocalizations	
Low-pitched vocalizations	Short or long grunts
High-pitched vocalizations	Screams, squeals or grunt-squeals

[1] This behavior was only scored for the restrained pig.
[2] These behaviors were only scored for the accompanying pigs.
[3] This behavior was only scored in those tests where both the restrained pig and accompanying pig were present.

2.6. Saliva collection and cortisol analysis

Saliva samples were collected in the home pen from the test pig(s) before (t = -30), just before (t = 0), directly after the test (t = 15) and 30, 45 and 60 min after the start of the test by allowing the pig(s) to chew on cotton buds (VWR International, Amsterdam, The Netherlands) until the buds were thoroughly moistened. The cotton buds were placed in test tubes (Sarstedt, Etten-Leur, The Netherlands) and stored on ice. At the end of the test day, tubes were centrifuged for 10 min at 2000 g to get the saliva from the cotton buds and the saliva was stored at -20 ºC until further analysis. Cortisol concentration (ng/ml) was measured in duplicate using a radioimmunoassay kit (COAT-A-COUNT®, Siemens Healthcare Diagnostics, Los Angeles, USA) which has been modified and validated for pigs (Ruis et al., 1997). All samples were analyzed in one assay (Intra-Assay CV was 5.0 %).

2.7. Heart rate and heart rate variability (HRV)

The heart rate of the pigs was measured non-invasively with the telemetric system Polar S810i (Polar Elektro Öy, Finland). It consisted of a flexible chest belt integrated with two electrodes and a radio transmitter for wireless data transmission, a data receiver and data logger in the form of a watch, an interface for downloading the data to a computer, and corresponding software (Polar Precision Performance v. 4.03.040). This system has successfully been used in previous studies, including pig studies (e.g. Langbein et al., 2004; Kingsley et al., 2005; Von Borell et al., 2007; Zebunke et al., 2011).

After the first saliva sample (t = -30 min) was obtained from the gilt, the heart rate belt was put on in such a way that the electrodes were placed in the middle of the chest directly behind the fore legs. In order to increase electrical conductivity, electrode gel (AUV Groothandel, Cuijk, The Netherlands) was applied on the belt and the pig beforehand. The watch was placed in a case and the case was fixed onto the belt after which the measurement of the heart rate was started by measuring each consecutive heartbeat. To ensure that the belt and case remained in place, they were fixed to the pig with veterinary wrap (AUV Groothandel, Cuijk, The Netherlands) and tape (AUV Groothandel, Cuijk, The Netherlands) and the test gilt was separated from her pen mates by a wire partition placed in their home pen to avoid chewing on the belt by the pen mates (Von Borell et al., 2007). The gilt (and accompanying boar) was (were) brought to the test room after the second saliva sample (t = 0 min) was obtained. After 15 min, the gilt was placed back behind the wire partition (and the accompanying boar back with its pen mates). The wire partition and belt were removed after the last saliva sample (t = 60 min) was obtained which was also the end of the heart rate measurement.

Each measurement was divided into fifteen 5-min periods (three 5-min periods of basal recordings (B1-B3), three 5-min periods during the test (T1-T3) and nine 5-min periods of recovery after the test (R1-R9)), because a 5-min time window has been proposed as a useful window for HRV analyses (Von Borell et al., 2007). Pigs had to walk from their home pen to the test room and back again after the test. To avoid possible noise from this walking (Von Borell et al., 2007), the last 5-min period of the basal period ended three min before the start of the test and the first 5-min of the recovery period started three min after the end of the test. With the use of the Polar Precision Performance Software, each 5-min period was visibly inspected and based on that inspection it was decided whether it could be used for subsequent analysis. This resulted in excluding the 5-min periods which were full of errors. As HRV parameters are very sensitive to errors in the beat to beat measurement (Berntson and Stowell, 1998; Marchant-Forde et al., 2004), a 5-min period was only included in the HRV analysis if it did not contain three or more consecutive errors classified by Marchant-Forde et al. (2004) and also did not contain more than 5 % of these errors as suggested by Von Borell et al. (2007). Subsequently, the errors in these included 5-min periods were corrected using the Polar Precision Performance Software with standard settings. Thereafter, the average heart rate (in beats per min (bpm)) and the following HRV parameters in the time domain were calculated from the corrected 5-min periods: the root mean square of successive beat to beat interval differences (RMSSD (in ms)) which is an indicator of parasympathetic activity (De Jong et al., 2000; Von Borell et al., 2007), the standard deviation of the mean beat to beat interval (SDNN (in ms)) which is an indicator of sympathetic and parasympathetic activity and, thus, of overall variability (De Jong et al., 2000; Von Borell et al., 2007), and the RMSSD/SDNN ratio which is an indicator of the balance between sympathetic and parasympathetic activity (Langbein et al., 2004).

2.8. Statistical analyses

SAS (SAS 9.2, SAS Institute Inc.) was used for statistical analyses. The behaviors in Table 1 that were recorded as states were expressed as percentages of time and the behaviors that were scored as events were expressed as absolute frequencies. The data of the restrained and accompanying pigs were analyzed separately. Preliminary analyses showed no effects of testing order (i.e. order in which the tests were performed on a day) or day of testing. These factors were, therefore, not included in the final models. Results are presented as means ± SEM.

2.8.1. Restrained pigs

During the 15 min test, the tail of the restrained pigs was in a curl for almost the entire test (overall mean 99.98 ± 0.02 % of time) and tail low did not occur. The different tail postures tail in curl, tail wagging and tail low, were, therefore, not statistically analyzed. The behaviors exploring cage floor and exploring cage walls were summed into a new behavior, i.e. exploring cage. The effect of treatment (no support or support), backtest classification (HR or LR) and their interaction on the behaviors of the restrained pigs were analyzed with a mixed linear model with treatment, backtest classification, their interaction and round as fixed effects and pen, nested within round (two rounds), as random effect. Significant interactions were further investigated with post hoc pairwise comparisons using the differences of the least square means. Urinating during the 15 min restrain test was analyzed as a 0/1 trait using a generalized linear mixed model with a logit link and binary distribution and with the same fixed and random effects as above. The behavior social contact could only occur during the support treatment. This behavior was, therefore, analyzed with the same model used for the other behaviors, but excluding the fixed effect of treatment. The low- and high-pitched vocalizations were analyzed separately for each treatment, because in the support treatment the vocalizations were scored as a total of the two pigs compared to one pig in the no support treatment. The model used here was the same as that used for the behavior social contact.

The salivary cortisol concentrations were analyzed with a repeated mixed linear model with the same fixed and random effects as above and including a fixed effect of time (t = -30, 0, 15, 30, 45 and 60 min) and its interactions. Pen nested within round and time and pig nested within pen, round, treatment and backtest classification were included as random effects. Prior to this analysis, the salivary cortisol concentrations were log transformed to obtain normally distributed residuals. One pig had an unusually high concentration of 20.0 ng/ml at t = 15 min. This data point was considered to be an outlier (overall mean ± SEM of all other pigs: 3.1 ± 0.2 ng/ml) and this pig was, therefore, excluded from the cortisol analysis.

For each 5-min period, the heart rate could be calculated from on average 7 pigs per treatment group (i.e. 12 HR gilts tested with a pen mate present, 12 HR gilts tested without a pen mate present, 12 LR gilts tested with a pen mate present and 12 LR gilts tested without a pen mate present) and the HRV parameters could be calculated from on average 5 pigs per treatment group. Even though this meant that we could not use about half of all the 5-min periods, we did analyze and present the remaining results, because they can still give an indication to whether treatment and backtest classification affect heart rate and HRV. The average heart rate and RMSSD, SDNN and RMSSD/SDNN ratio calculated from these 5-min periods were analyzed with the same model as used for the salivary cortisol concentrations, except that the effect of time consisted here of the fifteen 5-min periods. Prior to this analysis, the heart rate, RMSSD and SDNN were log transformed to obtain normally distributed residuals.

2.8.2. Accompanying pigs

Of the 24 accompanying pigs, only two, one HR and one LR, tried to escape from the arena. Escape behavior was, therefore, not statistically analyzed. Furthermore, the tail postures tail in curl, tail wagging and tail low were also not statistically analyzed, because the accompanying pigs had their tail in a curl for most of the 15 min (overall mean 97.3 ± 2.7 % of time). The remaining behaviors of Table 1 were analyzed with the same model as used for the restrained pigs, but excluding the fixed effect of treatment. The percentage of time spent lying/sitting was arcsine square root transformed prior to analysis to obtain normally distributed residuals. The salivary cortisol concentrations were analyzed with the same model as used for the salivary cortisol concentrations of the restrained pigs, but excluding the fixed effect of treatment here as well. In addition, the salivary cortisol concentrations were log transformed prior to analysis to obtain normally distributed residuals.

3. Results

3.1. Restrained pigs

3.1.1. Behavior

The presence of a pen mate affected the behavior of the restrained HR and LR pigs differently. This is, for instance, indicated by the significant interaction between treatment and backtest classification for the time spent standing alert ($F_{1,33} = 5.13$, $P = 0.03$). Post hoc pairwise comparisons showed that LR pigs spent more time standing alert than HR pigs when tested alone and that the presence of the accompanying pen mate profoundly reduced the time spent standing alert by the LR pigs, but not by the HR pigs (Fig. 2A). Moreover, an effect of treatment ($F_{1,33} = 3.60$, $P = 0.07$), backtest classification ($F_{1,33} = 4.32$, $P = 0.046$) and their interaction ($F_{1,33} = 5.10$, $P = 0.03$) was found for the percentage of time ears back. Post hoc pairwise comparisons showed that, similarly to the time spent standing alert, the percentage of time ears back was higher for the LR pigs than for the HR pigs when tested alone and that the presence of the accompanying pen mate substantially reduced the percentage of time ears back in the LR pigs, but not in the HR pigs (Fig. 2B). In contrast, HR pigs seemed to spend more time on escaping the cage than LR pigs when tested alone and the presence of the accompanying pen mate seemed to reduce the time spent escaping by the HR pigs and not by the LR pigs, but this was not significant (interaction between treatment and backtest classification, $P = 0.35$) (Fig. 2C). Furthermore, HR pigs were more likely to urinate than LR pigs ($F_{1,33} = 5.56$, $P = 0.02$) and pigs tended to urinate more during the test when the accompanying pen mate was present ($F_{1,33} = 3.14$, $P = 0.09$) (Fig. 2D). This seemed to be more pronounced for the HR pigs, but this difference was not significant (interaction between treatment and backtest classification, $P = 0.32$). The percentage of time spent lying and sitting (Fig. 2E) and the frequency of defecating (Fig. 2F) were not affected by treatment ($P \geq 0.89$), backtest classification ($P \geq 0.48$) or their interaction ($P \geq 0.25$). In addition, percentage of time spent standing (HR pigs: support: 67.6 ± 5.2 vs. no support: 62.7 ± 7.0, LR pigs: support: 57.0 ± 5.7 vs. no support: 58.9 ± 5.1), walking (HR pigs: 2.3 ± 0.4 vs. 2.9 ± 0.5, LR pigs: 2.0 ± 0.2 vs. 2.5 ± 0.4), and exploring the cage (HR pigs: 28.3 ± 2.3 vs. 31.6 ± 2.1, LR pigs: 28.8 ± 2.6 vs. 29.7 ± 3.9) was also not affected by treatment ($P \geq 0.22$), backtest classification ($P \geq 0.23$) or their interaction ($P \geq 0.56$). Social contact behavior between the restrained and accompanying pig, which could only occur during the support treatment, was also not different between HR and LR pigs (1.9 ± 0.4 vs. 2.2 ± 0.4 % of time, $P = 0.66$). In the support treatment, the vocalizations could not be scored individually for both pigs and were, therefore, analyzed for each treatment separately. Within each treatment, HR pigs produced more low-pitched vocalizations than LR pigs (support: 249.1 ± 14.9 vs. 174.8 ± 24.2, $F_{1,11} = 6.57$, $P = 0.03$, no support: 195.3 ± 21.6 vs. 121.3 ± 19.0, $F_{1,11} = 7.45$, $P = 0.02$), but HR and LR pigs did not differ in the number of high-pitched vocalizations produced (support: 161.3 ± 29.0 vs. 152.3 ± 24.0, $P = 0.74$, no support: 148.1 ± 36.9 vs. 115.0 ± 30.5, $P = 0.50$).

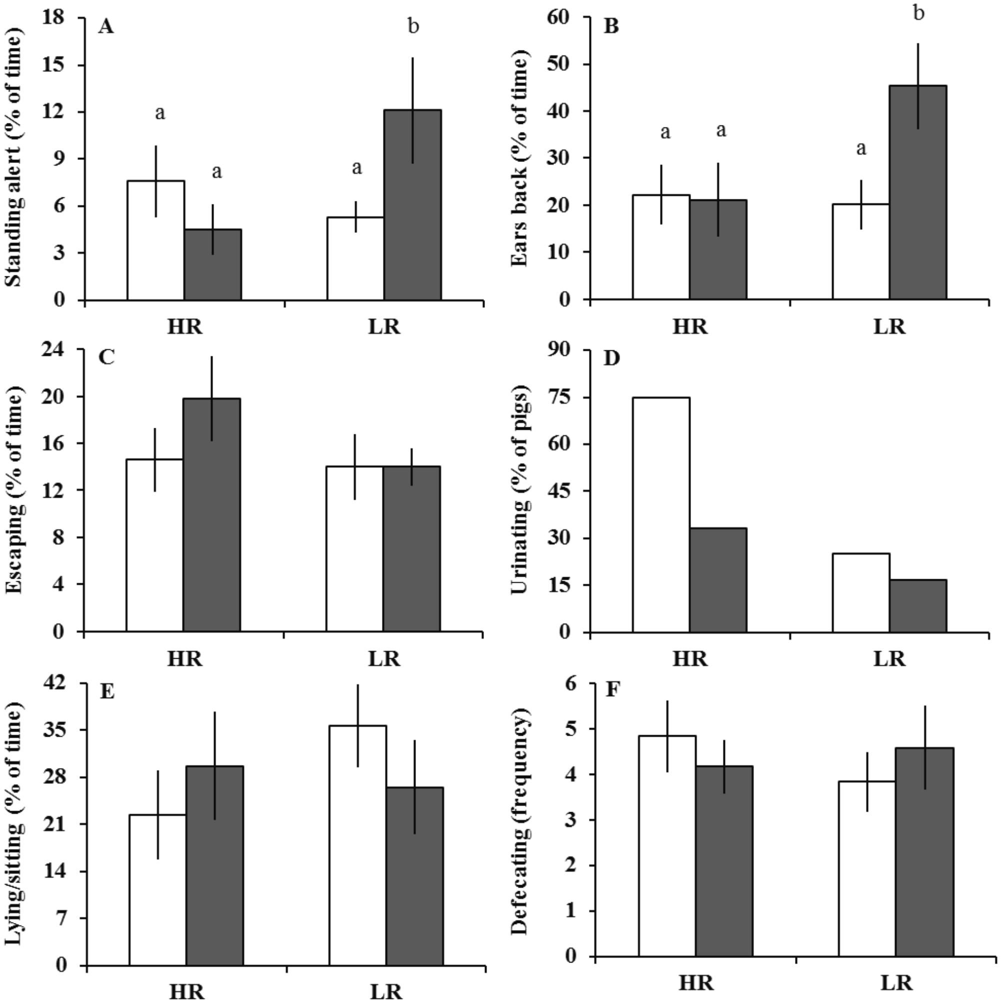

Fig. 2. Behaviors, standing alert (panel A), ears back (panel B), escaping (panel C), urinating (panel D), lying/sitting (panel E) and defecating (panel F), of the restrained pigs with a high-resisting (HR) or low-resisting (LR) backtest classification during the 15 min restraint test and with an accompanying pen mate present (open bars) and without a pen mate present (gray bars). Differences between means are indicated by small letters ($P < 0.05$).

3.1.2. Salivary cortisol

Salivary cortisol concentrations were not affected by treatment (P = 0.89), backtest classification (P = 0.10) or their interaction (P = 0.87) (Fig. 3A and 3B). Salivary cortisol concentrations were, however, affected by time ($F_{5,55}$ = 10.09, P < 0.001). Post hoc pairwise comparisons showed that concentrations increased from t = -30 to t = 30 min after which they decreased again to starting concentrations at t = 60 min (Fig. 3A and 3B). Concentrations were not different between t = 0 and t = 15 min (Fig. 3A and 3B).

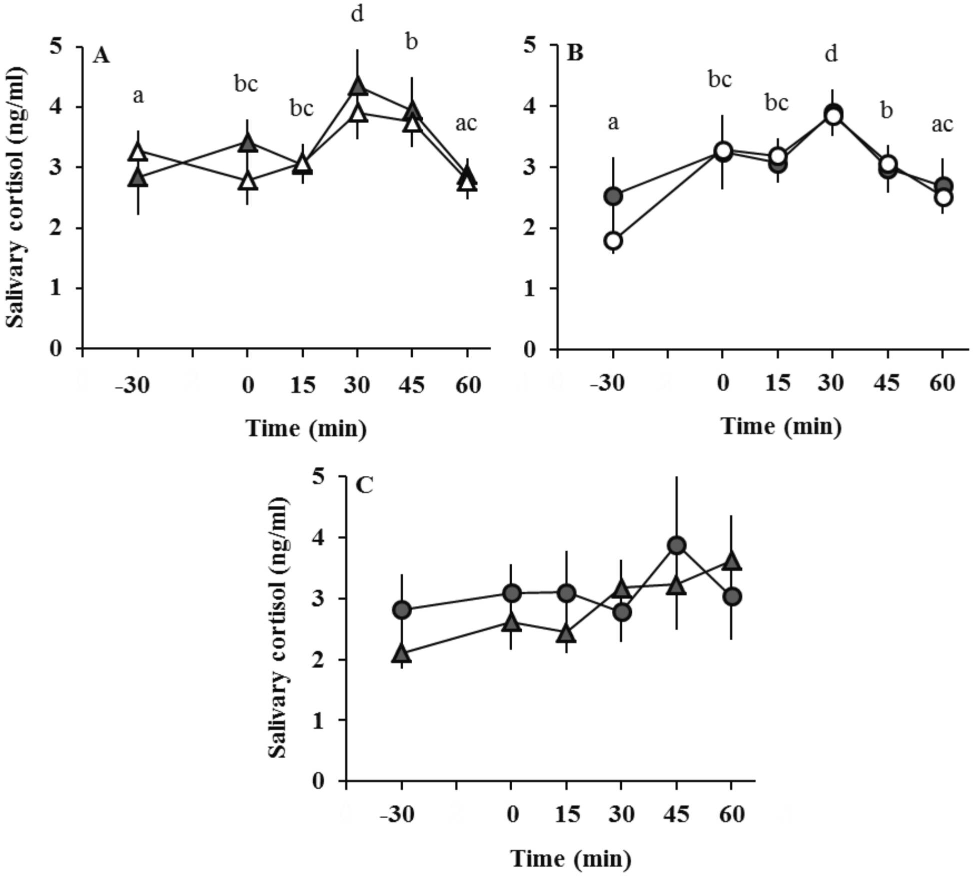

Fig. 3. Salivary cortisol concentrations measured 30 min before, just before (0) and 15, 30, 45 and 60 min after the start of the 15 min restraint test of the restrained pigs with a high-resisting (panel A) or low-resisting backtest classification (panel B) and with an accompanying pen mate present (white symbols) and without a pen mate present (gray symbols). Panel C shows the salivary cortisol concentrations of the accompanying pen mates with a high-resisting (triangles) or low-resisting (circles) backtest classification. Differences between means are indicated by different small letters (P < 0.05).

3.1.3. Heart rate and RMSSD, SDNN and RMSSD/SDNN ratio

The heart rate and RMSSD were not affected by treatment ($P \geq 0.88$), backtest classification ($P \geq 0.23$) or their interaction ($P \geq 0.36$) (Fig. 4A, B, C and D). Time, however, affected both the heart rate ($F_{14,152} = 23.44$, $P < 0.001$) and RMSSD ($F_{14,138} = 19.50$, $P < 0.001$). Post hoc pairwise comparisons showed that the heart rate decreased from B1 to B3 during the basal measurements. The heart rate was increased during the first 5 min of the test, but it decreased thereafter. Furthermore, the heart rate was higher at the beginning of recovery compared to the end of the test period, but decreased thereafter to a heart rate even below basal conditions (Fig. 4A and B). The RMSSD did not differ within the three basal and within the nine recovery periods of 5 min. Measurements were also not different between the basal and recovery period. The RMSSD was, however, higher during the test compared to both the basal and recovery period. The RMSSD was not different between the three 5-min periods of the test (Fig. 4C and D). The SDNN was affected by time ($F_{14,138} = 19.48$, $P < 0.001$) and by the interaction between treatment, backtest classification and time ($F_{14,67} = 2.68$, $P < 0.01$) (Fig. 4E and F). Similarly to the RMSSD, the SDNN was generally higher during the three test measurements compared to the basal and recovery measurements. Within the test period, the LR pigs that underwent the restraint test without an accompanying pen mate present had a higher SDNN during the first and last 5-min period compared to the other treatment groups. Treatment did not affect the SDNN of the LR pigs during the basal and recovery period, but did affect the SDNN of the HR pigs during the basal period. During the basal period, HR pigs that would undergo the restraint test with an accompanying pen mate present had a lower SDNN during the first basal period compared to HR pigs that would undergo the test without a pen mate present. Treatment did not affect the SDNN of the HR pigs during the recovery period (Fig. 4E and F). No effect of treatment, backtest classification, their interaction, time or any other interactions was found on the RMSSD/SDNN ratio ($P \geq 0.11$) (Fig. 4G and H).

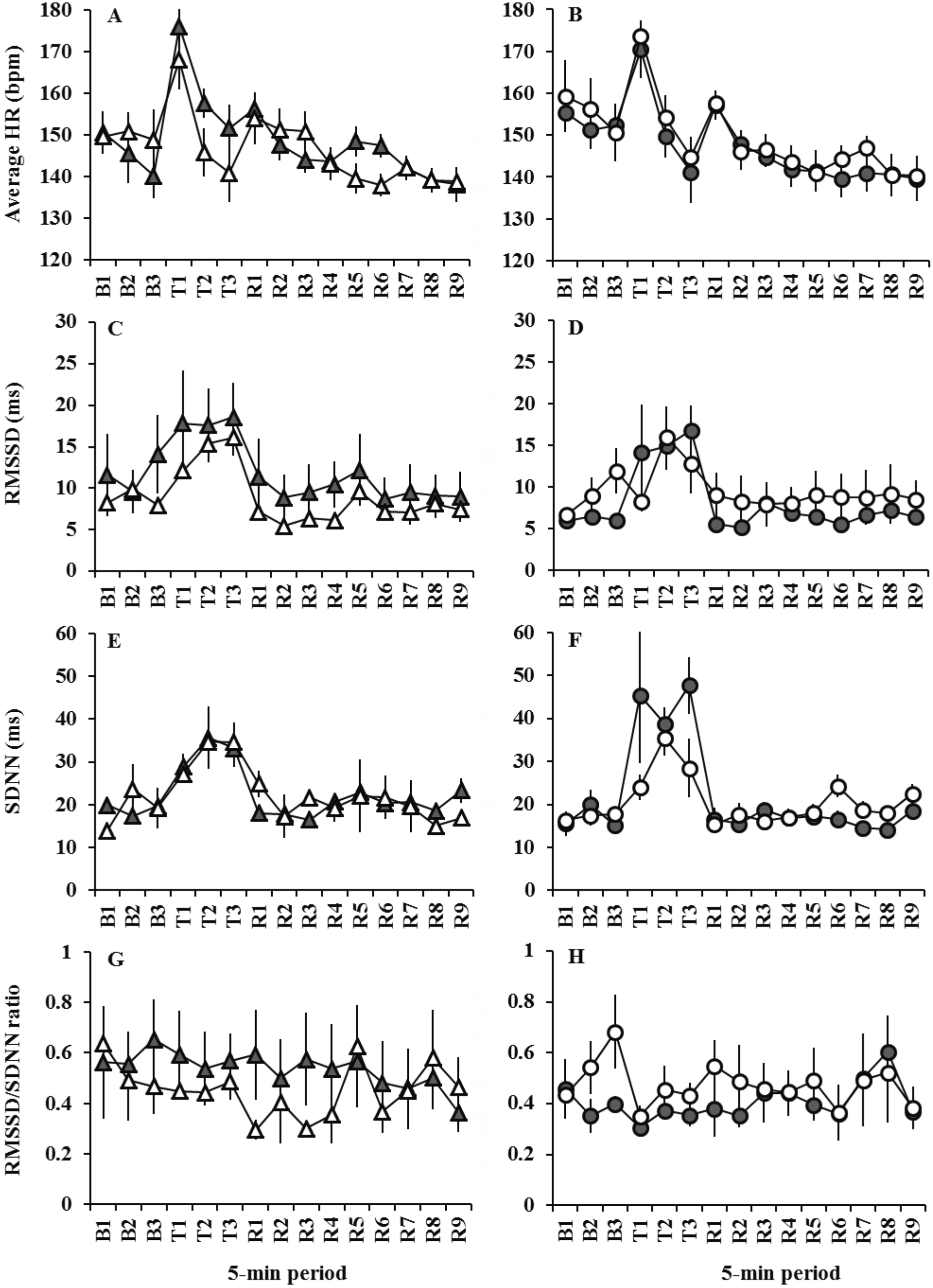

Fig. 4. Average HR (heart rate) (panels A and B) and the heart rate variability parameters RMSSD (root mean square of successive differences) (panels C and D), SDNN (standard deviation) (panels E and F), and the RMSSD/SDNN ratio (panels G and H) measured during 15 min of basal conditions before the test (B1-B3), during the 15 min restraint test (T1-T3) and during 45 min of recovery after the test (R1-R9) of the restrained gilts with a high-resisting (triangles) or low-resisting (circles) backtest classification and with an accompanying pen mate present (white symbols) and without a pen mate present (gray symbols).

3.2. Accompanying pigs

Table 2 shows the behaviors of the accompanying HR and LR pigs during the 15 min test. Backtest classification did not affect their behavior. In addition, the salivary cortisol response of the accompanying pigs was also not affected by backtest classification (P = 0.91), time (P = 0.69) or the interaction between backtest classification and time (P = 0.30) (Fig. 3C).

Table 2
Means and SEM of the behaviors of the accompanying pigs with a high-resisting (HR) or low-resisting (LR) backtest classification

Behavior (% of time)[1]	HR pigs	LR pigs	P-value
Postures and locomotion			
Standing	55.6 ± 2.5	56.3 ± 3.3	0.85
Standing alert	4.7 ± 1.1	3.5 ± 1.1	0.51
Lying/sitting	3.3 ± 2.4	3.4 ± 2.1	0.77
Walking	36.5 ± 2.8	36.8 ± 2.7	0.94
Behaviors			
Exploring cage walls	22.4 ± 2.5	23.9 ± 2.5	0.62
Exploring arena	29.0 ± 3.0	29.5 ± 2.5	0.89
Exploring arena door	2.0 ± 0.4	2.0 ± 0.5	0.95
Social contact	0.5 ± 0.2	0.7 ± 0.2	0.34
Defecating *(frequency)*	8.3 ± 1.0	8.4 ± 0.9	0.85
Urinating *(% of pigs)*	16.7	8.3	0.50
Ear posture			
Ears back	15.4 ± 4.5	14.6 ± 2.7	0.82

[1] Behaviors are presented as percentages of time unless indicated otherwise.

4. Discussion

In this study, we investigated the effect of social support, i.e. the presence of a pen mate, on the behavior, salivary cortisol and heart rate (variability) response of pigs with diverging coping styles (i.e. high-resisting (HR) or low-resisting (LR)) during a 15 min restraint test in a weighing cage. The pig's coping style was assessed with a backtest at a young age.

Social support has been defined as the ability of one (or more) social partner(s) to lower the stress response of an individual that is subjected to a stressful situation (Kikusui et al., 2006; Hennessy et al., 2009). In this study, 15 min restraint in a weighing cage was chosen as a stressful situation, because it was shown to be stressful in a previous study (Bolhuis et al., 2006). During the 15 min restraint test, pigs showed behaviors such as escape and standing alert behavior. Pigs also produced many high-pitched vocalizations, defecated and positioned their ears backwards relatively much. Furthermore, pigs were also aroused by the test indicated by their salivary cortisol and heart rate responses. Together, these results indicate that the pigs experienced the restraint test indeed as stressful (Boissy, 1995; Mendl et al., 1997; Ramos et al., 1998; Mendl and Paul, 2004; Paul et al., 2005; Manteuffel et al., 2007; Mormède et al., 2007; Düpjan et al., 2008; Boissy et al., 2011; Reimert et al., 2013a). To be able to give social support, the accompanying pen mate should not be stressed (Rault, 2011). The behavior displayed by and the physiological response of the accompanying pen mates showed that they were hardly stressed by the situation. This is also supported by the lack of an effect of coping style on their behavior, because differences in coping with a situation have been suggested to emerge only when that situation is sufficiently stressful (Koolhaas et al., 2010; Reimert et al., 2013b). The accompanying pen mates were, thus, not hindered by stressful conditions to give support. We hypothesized that LR pigs would benefit more from social support than HR pigs, because LR pigs are more attentive to environmental cues than HR pigs and, thus, could actually use the presence of the pen mate to feel less stressed. The results showed that LR pigs indeed spent less time standing alert and had their ears back less often during the 15 min restraint test with an accompanying pen mate present than without a pen mate present. On the other hand, HR pigs seemed to spend less time on escape behavior and seemed to urinate more with an accompanying pen mate present than without a pen mate present, but this was not significant. The vocalizations could, unfortunately, not be scored for each pig individually and therefore we cannot discuss whether the presence of a pen mate affected the number of low- and high-pitched vocalizations. Nevertheless, the effect of coping style could be analyzed and the results showed that in both test situations HR pigs produced more low-pitched vocalizations than LR pigs. Although the results are modest, they do support our hypothesis and indicate that it might be relevant to take personality or coping style into account in future studies on social support. Moreover, the behavioral differences between the HR and LR pigs are partly in line with other studies. Jansen et al. (2009) and Bolhuis and Schouten (2002) found, for instance, that LR pigs also showed more standing alert in response to a stressful situation compared to HR pigs, whereas HR pigs responded with more escape behavior (Bolhuis and Schouten, 2002) and more vocalizations (Ruis et al., 2001a; Bolhuis and Schouten 2002; Geverink et al., 2002; Jansen et al., 2009; Reimert et al., 2014). Pigs with a HR coping style, thus, show a more (pro)active response to a stressful situation, whereas pigs with a LR coping style show a more passive or reactive response, which is in line with coping style studies in other animals (Carere et al., 2010; Koolhaas et al., 2010). To our knowledge, the found differences in ear posture and urinating between HR and LR pigs have not been reported before. The posture of the ears could be regarded as a more reactive behavior and pigs may use their ears to pick up more cues from the environment (Manteuffel, 2006). If so, the difference found in ears back between both treatments for the LR pigs, but not for the HR pigs, fits well within the existing differences between HR and LR pigs. Several studies proposed that proactive animals (i.e. HR pigs) have a higher sympathetic reactivity in response to stress, whereas reactive animals (i.e. LR pigs) were thought to have higher reactivity of the HPA-axis (e.g. Hessing et al., 1994; Koolhaas et al., 1999; Ruis et al., 2001a). This could explain the difference in urinating between the HR and LR pigs, because stress induced urinating has been suggested to be under the control of the sympathetic nervous system (Hall, 1934). There are, however, also studies that do not find a difference between HR and LR pigs in the reactivity of these systems (e.g. Geverink et al., 2002, Reimert et al., 2014; this study) which indicates that the relationship between coping style and these systems is not yet clear (Coppens et al., 2010; Koolhaas et al., 2010). At present, we, therefore, cannot explain the difference in urinating between HR and LR pigs.

In addition to behavior, we also measured salivary cortisol and heart rate (variability) (HRV). Salivary cortisol was not affected by treatment or coping style. As already explained above, the (re)activity of the HPA-axis and, thus, cortisol, does not necessarily have to do differ between proactive and reactive animals despite earlier findings. Compared to the basal and recovery period, the average heart rate was only elevated during the first 5 min of the restraint test, whereas the RMSSD and the SDNN were elevated during the entire 15 min of the test. The RMSSD/SDNN ratio was, however, not affected by the test. During scoring of the video recordings it was observed that pigs were most active at the beginning of the test which might explain the increased heart rate during the first 5 min only. The increase in RMSSD and SDNN with no increase in the RMSSD/SDNN ratio during the entire test indicates that both the sympathetic and parasympathetic branches of the autonomic nervous system were activated without dominance of one over the other (Zebunke et al., 2011). Activation of both branches seems odd, because during stress and physical activity the sympathetic branch normally dominates the parasympathetic branch (Von Borell et al., 2007; Lay Jr et al., 2009) and in another study, the RMSSD and SDNN were actually reduced during stress (Mohr et al., 2002). Zebunke et al. (2011, 2013), however, interpreted an increase in both the heart rate and SDNN as indicators of arousal and, at the same time, an increased RMSSD as an indicator of positive affect or orientation. Considering the behaviors of the pigs in this study, they were not in a positive emotional state during the test, but being in a state of arousal and oriented to their surroundings is likely. In this study, treatment and coping style effects were not found on the heart rate, RMSSD and RMSSD/SDNN ratio, but the SDNN of the LR pigs tested alone was higher in the first and last 5 min of the test compared to the SDNN of the LR pigs tested with a pen mate present and to the SDNN of the HR pigs tested either alone or with a pen mate present. If the SDNN is an indicator of arousal, the LR pigs could have been less aroused during the test when a pen mate was present than when tested alone, which in line with the behavioral results indeed indicates that LR pigs had benefited more than HR pigs from social support. Studies that investigated differences between proactive and reactive animals on HRV parameters are, however, scarce (Von Borell et al., 2007) and inconsistent. For instance, Korte et al. (1998) found that reactive laying hens had a higher RMSSD than proactive laying hens in response to a restraint test, whereas Ruis et al. (2001a) found that proactive pigs had a higher RMSSD than reactive pigs in response to a novel environment test and novel object test. In addition, in another study by Ruis et al. (2002) the RMSSD did not differ between proactive and reactive pigs, but proactive pigs did have a higher SDNN than reactive pigs in response to a novel object test, but not to a novel environment test. Furthermore, differences in the average heart rate are also not consistent between proactive and reactive pigs (Hessing et al., 1994; Geverink et al., 2002). From these results it may be concluded that these cardiovascular variables are not part of the coping style trait characteristics, but rather seem to reflect the arousal state which in turn depends on the demands of the environment. More research is, however, needed to make this conclusive.

We expected to find more effects of social support on the behavioral and physiological responses of the gilts, because Bolhuis et al. (2006) used the same stress test and they found effects of social support on standing alert and escape behavior, defecating, the salivary cortisol response, the heart rate during the stress test and on the RMSSD during the recovery period. In Bolhuis et al., the tested pigs were individually housed, but were visited daily by a conspecific or person for four weeks before the test took place, whereas in this study the tested pigs were housed in groups. It could be that the tested pigs in the study of Bolhuis et al. really bonded with the visiting pig or person which made social support more effective (Rault, 2012). It is, however, not yet known whether pigs do form strong bonds with particular pigs (Durrell et al., 2004) or persons. In addition, in Hameister et al. (2012) piglets were, just as in our study, housed in groups and they also found more effects of social support than we found, even when support was given by an unfamiliar piglet. Before the restraint test, pigs were physically, but not visually, isolated from their pen mates by a wire partition to avoid chewing on the heart rate belt. This procedure could have made the gilts already stressed which is supported by the increase in salivary cortisol from $t = -30$ min to $t = 0$ min and the increase in heart rate from B1 to B3. Consequently, it could be that the gilts started the restraint test so stressed that support did not affect them anymore. This is, however, not likely, because some effects of support were found. In this study, we had no opportunities to make uniform gender groups. We decided, therefore, to have, at least, all the restrained pigs of one gender which resulted in female restrained pigs and male accompanying pigs. As the pigs were too young to be sexually mature (Brooks and Smith, 1980; Graves, 1984; Babol et al., 2004), we assumed that gender would not affect the results. In retrospect, this difference in gender might, in fact, have affected the results. Perhaps boars are less able to provide support or gilts do not accept support given by the other gender. This suggestion could be supported by the observation that actual social contact between the gilt and boar was low in this study. In addition, gender differences in giving and receiving social support have also been found in, for instance, rats (Westenbroek et al., 2003, 2005) and guinea pigs (Kaiser et al., 2003). Furthermore, the fact that a boar was chosen as accompanying pen mate could explain the result that more gilts urinated in the support situation compared to the no support situation, because a courtship behavior in ungulates is urination by the female upon the approach of a male which subsequently shows flehmen behavior (Gassett et al., 1998). Display of courtship behavior by sexually immature animals might seem unlikely, but the gilts and boars might have reached puberty earlier than they usually do, because they were housed together from birth onwards (Gelez et al., 2004). We suggest, therefore, to use pigs of one gender only in future studies on social support.

5. Conclusions

Although modest, the results of this study do suggest that pigs are able to benefit from social support, but this seemed to be dependent on the coping style of the pigs, because effects were only significant for pigs with a low-resisting coping style. This fits well into the framework of low-resisting pigs being more reactive and open to environmental cues.

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CHAPTER 8

General Discussion

The aim of this thesis was to investigate to what extent social processes could affect welfare, including positive and negative emotional states, in pigs. Firstly, it was investigated whether fear and stress could be reduced by a breeding method based on heritable effects on the growth of group members or by environmental enrichment. With this breeding method, pigs can be divergently selected to genetically affect the growth of their pen mates either relatively positively or negatively and these genetic effects on each other's growth could possibly be explained by effects on each other's emotional state. Therefore, the extent to which pigs might affect the emotional state of other pigs was studied through investigating emotional contagion and social support.

Breeding and environmental enrichment

Pigs in intensive husbandry systems are generally subjected to several standard management procedures such as tail docking, mixing with unfamiliar pigs and relocation, and these management procedures have been shown to induce acute stress or fear in these pigs (e.g. Noonan et al., 1994; Stookey and Gonyou, 1994; Von Borell, 2001; Hemsworth, 2003; Dudink et al., 2006). Moreover, these pigs are usually also housed in rather barren conditions which may induce a state of chronic stress in pigs (Lyons et al., 1995; Beattie et al., 2000; De Jong et al., 2000; Munsterhjelm et al., 2009). Strategies to reduce (chronic) fear and stress in intensively housed pigs are therefore required. In this thesis, two strategies were investigated: an alternative breeding method and the provision of environmental enrichment.

It seems possible to directly breed against fearfulness or stress sensitivity, but for various reasons this will likely not be implemented on a commercial basis (Ramos and Mormède, 1997; Rodenburg et al., 2004; Boissy et al., 2005; Turner, 2011; Bijma, 2012; Canario et al., 2013). In contrast, the breeding method investigated in this thesis - based on heritable effects of pigs on their group members' growth (Bergsma et al., 2008, 2013; Chen et al., 2007) - could be used commercially (Bijma et al., 2007; Bijma, 2012) and with this breeding method fearfulness or stress may be indirectly reduced as well. In this thesis it was hypothesized that if pigs are sensitive to the emotional state of their pen mates, in particular to negative emotions such as fear and stress, this could lead to a situation of repeated or chronic stress which in turn negatively affects the growth of the whole group (Hemsworth, 2003). If so, pigs that have a relatively negative indirect genetic effect on the growth of their pen mates may then be pigs that become easily fearful or stressed, whereas pigs that have a relatively positive indirect genetic effect on the growth of their pen mates may be pigs that are less sensitive to fear or stress. Therefore, this breeding method could be a strategy to indirectly reduce fear or stress in intensively housed pigs. To test this, pigs selected to have either a relatively positive (+SBV) or negative (–SBV) indirect genetic effect on the growth of their pen mates were subjected to a series of tests commonly used to study fear and stress (Stookey and Gonyou, 1994; Forkman et al., 2007). Chapter 2, 3 and 4 show that before weaning +SBV pigs were faster to touch a novel feeder in a group-wise novel object test and spent more time near a person in a group-wise human approach test than –SBV pigs. After weaning, +SBV pigs were faster to touch a rope in a group-wise novel object test and showed more locomotion in the individual novel environment test after a bucket was introduced in the arena than –SBV pigs. In addition, +SBV pigs were found to have overall lower leukocyte, lymphocyte and haptoglobin concentrations than –SBV pigs between 8 and 22 weeks of age which, in line with the behavioral results, may indicate that the +SBV pigs had experienced less stress. The absolute value of each difference found between the +SBV and –SBV pigs may be small (e.g. +SBV pigs were just 22 s faster than –SBV pigs to touch the novel feeder in a test of 600 s), but that could be due to the fact that the estimated contrast in growth between the +SBV and –SBV pigs was also small, i.e. an estimated contrast of 3 kg at the end of the finishing phase (Camerlink et al., 2013) which is small given the variation normally found in slaughter weights (Gnaedinger et al., 1963; Gispert et al., 2010). It was, however, the largest contrast possible with the available population of boars and sows and the contrast was calculated to be significant (Camerlink et al., 2013). Therefore it was assumed that behavioral differences, if present, would be found. Besides these results, Camerlink et al. (2013) found +SBV pigs to be less aggressive towards their pen mates than –SBV pigs upon reunion after a 24 h regrouping test at nine weeks of age and they suggested that this could have been due to the fact that +SBV pigs experienced less stress upon reunion. Moreover, +SBV pigs were also found to show less biting behavior (i.e. aggressive biting, ear biting and biting on the materials (jute sack and chain with ball) provided) and to inflict less tail damage between eight and 23 weeks of age (Camerlink et al., submitted). This type of biting behavior is proposed as an outlet of stress (Schrøder-Petersen and Simonsen, 2001). Taking all these results together, it seems that +SBV pigs are pigs that respond less stressful in different test situations and they may overall be less sensitive to stress. As both types of pigs were housed with other pigs of the same SBV class, it can be questioned whether +SBV pigs are indeed pigs that are biologically less sensitive to stress or that they just experience less stress because their social environment is less stressful (e.g. +SBV pigs perform less biting behavior and are thus also less bitten which may result in feeling less stressed). Biting pen mates may be considered as redirected foraging or explorative behavior (Van Putten and Dammers, 1976; Taylor et al., 2010) which could explain why pigs hardly perform this behavior in enriched environments (e.g. Beattie et al., 2000; Bolhuis et al., 2006a). Also the pigs studied in chapter 3 and 4 showed much less biting of pen materials and pen mates when housed in pens enriched with straw bedding (Camerlink et al. submitted; Ursinus et al., in press). If the changes in +SBV pigs in fearfulness and physiology would solely be an effect of a less stressful (social) environment due to a lower incidence of biting behavior, the SBV effects should be much lower in enriched environments. However, as can be seen in Fig. 1, SBV effects were similar in barren and enriched environments. Hence, +SBV pigs are likely pigs that are less easily stressed than –SBV pigs. Including indirect genetic effects into the breeding program thus seemed to have affected the pigs’ capacity to deal with stress. Interestingly, group selection experiments with laying hens seem to have affected laying hens’ sensitivity to stress or fear as well. For example, in a series of experiments by Muir and colleagues laying hens of the same sire family were kept as a group in cages. Groups of hens with the highest survival rates and egg production were accordingly selected and used to breed a next generation (described in Muir, 1996). In subsequent generations, these hens showed less feather pecking (Craig and Muir, 1996; Cheng et al., 2001a), had lower dopamine concentrations (Cheng et al., 2001b, 2003), a lower H:L ratio (~ N:L ratio), and had a more efficient cell-mediated immunity (Cheng et al., 2001c) than a control line of hens that were selected for low group survival and production. Cheng and Muir (2005) proposed, therefore, that selection on high group survival and egg production resulted in hens that were able to cope better with novel environments and were less susceptible to social and non-social stress. In another series of selection experiments, laying hens were selected on both individual performance and low group mortality. Selection candidates were housed individually and were monitored for individual egg production. The sisters of the selection candidates were housed per family in a group and in these groups, the mortality was monitored. For the next generation, selection candidates with sufficient individual egg production and low mortality in their sister group were selected to create a low mortality line. A control line was maintained by selecting laying hens on individual egg production only (described in Ellen et al., 2007). Laying hens of the second or fourth generation of the low mortality line were found to be less fearful than the hens of the control line in an individual manual restraint test (Bolhuis et al., 2009), an individual novel environment test (Rodenburg et al., 2009), an individual maze test and human approach test (Nordquist et al., 2011), and in a group-wise human approach test (Bolhuis et al., 2009). The results of the pig and chicken experiments thus suggest that the (absence of) experience of (chronic) stress is of great importance to group housed animals and that it can be targeted by breeding. The pig selection experiment described in this thesis is, apart from a small-scale pilot study (see Rodenburg et al., 2010), the first large scale experiment investigating differences between +SBV and –SBV pigs. The differences are such that further selection experiments with more generations are likely worthwhile. However, the decision to continue selecting pigs using this breeding method rests largely at the breeding companies. To continue, positive effects on performance should be present as breeding companies operate on economic incentives. In the experiment, performance parameters were measured, but against expectations +SBV pigs tended to have lower body weights than –SBV pigs at the end of the finishing phase (Camerlink et al., accepted). During the experiment, however, the pigs were for ethical reasons far more closely monitored than most pigs on commercial farms and therefore received faster and most likely better care. For instance, bitten tails were alternately treated with PHB spray and Stockholm tar and pigs with a severe tail wound were taken out of the experiment. Moreover, all pens were provided with two hands of wood shavings per day from six weeks of age and a jute sack from eight weeks of age to prevent excessive tail biting, the latter known to result in a reduction of biting behavior of approximately 50 % (Ursinus et al., submitted). Growth performance may be profoundly reduced in victims of tail biting and other damaging behaviors (Camerlink et al., 2012; Sinisalo et al., 2012). As the –SBV pigs received more care than the +SBV pigs for tail damage caused by harmful behavior of pen mates (Camerlink et al., accepted), it is likely that their growth was better than expected which consequently led to result found. If so, repeating the experiment under commercial farm conditions would probably yield substantial growth differences between +SBV and –SBV pigs in the expected direction. It is then also not unreasonable to assume that +SBV and –SBV pigs would show more and/or larger differences in behavior and physiology. To be sure, however, the experiment has to be performed first. Even so, the behavioral and physiological results do indicate that further selection on pigs with relatively positive indirect genetic effects on the growth of their pen mates could lead to less stressful pigs and thus to a welfare improvement.

Welfare improvements by reducing (chronic) fear or stress can also be obtained by providing pigs with environmental enrichment (Van de Weerd and Day, 2009). The results of chapter 3 and 4 of this thesis and the results of the studies of Camerlink and coworkers generally support this. For instance, pigs provided with environmental enrichment responded less fearful to a novel object or person present in their home pen (chapter 3) and also had overall lower haptoglobin concentrations and N:L ratios than barren housed pigs (chapter 4). In addition, enriched pigs also showed less harmful oral manipulations such as tail biting and ear biting, but more comfort behavior than barren housed pigs (Camerlink et al., submitted). It can thus be concluded that both strategies - breeding and housing adjustments - can be used to improve pig welfare. As no interactions were found between effects of SBV for growth and effects of housing condition, results point to additive effects of both strategies. These additive effects are for instance evident in the latency to touch a rope in the novel rope test (chapter 3), in the haptoglobin concentrations (chapter 4), and in the tail damage scores (Camerlink et al., submitted) (Fig. 1). In each graph, the most optimal result with respect to its interpretation for welfare was found for the +SBV pigs in enriched housing and the worst result for the –SBV pigs in barren housing.

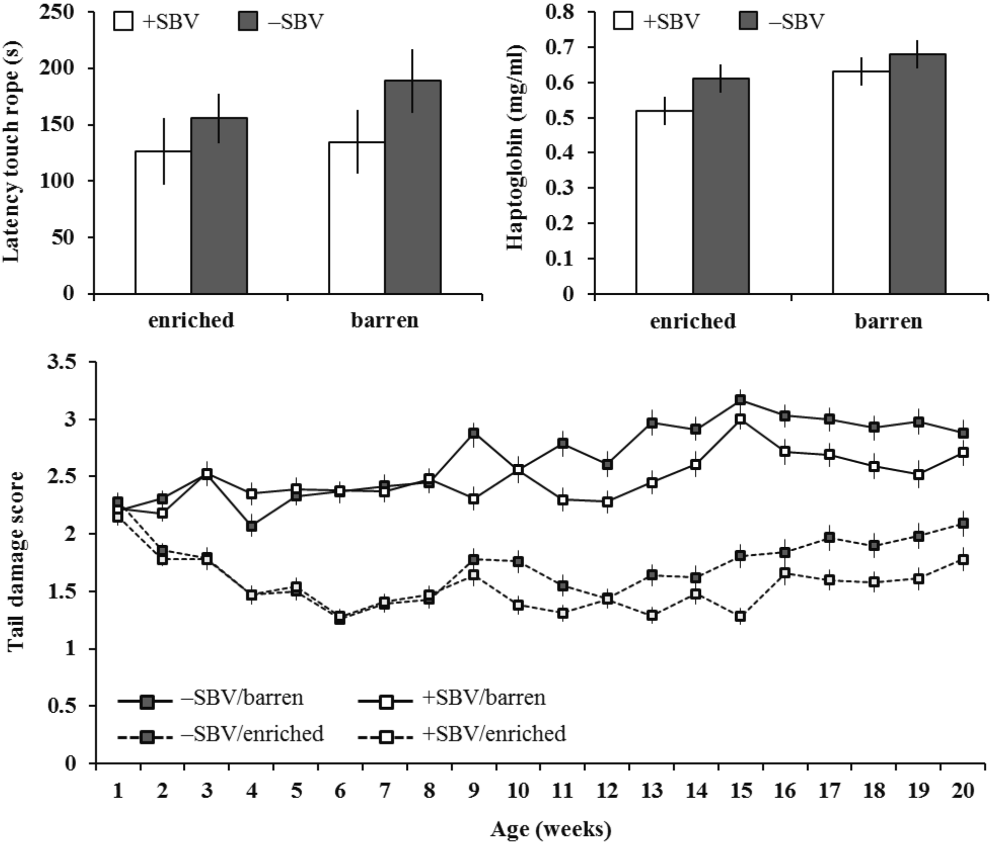

Fig. 1. Latency to touch a rope in the novel rope test (upper left, adapted from chapter 3), overall haptoglobin concentrations (upper right, adapted from chapter 4) and tail damage scores (bottom, adapted from Camerlink et al., submitted) of +SBV and –SBV pigs housed in barren or enriched pens.

These results indicate that changes in housing alone would not yield optimal welfare conditions, but that "changes" in the pigs are also needed, at least as presented by this breeding method. So, selection on +SBV pigs may also be useful for pigs housed in organic farms (Sutherland et al., 2013) or in specially designed new housing systems (e.g. De Greef et al., 2011). Changing animals through breeding so that they better match with their environment might, however, be opposed by society (Sandøe et al., 1996; Millet et al., 2005; Star et al., 2008). This could occur due to misconceptions about breeding. For instance, some people might believe that breeding is equivalent to genetic modification, i.e. the process of transferring genetic material from one species to another, while that is not the case

(Verhoog, 2003; Hu et al., 2004; Macnaghten, 2004). In addition, people might also regard breeding as being detrimental for animal welfare, whereas research, including the research presented here, indicates that breeding can also lead to positive effects on welfare (Lawrence et al., 2004; Kanis et al., 2005; Oltenacu and Algers, 2005). By better informing the general public through e.g. science society-dialogues (Gamborg and Sandøe, 2005; Miele et al., 2011), such misconceptions could be cleared up and in this way (new) breeding (methods) may be easier accepted. Here, it should also be noted that welfare improvements through environmental enrichment only apply to the animals that have been provided with it, whereas welfare improvements through breeding can increase with each subsequent generation. To take the tail damage scores in Fig. 1 as an example, enrichment reduced scores with ca. 1 point, whereas +SBV pigs only had half a point lower scores than –SBV pigs. However, if this difference would be present in each subsequent generation, no damaged tails would be found in the +SBV pigs after five generations. This effect thus presents an advantage of breeding over providing environmental enrichment. At the same time, it should be realized that it is not known yet to what extent +SBV and –SBV pigs of later generations will differ. At present, stress is likely a major factor affecting growth in pigs, but it could be that selection of pigs with a positive effect on the growth of their pen mates lead to pigs in which stress is no longer a factor affecting growth. In that situation, there may be other factors present then that do constrain growth. Therefore, it is very important to closely monitor future generations of SBV pigs, also to avoid breeding in a way that is not ethically acceptable (see Star et al., 2008). Moreover, in this thesis differences between +SBV and –SBV pigs were mainly investigated from a negative perspective (e.g. novelty tests and aggressive and other harmful behaviors), but hardly from a positive perspective which in relation to social support and positive emotional contagion may, if tested, lead to very interesting results as well. Hence, the study of welfare consequences of selection on SBV for growth in pigs warrants further research.

Emotional contagion, social support and SBV for growth

Emotional contagion was proposed as one of the mechanisms through which pigs could affect each other’s growth. Emotional contagion in pigs was investigated in chapter 5 and 6 of this thesis, both during anticipation and during experience of a positive or negative situation. Anticipatory behavior was induced by classical or Pavlovian conditioning, coupling a cue to each situation. This type of conditioning requires training and therefore emotional contagion could not be studied in many pigs. On the other hand, a sufficient contrast in SBV for growth could not be obtained for a small number of pigs. Hence, emotional contagion was not studied in pigs differentially selected on SBV for growth.

Chapter 5 shows that the training pigs had associated the cue with the subsequent situation, but it seemed that the association was best established at the end of the training period. During the test for emotional contagion, their naive pen mates tended to show less tail in curl and more tail low during the display of cues which the training pigs had learned to associate with the negative situation. As tail low is associated with a negative emotional state (e.g. Kiley-Worthington, 1976; Noonan et al., 1994), naive pigs could have indicated with this behavior that they had received ‘bad news’ from the training pigs regarding the subsequent situation and became anxious about that situation as well. However, to draw a more definite conclusion about emotional contagion during anticipation more evidence is needed. Unfortunately, in a follow up experiment (chapter 6), training pigs showed overall even less differences in anticipatory behaviors during the display of cues preceding a positive or negative situation, although individual pigs did seem to have learned the meaning of the cues (see section anticipation differences). Furthermore, during the test for emotional contagion the naive pen mates did not differ in any of the behaviors scored during the display of the two cues. Therefore, emotional contagion during anticipation was not further analyzed in chapter 6. At present, it remains inconclusive whether emotional contagion during anticipation occurs in pigs. Through announcement of positive situations it is possible to induce positive emotions in animals during anticipation which is positive for their welfare (Dudink et al., 2006; Moe et al., 2009) and through emotional contagion a whole group of animals could benefit from these positive welfare effects without the need of inducing positive anticipation in each individual animal. To accomplish this for pigs, first a better understanding of how pigs express their expectations towards a valenced (i.e. positive or negative) situation is needed.

In contrast to the anticipation phase, during exposure to the positive and negative situation, training pigs were clearly in a positive and negative emotional state, respectively, both in chapter 5 and 6, and pigs did not seem to habituate to the situations over the course of the experiment. Moreover, the results of chapter 5 and 6 also indicate that the naive pigs were in a positive emotional state during the positive situation of the training pigs (chapter 5) and (more) in a negative emotional state during the negative situation of the training pigs (chapter 5 and 6). To interpret these results as emotional contagion, the emotional state of the naive pigs should have been elicited by (the emotional state of) the training pigs and not by something else. During the positive situation, play and thereby a positive emotional state could have been elicited in the naive pigs by hearing straw being moved around or by the smell of peat and chocolate raisins. If so, then their emotional state was not related to the emotional state of the training pigs which argues against emotional contagion. However, straw was also present in the home pen and there it did not induce play behavior each time it was moved around. In addition, the smell of peat and chocolate raisins was also present during the negative situation and during that situation naive pigs did not play either. Therefore, it is not likely that the straw or peat itself had elicited a positive emotional state in the naive pigs. As naive pigs were habituated to the test room and no other stimuli were present except for the training pigs (Edgar et al., 2012), it is reasonable to assume that the positive emotional state of the naive pigs was elicited through emotional contagion. Similarly, there are no other obvious reasons than emotional contagion that could have elicited the negative emotional state of the naive pigs during the negative situation of the training pigs. Hence, it can be concluded that emotional contagion occurs between pigs. In the experiments in chapter 5 and 6, training and naive pigs could not see each other during the negative and positive situation experienced by the training pigs. Consequently, emotional contagion likely had occurred through auditory and/or olfactory signals. Expressions of emotional states through different signals and their relevance for the sender and receiver will be discussed in more detail later on (see section indicators of positive and negative emotional states in pigs).

With this information, it is thus possible that emotional contagion can be a mechanism underlying SBV for growth. Chapter 2, 3 and 4 have shown that –SBV pigs are more easily stressed or more fearful than +SBV pigs. So, a –SBV pig is probably more affected by a negative event than a +SBV pig and consequently shows a more intense reaction. Another –SBV pig in the same pen - as pigs were housed together with pigs of the same SBV class - may observe this reaction and this pig is then also negatively affected, hereby possibly creating a snowball effect ultimately leading to a lower performance of the whole group as sustained fear or stress have been associated with reduced growth (Hemsworth, 2003). In contrast, a +SBV pig may be less affected by a negative situation and will consequently show a less intense reaction. Another +SBV pig in the same pen will observe this less intense reaction, but because it is a +SBV pig this reaction will have little effect on its own emotional state and thus also little effect on its growth. On the other hand, +SBV pigs might also be more sensitive to positive emotions than –SBV pigs and they may thus affect the growth of their pen mates positively through positive emotional contagion. Although I am not aware of any studies showing that positive emotions can actually promote growth, the found effects of positive emotions on welfare and health (e.g. Richman et al., 2005; Boissy et al., 2007) may indirectly lead to positive effects on growth as well. Positive emotions were, however, not directly studied in the +SBV and –SBV pigs and therefore one can only speculate about it. Positive emotional contagion can occur through play behavior (Held and Špinka, 2011; Špinka, 2012; chapter 5). During home pen observations, +SBV pigs did, however, not show more play behavior than –SBV pigs (Camerlink et al., submitted), although it should be noted that the scan sampling method applied during the home pen observations is not particularly suitable for behaviors with a short duration like play. During the same home pen observations, +SBV pigs were found to show more comfort behavior and this behavior has been associated with positive emotional states (Bracke, 2011; Zimmerman et al., 2011) and might even be contagious (Schenkel, 1966; Hoppitt et al., 2007), but whether it also stimulates growth, is not known.

Besides emotional contagion, social support could also be a mechanism underlying SBV for growth in pigs. The results of chapter 7 and other studies (Fraser, 1974; Arnone and Dantzer, 1980; Geverink et al., 1998; Ruis et al., 2001; Bolhuis et al., 2006b; Hameister et al., 2012) suggest that pigs are able to provide support to stressed pigs, thereby reducing their stress response. Stress negatively affects growth (Hemsworth, 2003), but if +SBV pigs are pigs that are able to buffer stress or fear responses of other (+SBV) pigs, they could, via this stress buffering effect, have a positive effect on the growth of their pen mates (Feldman et al., 2000). At present it is, however, not clear how social support could work in pigs. For instance, is the mere presence of another pig sufficient to reduce stress responses or is positive physical contact between the supporting and stressed pig needed as well (Uvnäs-Moberg, 1998; Rault, 2012)? In the study of Bolhuis et al. (2006b), a familiar accompanying person interacted with the restrained pig during the whole 15 min test by gently stroking and talking to the pig. The stress response of these pigs was clearly reduced in comparison to the stress response of the pigs subjected to the restrained test alone. In the social support experiment in chapter 7, the accompanying and restrained pigs engaged little in any type of physical contact and effects of social support were less clear than in Bolhuis et al. (2006b). Positive physical contact between the support giver and support receiver may thus be more effective for social support than mere presence. In pigs, positive physical contact may be expressed through nosing behavior (Boissy et al., 2007; Camerlink et al., 2012; Camerlink and Turner, 2013) and receiving it has even been found to have positive effects on growth (Camerlink et al., 2012). So, by performing nosing behaviors +SBV pigs could positively affect the growth of their pen mates directly or more indirectly via social support. Home pen observations revealed, however, no difference in nosing between the +SBV and –SBV pigs (Camerlink et al., submitted), but as it is rather difficult to score this behavior properly (Camerlink et al., 2012), +SBV and –SBV pigs may have differed in nosing behavior. Social support is also found to be more effective if the supporting animal is not stressed itself (Kiyokawa et al., 2004; Rault, 2012). Hence, +SBV could also be more able than –SBV pigs to give social support, just because they are less fearful or less stressed pigs.

In sum, pigs could affect each other's growth negatively through emotional contagion of negative emotional states, but how pigs could affect each other's growth positively is less evident.

No two pigs are alike

As discussed above, +SBV pigs are quite different from –SBV pigs in their behavior and physiology. This has not only implications for their own welfare, health and productivity, but also for that of their pen mates because pigs do pay attention to their pen mates' behavior and/or emotional state which then may subsequently influence their own behavior and/or emotional state (Held et al., 2001; chapter 5, 6 and 7). In this thesis, differences were not only found between +SBV and –SBV pigs, but also between pigs with a proactive (or high-resisting (HR)) and reactive (or low-resisting (LR)) coping style, between gilts and barrows and gilts and boars, and between individuals in learning to anticipate a positive or negative situation. As the social group pigs live in can both be beneficial and detrimental for pig welfare, health and productivity (e.g. Hessing et al., 1993; Sachser et al., 1998; Tuchscherer et al., 1998; Turner et al., 2013), the implications of these individual differences for group functioning will be discussed here.

Coping style differences

In chapter 2, 3 and 7 of this thesis, pigs that struggled and vocalized relatively much in a backtest (i.e. high-resisting (HR) pigs) were found to be more active and produced more vocalizations in a series of novelty tests later on (chapter 2 and 3) and in a restraint test (chapter 7) than pigs that hardly struggled and vocalized in a backtest (i.e. low-resisting (LR) pigs). In contrast, LR pigs showed more passive behaviors such as standing alert and ears back than HR pigs (chapter 7). These differences are to a great extent consistent with other studies that have related backtest responses to responses in other situations later in life. These studies generally report HR pigs to behave more actively and LR pigs more passively in the situations tested (e.g. Hessing et al., 1994a; Bolhuis and Schouten, 2002; Geverink et al., 2002; discussion chapter 2, 3 and 7). In addition, the difference in social support between the HR and LR pigs in chapter 7 may be due to a difference in cue dependency which is also in line with another study (Bolhuis et al., 2004). No differences were, however, found between HR and LR pigs in their salivary cortisol response to the novel environment test and restraint test (chapter 3 and 7) or in their heart rate response to the restraint test (chapter 7), but as discussed in these chapters coping style differences do not have to be present on a neuroendocrinological level (cf. Coppens et al., 2010; Koolhaas et al., 2010). In chapter 4, it was found that HR pigs seem to have a higher innate immune activity than LR pigs. Other studies have also investigated immune reactivity of HR and LR pigs (see discussion chapter 4), but these studies investigated specific immune responses rather than innate immunity. The study in chapter 4 is therefore the first study to demonstrate a difference in innate immunity between HR and LR pigs which may indicate that HR and LR pigs have different strategies to deal with immune challenges.

In chapter 2, it was found that +SBV and –SBV pigs did not differ in their response to the backtest. Moreover, in chapter 3 and 4, no interactions were found between SBV for growth and backtest classification. This suggests that including SBV for growth into the selection program does probably not affect how a pig copes with a stressor, so neither more actively nor more passively, but, as discussed earlier, including SBV for growth into the selection program may target a pig's sensitivity to stress. If so, coping styles and SBV for growth may be two independent response dimensions, which resemble the two-tier model of Steimer and coworkers (1997). In this model, the response of an animal to a stressor can be classified as more active or more passive (i.e. referring to the type or quality of the response) and, at the same time, as more or less emotional (i.e. referring to the intensity or magnitude of the response), leading to animals being either shy (passively coping and highly emotional), panicky (actively coping and highly emotional), bold (actively coping and little emotional), or docile (passively coping and little emotional) (Koolhaas et al., 2010). Research in mice and rats (Koolhaas et al., 2010) and dairy cattle (van Reenen, 2012) seems to support this model.

Based on chapter 2, 3 and 4 and the results of Camerlink and co-workers, selection on +SBV pigs may represent a more desirable breeding strategy than current breeding strategies, but selection on pigs of one particular coping style seems, although suggested to be possible (Velie et al., 2009; Iversen et al., in preparation), not a desirable strategy. This is, because results of several studies indicate that housing pigs with varying coping styles together is most optimal with respect to pig welfare and productivity. For instance, Hessing et al. (1994b) found that several performance parameters such as average daily gain and carcass quality were higher in pigs from mixed pens than in pigs from pens which consisted of only HR or only LR pigs. Moreover, uniform HR pens are likely to suffer from more social stress due to aggression than uniform LR pens or mixed HR/LR pens, because HR pigs are more aggressive and have more difficulties to inhibit this aggression (Hessing et al., 1994b; Ruis et al., 2002; Bolhuis et al., 2005a, 2006a). In contrast, uniform LR pens might suffer from more social stress due to oral manipulations than uniform HR pens or mixed HR/LR pens, because LR pigs express this behavior more than HR pigs (Bolhuis et al., 2005b, 2006a). Pig welfare and productivity are, however, also affected by emotional contagion and social support, but at present no conclusions can be drawn as to whether mixed HR/LR pens do also better with respect to these two social processes, because emotional contagion was not studied in relation to coping style in chapter 5 and 6, and mixed HR/LR pairs were not tested for social support in chapter 7. Moreover, the differences in immune function between HR and LR pigs may have implications for their own health as the higher cell-mediated specific immunity in HR pigs (Hessing et al., 1995; Schrama et al., 1997; Bolhuis et al., 2003) may account for HR pigs being less susceptible to a salmonella infection (Van Erp-van der Kooij, 2003), but how that relates to the health of the group and whether pig health is different in uniform HR or LR pens or mixed HR/LR pens cannot be determined without more research. Therefore, it would be interesting to further investigate the effects of coping style on group functioning in pigs.

Gender differences

Differences between females and males can be found in many animal species. Most apparent are perhaps the differences in appearance, but females and males also differ in other features as such as behavior (Collaer and Hines, 1995) or learning and memory (Jonasson, 2005; Cahill, 2006). Pigs form no exception to this as many differences are found between female and male pigs as well (e.g. Adkins-Regan et al., 1989; Dorries et al., 1995; Baxter et al., 2012). Moreover, in chapter 2 and 3 of this thesis, gilts and barrows were found to differ substantially in their response to several novelty tests with barrows responding more fearfully and, based on the results of chapter 3 and 4, gilts and barrows may also differ in stress physiology. From these differences, it could be hypothesized that as barrows express stronger fear responses than gilts, emotional contagion of negative emotions such as fear may occur more frequently or easier in all barrow groups. If so, uniform barrow groups likely suffer more from stressors than other gender groups and that may negatively affect their welfare, health and productivity. In modern day intensive pig husbandry systems, pigs are kept with their mother and litter mates from birth to weaning (Gonyou, 2001), but after weaning they may be regrouped into uniform or other mixed gender groups (Hintze et al., 2013), depending on the management procedures of the farm. Hence, different management strategies are likely needed for the different group compositions (i.e. uniform barrow, uniform gilt and mixed gender groups) as uniform barrow groups may for instance benefit more than other gender groups from keeping stressors to a minimum.

Although the differences between barrows and gilts and their implications with respect to group functioning are still of importance for current husbandry practices, differences between boars and gilts will likely receive more attention as castration will no longer be performed in the Netherlands from 2015 onwards and in other countries of the European Union from 2018 onwards (European Commission, 2010; Van Wagenberg et al., 2013). Indeed, differences between boars and gilts have already been studied in relation to aggressive behavior (e.g. Rydhmer et al., 2006; Boyle and Björklund, 2007), mounting behavior (e.g. Boyle and Björklund, 2007; Hintze et al., 2013) and performance parameters (e.g. Andersson et al., 2005; Agostini et al., 2013) as these are considered to be most relevant with respect to keeping boars for meat production. This thesis shows, however, that the emotional state of the pigs is also of importance on an individual and group level. Boars and gilts may also differ in this respect as a difference in gender between chapter 5 (boars and gilts) and chapter 6 (gilts) may be one explanation for the different results found and gender was suggested as an explanation for the social support results in chapter 7 (see discussion there). So, to be able to offer the best housing conditions to boars and gilts from 2018 onwards, uniform boar and gilt groups and mixed boar and gilt groups should be studied simultaneously using an integral approach, i.e. not only studying mounting behavior, but also their response to novelty, performance parameters such as growth, use of environmental enrichment, etc. In this way, it can be assessed what is best for the pigs, what the farmers prefer and what is feasible to manage and thereby the most optimal strategy for all involved.

Anticipation differences

In chapter 5, pigs were trained in a ten-day period to associate one cue (i.e. a combination of a visual and auditory cue) with a positive situation and another cue with a negative situation. The results of chapter 5 indicated that the pigs had attributed a different meaning to the two cues, but they did, as far as could be observed, not clearly express their emotional expectation toward the situation during anticipation in a uniform manner. Some adaptations to the design might change this and that led to the design used in chapter 6. One of the adaptations was a longer training period. Although the results of chapter 5 indicated that the training pigs had learned the association between the cue and the subsequent situation, a longer training period might result in more behavioral or more stable differences between positive and negative anticipation. The training period was therefore extended to three weeks in chapter 6. In chapter 5, training pigs could see and touch the doors that gave entrance to the situations during anticipation which could have led to a difference in the door investigatory behaviors and head oriented behaviors between positive and negative anticipation. With an extra door between the anticipation compartment and the compartments of the situations, these location-directed behaviors might disappear and pigs might instead show more affective behaviors. Moreover, to strengthen the association between the cue and the situation, the cue was also played during the situation and not only during the anticipation period. Hereto, the cues were changed from a bicycle bell and siren into a piano piece and march music. It was not expected that this would affect the learning abilities of pigs as studies have shown that pigs are able to learn to respond to cues of various sounds (e.g. Dantzer and Mormède, 1976; De Jonge et al., 2008; Imfeld-Mueller et al., 2011). Analyzing the results of chapter 6, however, seemed not to have improved anticipation overall, but there were some interesting individual differences. For instance, both pigs from one pen spent more time standing alert during anticipation of the negative situation than during anticipation of the positive situation. In contrast, in another pen one pig spent more time exploring the compartment floor during anticipation of the negative situation than during anticipation of the positive situation, whereas its pen mate did not anticipate the situations using this behavior. Moreover, there were also behaviors, such as ears back, that both pigs from a pen did not differently express between anticipation of the negative and positive situation (Fig. 2).

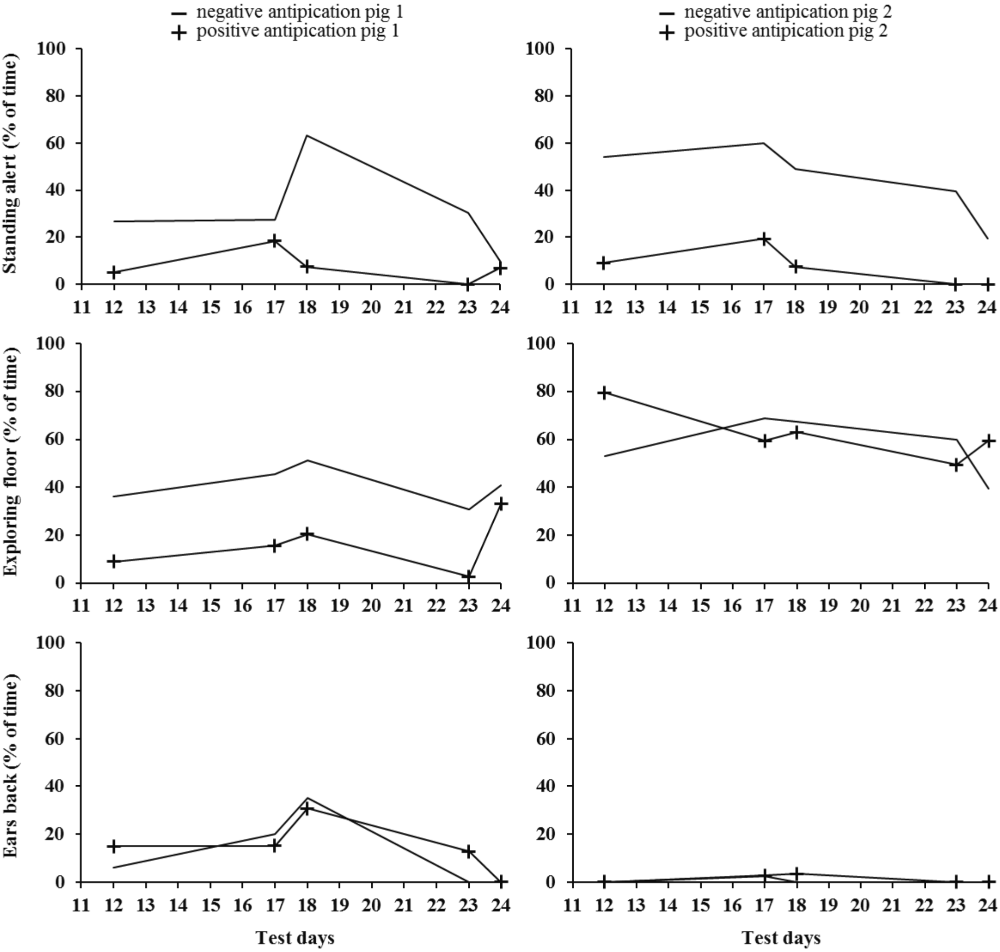

Fig. 2. Time spent by the two training pigs of three different pens on standing alert (upper graphs), exploring the compartment floor (middle graphs) and ears back (lower graphs) during anticipation of the positive situation and anticipation of the negative situation measured on test (training) days 12, 17, 18, 23 and 24.

These examples show that pigs are capable of learning to anticipate two different situations based on sound cues, but that pigs vary greatly in their way of expressing it. It could also be that pigs have a general way of expressing their expectations towards a positive and negative situation, but that is then something which was not measured in chapter 6. Results of a study by Gimsa et al. (2012) suggested that positive anticipation coincides with sympathetic activation. Another study, however, did not find a difference in heart rate and heart rate variability between pigs anticipating a positive or negative event (Imfeld-Mueller et al., 2011). This inconsistency may support the results of chapter 6 in that pigs indeed have very individual responses reflecting positive and negative anticipation. Moreover, also in other studies where pigs had to learn a certain task, substantial individual differences were found (e.g. Bolhuis et al., 2004; Jansen et al., 2009; Murphy et al., 2013). These individual differences in learning abilities were all found in a specially designed experimental set up, but it is likely that also in the home pen pigs express individual differences in learning. In farming practices, pigs might learn to expect or anticipate a visit from the caretaker when the caretaker always comes at the same time of day or pigs might learn to anticipate the arrival of food when the food is preceded by a particular sound. If pigs would express these expectations differently by showing different behaviors, this might lead to confusion in the pen mates as they might associate these behaviors with a different context. For example, if a pig would anticipate food by showing standing alert behavior, another pig might interpret this as a sign of danger and consequently experiences acute fear or stress, while that is not needed. Through these individual differences, pigs might thus affect each other’s welfare. Therefore, more knowledge of how pigs express their expectations about their environment and how they perceive the behavior and intentions of their pen mates, could help to improve pig welfare (Held et al., 2002).

Indicators of positive and negative emotional states in pigs

In this thesis, positive and negative emotional states were inferred from physiological and behavioral indicators. Both types of indicators have their advantages and disadvantages. Physiological indicators have the advantage that the obtained values are not disputable, at least as long as the method (e.g. a laboratory assay) of obtaining the values has been correctly executed. To illustrate this, a cortisol concentration of 2.5 ng/ml is a concentration of 2.5 ng/ml and a heart rate of 150 bpm is a heart rate of 150 bpm. There is no question about that. However, their interpretation with respect to emotional states can be ambiguous. For instance, an increase in (salivary) cortisol could point to stress (Mormède et al., 2007; Merlot et al., 2011), but cortisol increases have also been found in situations of neutral or even positive emotional states (Paul et al., 2005; Koolhaas et al., 2011). Similarly, an increased heart rate may indicate a negative emotional state, but may also indicate a positive emotional state (Paul et al., 2005; Kreibig, 2010). Such physiological indicators can therefore not be used on their own, but they may still be informative in combination with the context in which they were measured or in combination with other physiological or behavioral indicators (Paul et al., 2005). In addition, the physiological measurement itself can already induce an emotional state (e.g. blood sampling often induces stress) which might complicate the interpretation of the values later on (Möstl and Palme, 2002; Merlot et al., 2011). Moreover, physiological indicators can be quite costly to analyze. Hence, the practical use of physiological indicators is limited.

In contrast to physiological measures, behavioral measures can be obtained directly through observation. Inferring emotional states from behavioral indicators is therefore more convenient for practice than using physiological indicators. Furthermore, behaviors can be observed without disturbing the animal which does therefore not affect the subsequent interpretation (Dawkins, 2004). Moreover, behavior is suggested to be the outcome of all the (sub)consciously decisions made by an animal and is even suggested to represent an animal’s emotional state (Dawkins, 2004). If so, this would very much support the use of behavioral indicators to assess emotions in pigs. The question is then, which behaviors are reliable indicators of pig emotional states? Comparing the behaviors displayed by the pigs in the individual novel environment test in chapter 2 and 3 and the behaviors displayed by the pigs in the positive and negative situation in chapter 5 and 6 and in the weighing cage in chapter 7 shows that standing alert, escape attempts, relatively much defecating and urinating, high-pitched vocalizations and ears backwards are clear behavioral indicators of a negative emotional state and that play behavior, tail wagging and ‘play barks’ are clear behavioral indicators of a positive emotional state. From this list, it is evident that there are more known indicators of a negative than for a positive emotional state. This bias may be present, because the study of positive emotions in animals is still in its infancy and therefore we have less knowledge of positive emotions and how to measure them as compared to negative emotions, but this bias may also be present, because negative emotions are more intensely expressed than positive emotions (Boissy et al., 2007). That negative emotional states are more intensely expressed could be related to survival as survival chances for individuals are clearly increased by the activation of fight or flight responses upon sensing danger of a predator nearby, but there are no immediate survival benefits by expressing positive emotions (Fraser and Duncan, 1998). In addition, without clear expressions of negative emotions such as fear or danger, individuals within a group would not be triggered to become alert of the danger as well which then lowers the survival of the whole group (Spoor and Kelly, 2004). So an intense expression of negative emotions is beneficial to both individuals and groups.

Pigs in intensive farming systems are kept in closed pens and at a high stocking density (Spoolder et al., 2000; Van de Weerd and Day, 2009). It is therefore likely that the expression of an emotional state by one pig is observed by other pigs which may then become emotionally affected by that emotional state as well. That this can happen, is shown in chapter 5 and 6 of this thesis. However, emotional contagion does not have to occur each time emotions are expressed. For instance, a pig may understand at some cognitive level that any stress or fear expressed by a pen mate is not relevant for its own situation and it will therefore not be affected by that negative emotion (Edgar et al., 2012). The pen mate, in turn, may observe that this pig is unaffected by its negative emotional state which the pen mate may even take as social support, intended or not. That social support can occur was shown in chapter 7 of this thesis. This raises an interesting feature of emotional expressions. Is an expression just an expression of one's own emotional state or is the emotion expressed to elicit a response in others? And do others only respond when it was intended to do so or do they respond in any situation? Literature shows that there are situations in pigs where the expression of emotional states is specifically intended for others and that others also respond to this. For instance, piglets may express their state of hunger by emitting specific high and deep grunts and upon hearing these grunts sows respond by exposing the udder (Algers and Jensen, 1985). In addition, piglets may also express their emotional states or needs through high-pitched vocalizations to elicit a response from the sow (Weary et al., 1996; Illmann et al., 2008). Although high-pitched vocalizations are also indicative of a negative emotional state in older pigs (e.g. Manteuffel et al., 2007; Düpjan et al., 2008; this thesis), it is not clear whether these older pigs emit these vocalizations with the intent to elicit a specific response in other pigs as well. In a study on emotional contagion of Düpjan et al. (2011), six-week old test pigs did not negatively respond to playbacks of high-pitched vocalizations of other pigs of the same age in a test room. They proposed that this might have occurred, because these vocalizations are only relevant for mothers and not for pigs of similar age. If so, the test pigs did not respond to the high-pitched vocalizations, because they did not consider these vocalizations as indicative of possible danger that could affect themselves (Düpjan et al., 2011). In chapter 6 of this thesis, the number of high-pitched vocalizations was for instance higher in the situation with the naive pigs present than in the situation with only training pigs present in the test room. Although it is possible that all the vocalizations were emitted by the training pigs, it seems more likely that part of these high-pitched vocalizations also came from the naive pigs. Whether they produced these vocalizations in response to the situation of their pen mates or in response to their own situation cannot be determined, but the former is certainly a possibility in the light of emotional contagion. If so, high-pitched vocalizations are not only relevant for mothers, but also for peers. Considering play behavior, play often induces play in other pigs. At present, there are several theories about the function of play behavior and these theories either consider play behavior as a preparation for certain situations later in life (e.g. training social skills) or as a means to obtain something which has a more immediate purpose (e.g. obtaining information on group mates) (Held and Špinka, 2011). Although some aspects of social play in pigs could be explained by these theories, they do not seem to cover all aspects of social play. For example, pigs may play together to train their social skills or to gather information about their dominance status, but the social play observed in the positive situation in chapter 5 and 6 is not directly explained by these theories. This is because training for social skills is generally considered to occur during the first weeks of life (Schouten, 1986) and these pigs were between 10 and 16 weeks of age and as they were familiar to each other they knew each other's dominance status. So, a pig might start to play with the intention to induce play in others, but this intention is not always evident. Similarly, it is not known whether tail wagging and ears back are just expressions of a pig's own emotional state or whether they are intended to and elicit responses in other pigs. This is because these two behaviors were discovered as indicators of emotional states in this thesis and they have thus not been studied before in relation to emotional states. It would therefore be very interesting to investigate whether pigs pay attention to the ear and tail postures of other pigs and whether pigs may communicate (emotional) information through different ear and tail postures. On the other hand, urine has been found to induce emotional responses in other pigs although such responses may not always be intended. Vieuille-Thomas and Signoret (1992) and Amory and Pearce (2000) found, for instance, that stressful experiences are reflected in the urine of pigs and that other pigs also respond aversively to that urine. Vieuille-Thomas and Signoret (1992) hypothesized that this could be a way of pigs to warn other pigs of possible danger, but they did not actually investigate that. That pigs responded aversively to the urine was explained by the presence of alarm pheromones in the urine (Vieuille-Thomas and Signoret, 1992; Amory and Pearce, 2000). Pheromones have not been studied in this thesis, but as pheromones do play an important role in pig communication (McGlone et al., 1987; Gonyou, 2001; Guiraudie et al., 2003) and as they have been implicated in emotional contagion (Brennan and Zufall, 2006; Dalton et al., 2013), a better understanding of emotional expressions in pigs and their effect on other pigs might be gained by studying pheromones in more detail.

In sum, more research is needed to better understand the social side of emotions and thereby strategies may be developed to improve pig welfare. For instance, sow pheromones could be used to reduce weaning stress (McGlone and Anderson, 2002) or routine management procedures such as castration or tail docking could be performed in a sound proof compartment so that other pigs are not affected by these procedures as long as it is not their turn.

General conclusions

The welfare, health and productivity of pigs are affected by features of their physical environment, but certainly also by their social environment. The importance of the social environment was shown by the differences found between the +SBV and –SBV pigs as these differences suggest that +SBV pigs may be pigs with greater abilities to cope with stress. In addition, the +SBV pigs may even be more 'happy' pigs but that needs to be studied further. Moreover, as the (composition of the) social environment likely plays a role in the degree to which positive and negative emotional contagion and social support occur, the social environment seems, in this respect, also a major factor influencing pig welfare. Thus, to improve pig welfare, health and productivity, attention can be given to features of the physical *or* social environment, but for most optimal outcomes attention should be given to both the physical *and* social environment.

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Summary

Pigs in intensive husbandry systems can experience acute fear or stress during routine management procedures such as castration, tail docking, mixing with unfamiliar pigs, and relocation. Pigs may also suffer from prolonged stress due to limitations in their environment. The absence of proper substrates for oral manipulation in most intensive husbandry systems, for instance, prevents pigs from performing highly motivated behaviors such as rooting and chewing. If pigs are sensitive to the stress expressed by their group mates, stress may spread within a group which then negatively affects the welfare, health and productivity of the whole group. On the other hand, a spread of positive emotions is likely beneficial for group performance. In this thesis, it was investigated to what extent social processes can affect pig welfare, including emotions. This was done by investigating a new breeding method where pigs were selected for their indirect genetic effect on growth, i.e. the heritable influence on their pen mates' growth. The effect of divergent selection for a relatively positive or negative indirect genetic effect on growth of pen members on pig behavior and physiology was studied. If pigs with relatively positive indirect genetic effects for growth are pigs that are less easily stressed, this breeding method might be a strategy to reduce stress in intensively raised pigs. Furthermore, it was investigated to what extent pigs can be affected by the emotional state of their pen mates on the basis of two social processes, emotional contagion and social support. If pigs are affected by the emotional state of their pen mates, it could explain how pigs can affect the growth of their pen mates as prolonged negative emotional states may diminish growth performance in pigs.

In chapter 2, 3 and 4 of this thesis, the effect of the new breeding method on pig behavior and physiology was investigated. Hereto, sows and boars were selected and mated in such a way that half of the offspring was estimated to have a relatively positive indirect genetic effect (+SBV) and the other half a relatively negative indirect genetic effect (–SVB) on the growth of their pen mates during the finishing period (i.e. from 25 kg to 110 kg (slaughter weight)). The offspring was subsequently subjected to a series of tests that have often been used to assess stress and fear in pigs. Tests were carried out before weaning (chapter 2) and after weaning (chapter 3 and 4). In chapter 2, it was found that +SBV piglets were faster than –SBV piglets to touch a novel feeder in a novel object test in the home pen at one week of age and they were more frequently present near a person in a human approach test in the home pen at two and a half weeks of age than –SBV piglets. No differences were, however, found between +SBV and –SBV piglets in their response to an individual novel environment test at three and a half weeks of age. Chapter 3 showed that +SBV pigs were faster than –SBV pigs to touch a novel rope in a novel object test in the home pen at six weeks of age and they showed less locomotion after a bucket was introduced in an individual novel environment test at 13 weeks of age. These pigs did, however, not differ in their response to a human approach test in the home pen at seven weeks of age or in their salivary cortisol response to the novel environment test. In chapter 4, the immune status of the +SBV and –SBV pigs was investigated. Overall, +SBV pigs had lower leukocyte, lymphocyte and haptoglobin concentrations than –SBV pigs. Collectively, these results suggest that +SBV pigs may indeed be pigs that are less easily stressed. After weaning, half of the pigs were housed in relatively barren pens and the other half in pens enriched with straw and wood shavings. Interestingly, the results of chapter 3 and 4 indicate that enrichment also has potential to reduce stress in pigs as enriched housed pigs were faster than barren housed pigs to touch the rope in the novel object test and to approach and touch the person in the human approach test. Moreover, enriched housing pigs also had lower cortisol concentrations than barren housed pigs in response to the novel environment test and they had overall lower haptoglobin concentrations and a lower neutrophil to lymphocyte ratio, but higher natural antibody titers specific for Keyhole Limpet Hemocyanin (KLH) than barren housed pigs. No interactions were found between SBV for growth and housing, suggesting additive effects of the breeding method and housing.

In chapter 2, all piglets were also subjected to a backtest to get an indication of their coping style, i.e. their mode of responding either more actively or passively to a stressor. Piglets that struggle and vocalize relatively much in this test can be classified as high-resisting (HR) or actively responding piglets and piglets that hardly struggle and vocalize can be classified as low-resisting (LR) or passively responding piglets. It was found that +SBV and –SBV piglets did not differ in their response to this test which could indicate that selection on SBV for growth may affect how a pig experiences a stressful situation, but likely not how it subsequently copes with that situation. Furthermore, the response of the piglets in the backtest was found to be related to their response in the other tests. Before weaning, HR piglets were found to walk more than LR piglets in the novel environment test and, after weaning, they were more frequently present near the person in the human approach test and more active (locomotor-wise and vocally) after the introduction of the bucket in the novel environment test than LR pigs. In addition, HR pigs had a higher alternative complement activity and, in the enriched pens, higher natural antibody titers specific for KLH than LR pigs. Besides differences between HR and LR pigs, differences were also found between castrated male pigs (barrows) and female pigs (gilts). Before weaning, barrows were, for instance, later than gilts to

touch the novel object and person, but they also produced more high-pitched vocalizations and defecated and urinated more in the novel environment test than gilts. Similarly, after weaning, barrows were later than gilts to touch the rope in the novel object test and later to approach and touch the person in human approach test, and they were more panicky than gilts in the novel environment test before bucket exposure and paid less attention to the bucket when it appeared. These results suggest that barrows reacted more fearfully to the tests than the gilts. Moreover, barrows had higher basal salivary cortisol concentrations and tended to have higher leukocyte, but lower haptoglobin concentrations than gilts, which may point to physiological differences in dealing with stress.

In chapter 5, 6 and 7, emotional contagion and social support were investigated. In chapter 5 and 6, emotional contagion was studied during anticipation and during experience of a positive and negative situation. In both chapters, two pigs per pen were trained to associate one cue with the positive situation (i.e. pairwise access to a relative large compartment filled with straw and peat in which chocolate raisins were hidden) and another cue with the negative situation (i.e. isolation in a relative small compartment combined with other negative handlings such as restraint with a nose sling) in a test room. Thereafter, two of their pen mates, habituated to the cues and test room but naive with respect to the situations, joined the training pigs to the test room. Both in chapter 5 and 6, evidence for emotional contagion was found and this was most clear while the training pigs were exposed to the positive (chapter 5) and negative situation (chapter 5 and 6). During anticipation, emotional contagion was overall less clear, but that could be due to very large individual differences in anticipatory behavior. Furthermore, two new possible indicators for emotional state were found in chapter 5 with tail wagging indicative for a positive emotional state and ears back for a negative emotional state.

Social support was studied in chapter 7. Pigs were individually restrained in a weighing cage in a test room for 15 min to induce acute stress. Half of these pigs were tested alone, whereas the other half was accompanied by a pen mate. The coping style of these pigs was also determined from their response to the backtest. From the results it was evident that restraint induced acute stress in all pigs (e.g. pigs vocalized much and had an increased heart rate and increased salivary cortisol response), but HR pigs expressed this acute stress more actively (e.g. more escape attempts and more vocal) and LR pigs more passively (e.g. more standing alert and ears back). Moreover, LR pigs seem to have benefitted more from social support than HR pigs as LR pigs had a lower stress response when a pen mate was present during the test than when tested alone as compared to HR pigs.

In conclusion, the results of chapter 2, 3 and 4 suggest that both the breeding method and environmental enrichment have the potential to reduce fear and stress in pigs and additionally, that breeding and enrichment combined will likely yield the best results for pig welfare, health and productivity. However, the experiments in these chapters entailed a single generation of divergent selection for indirect genetic effects on growth only. Therefore more research is needed to confirm this. Chapter 5, 6 and 7 provide evidence for emotional contagion and social support in pigs. Pigs may thus potentially affect the growth of their pen mates but also their health and welfare by affecting their emotional state.

Samenvatting

In de intensieve veehouderij kunnen varkens acute angst of stress ervaren tijdens de standaard uitgevoerde handelingen zoals castratie, staart couperen, mengen met onbekende varkens, en verplaatsen. Naast acute stress kunnen varkens ook last hebben van chronische stress door beperkingen in hun omgeving. Door de afwezigheid van goed substraat, zoals stro en zaagsel, zijn varkens bijvoorbeeld niet in staat om voor hen belangrijke gedragingen, zoals wroeten en kauwen, uit te voeren. Als varkens gevoelig zijn voor de stress van hun groepsgenoten, dan kan dat overslaan op de hele groep varkens met negatieve consequenties voor hun welzijn, gezondheid en productiviteit als gevolg. Aan de andere kant, als varkens gevoelig zijn voor positieve emotionele uitingen van hun groepsgenoten dan kan dat een positieve uitwerking hebben op de hele groep. In dit proefschrift is onderzocht in welke mate sociale processen het welzijn van varkens en hun emoties kunnen beïnvloeden. Dit is gedaan door de effecten op gedrag en fysiologie van een nieuwe fokmethode te onderzoeken. Bij deze fokmethode worden varkens gefokt op hun erfelijk effect op de groei van hun groepsgenoten. Varkens met een relatief positief genetisch effect op de groei van hun groepsgenoten zouden varkens kunnen zijn die minder snel gestrest zijn dan andere varkens. In dat geval zou deze fokmethode een manier zijn om stress in varkens in de intensieve veehouderij te verminderen. Daarnaast is onderzocht of varkens beïnvloed worden door de emoties van hun groepsgenoten op basis van twee processen, *emotional contagion* (een simpele vorm van empathie) en *social support* (het bieden van steun aan een individu tijdens een stressvolle ervaring, zodat dat individu zich minder gestrest voelt). Als varkens gevoelig zijn voor de emoties van hun groepsgenoten dan zou dat een verklaring kunnen zijn voor het erfelijke effect dat varkens hebben op de groei van hun groepsgenoten, omdat chronisch negatieve emoties een reducerend effect hebben op de groei van varkens.

In hoofdstuk 2, 3 en 4 van dit proefschrift is het effect van de nieuwe fokmethode op het gedrag en de fysiologie van varkens onderzocht. Hiervoor werden zeugen en beren op zo’n manier gepaard dat de helft van de nakomelingen een relatief positief genetisch effect (+SBV (*Social Breeding Value*)) en de andere helft een relatief negatief genetisch effect (–SBV) zou hebben op de groei van hun groepsgenoten tijdens de vleesvarkensfase (d.w.z. van 25 kg tot 110 kg (slachtgewicht)). Deze nakomelingen werden vervolgens onderworpen aan een aantal standaard stress- en angsttesten. In hoofdstuk 2 werd gevonden dat +SBV biggen sneller waren dan –SBV biggen in het aanraken van een onbekende voerbak in een *novel object test* uitgevoerd in het eigen hok op de leeftijd van één week oud. Verder waren +SBV biggen ook vaker aanwezig bij een persoon gedurende een *human approach test* uitgevoerd in het eigen hok op de leeftijd van tweeënhalve week. Op de leeftijd van drieënhalve week verschilden +SBV en –SBV biggen echter niet in gedrag in een individuele *novel environment test*. Hoofdstuk 3 liet zien dat +SBV varkens ook sneller een touw aanraakten dat was opgehangen in het eigen hok dan –SBV varkens in een *novel object test* op de leeftijd van zes weken. Gedurende een individuele *novel environment test* op de leeftijd van 13 weken liepen +SBV varkens minder rond in de arena dan –SBV varkens nadat halverwege de test een emmer in de arena werd neergelaten. Er was geen verschil tussen +SBV en –SBV varkens in een *human approach test* op de leeftijd van zeven weken noch in hun speekselcortisol respons op de *novel environment test*. In hoofdstuk 4 is het immuunsysteem van de +SBV en –SBV varkens onderzocht. Er werd gevonden dat +SBV varkens lagere leukocyt, lymfocyt en haptoglobine waardes hadden dan de –SBV varkens. Al deze resultaten samen suggereren dat +SBV varkens inderdaad varkens kunnen zijn die minder snel gestrest zijn. Na spenen (het scheiden van de biggen van de zeug ca. vier weken nadat de biggen geboren zijn) werden de varkens gehuisvest in twee verschillende typen hokken. De helft van de varkens werd gehuisvest in standaard, vrij kale hokken en de andere helft in hokken verrijkt met een laag stro en zaagsel. Interessant is dat de resultaten van hoofdstuk 3 en 4 erop wijzen dat ook verrijking mogelijk stress kan verlagen in varkens, omdat de varkens in de verrijkte hokken sneller dan de kaal gehuisveste varkens een touw aanraakten in de *novel object test* en sneller waren in het benaderen en aanraken van een persoon in de *human approach test*. Bovendien bleken de verrijkte varkens lagere cortisol waardes te hebben dan de kaal gehuisveste varkens in reactie op de *novel environment test,* en ze hadden ook lagere haptoglobine waardes en lagere neutrofiel lymfocyt ratios. Daarentegen hadden de verrijkte varkens hogere natuurlijke antistoffen tegen Keyhole Limpet Hemocyanin (KLH, een eiwit uit de Californische zeeslak dat vaak wordt gebruikt als antigeen model in immunologische studies) dan de kaal gehuisveste varkens. Er werden geen statistische interacties gevonden tussen de fokmethode en de huisvesting wat suggereert dat het effect van deze fokmethode en huisvesting additief zijn.

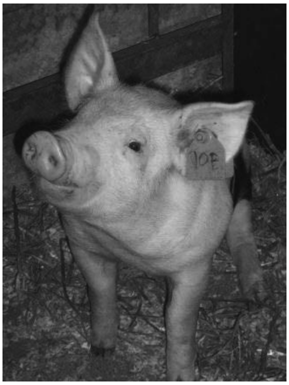
In hoofdstuk 2 werden alle biggen ook onderworpen aan een rugtest waarmee een indicatie van hun persoonlijkheid, d.w.z. een meer proactieve of meer reactieve respons op een stressor, verkregen kan worden. Biggen die veel spartelen en vocaliseren tijdens deze rugtest worden geclassificeerd als *high-resisters* (HR) en biggen die niet tot weinig spartelen en vocaliseren worden geclassificeerd als *low-resisters* (LR). De +SBV en –SBV biggen verschilden niet in hun reactie op deze rugtest wat zou kunnen betekenen dat het fokken op SBV voor groei een effect kan hebben op hoe een varken een stressvolle situatie ervaart, maar waarschijnlijk geen effect heeft op hoe een varken met die situatie omgaat. Verder werd gevonden dat de reactie van de varkens in de rugtest gerelateerd was aan hun reactie in de andere testen. In de *novel environment test* vóór spenen liepen de HR biggen meer dan de LR biggen, en na spenen waren de HR varkens meer aanwezig bij een persoon in de *human approach test* en bewogen en vocaliseerden ze meer in de *novel environment test* na de introductie van de emmer dan de LR varkens. Daarnaast hadden de HR varkens een hogere complement activiteit en hadden de verrijkte HR varkens hogere antistof titers tegen KLH dan de LR varkens. Naast deze verschillen tussen HR en LR varkens werden ook verschillen gevonden tussen vrouwtjes (gelten) en gecastreerde mannetjes (borgen). Vóór spenen waren de borgen bijvoorbeeld later dan de gelten in het aanraken van een nieuwe voerbak en van een persoon, maar de borgen vocaliseerden ook meer en poepten en plasten meer dan de gelten in de *novel environment test*. Ook na spenen waren de borgen later dan de gelten in het aanraken van een touw in de *novel object test,* en later in het benaderen en aanraken van een persoon in de *human approach test*. Bovendien waren de borgen meer in paniek dan de gelten in de *novel environment test* in de periode voor de emmer en minder geïnteresseerd in de emmer toen deze verscheen. Deze verschillen suggereren dat de borgen angstiger reageerden in deze testen dan de gelten. Ook werd gevonden dat de borgen een hogere basale speekselcortisol waarde, en hogere leukocyt waardes, maar lagere haptoglobine waardes, hadden dan de gelten. Deze resultaten kunnen erop wijzen dat borgen en gelten fysiologisch gezien anders omgaan met stress.

In hoofdstuk 5, 6 en 7 werden *emotional contagion* en *social support* onderzocht. In hoofdstuk 5 en 6 werd *emotional contagion* onderzocht tijdens anticipatie en tijdens een positieve en negatieve ervaring. In beide hoofdstukken werd aan twee varkens per hok geleerd om een signaal te koppelen aan een positieve ervaring (d.w.z. samen zijn in een relatief grote ruimte gevuld met een laag stro en turf waarin chocoladerozijntjes lagen) en een ander signaal aan een negatieve ervaring (d.w.z. sociale isolatie in een relatief kleine ruimte gecombineerd met andere negatieve handelingen zoals immobilisatie met een strop) in een testruimte. Daarna gingen twee van hun groepsgenoten, die gewend waren aan de signalen en de testruimte maar geen weet hadden van de positieve en negatieve ervaring, mee met de getrainde varkens naar de testruimte. Vervolgens werd het gedrag van de getrainde varkens en hun groepsgenoten geanalyseerd. Op basis van dit gedrag werd zowel in hoofdstuk 5 als 6 bewijs gevonden voor *emotional contagion* en dit was het duidelijkst tijdens de positieve (hoofdstuk 5) en negatieve ervaring (hoofdstuk 5 en 6). Tijdens anticipatie was *emotional contagion* niet echt duidelijk te zien, maar dat kan komen door grote individuele verschillen in gedrag van de varkens tijdens anticipatie.

Social support is onderzocht in hoofdstuk 7. Hiertoe werden varkens voor 15 min individueel in een veeweegschaal in een testruimte gezet om acute stress op te wekken. De helft van de varkens werd in hun eentje getest, maar bij de andere helft was een groepsgenoot aanwezig in de testruimte. Van ieder varken was ook de persoonlijkheid bepaald met de rugtest. De resultaten lieten duidelijk zien dat alle varkens gestrest waren tijdens de 15 min in de weegschaal omdat ze allemaal veel vocaliseerden en een verhoogde hartslag en speekselcortisol reactie hadden. Echter, de HR varkens gingen meer actief (b.v. meer ontsnappingspogingen en meer vocalisaties) en de LR varkens meer passief (b.v. meer immobilisatie en oren vaker naar achteren gepositioneerd) om met deze stress. Bovendien leken de LR varkens meer *social support* te hebben gehad van hun groepsgenoot dan de HR varkens, omdat de LR varkens een lagere stress respons lieten zien dan de HR varkens in aanwezigheid van een groepsgenoot dan wanneer er geen groepsgenoot bij was.

Concluderend, de resultaten van hoofdstuk 2, 3 en 4 suggereren dat zowel de nieuwe ‘sociale’ fokmethode als een verrijkte omgeving angst en stress in varkens kunnen verlagen en dat het toepassen van beide waarschijnlijk het beste is voor het welzijn, de gezondheid en de productiviteit van varkens. Echter, omdat het in de experimenten van deze hoofdstukken gaat om een eerste generatie van varkens geselecteerd met deze fokmethode, is meer onderzoek nodig om deze conclusie te kunnen bevestigen. Hoofdstuk 5, 6 en 7 bieden bewijs voor *emotional contagion* en *social support* in varkens. Varkens zouden dus mogelijk de groei, maar ook de gezondheid en het welzijn van hun groepsgenoten kunnen beïnvloeden via hun emotionele reacties.

Dankwoord



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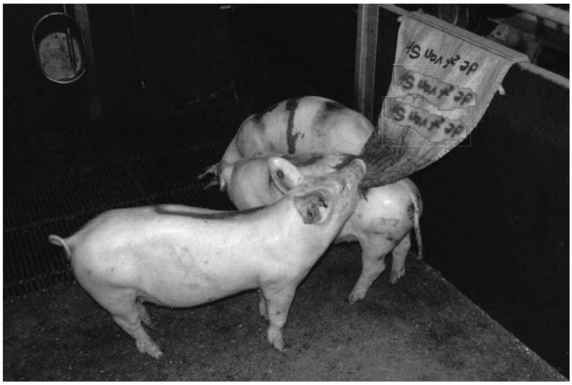
En ‘last, but not least’, de varkens. Jullie waren het niet altijd eens met het verloop van het experiment, maar jullie hebben je best gedaan en ik hoop dat in ieder geval een deel van jullie genoten heeft van het stro, de turf en de chocolade rozijntjes. Ik heb in ieder geval genoten van jullie!

Curriculum Vitae

Inonge Reimert was born on 22 June 1985 in Senanga in Zambia. Before her first birthday, she returned with her family to the Netherlands. She went to secondary school in Leeuwarden and thereafter to Groningen University. For both her Bachelor Biology and her Masters Behavioural Cognitive Neurosciences she obtained the degree *cum laude*. In her Masters, she investigated the effect of ambient temperature on lactation performance in voles. In addition, she studied the effect of in vitro fertilization on the neuromotor development of 18 months old infants. After her Masters, she did a PhD at the Adaptation Physiology Group (ADP) of Wageningen University to investigate the behavior and physiology of pigs divergently selected for indirect genetic effects on the growth of their pen mates. This research was part of the project ‘Seeking sociable swine? Incorporating social genetic effects into pig breeding programs to achieve balanced improvement in productivity and welfare’ which was financially supported by the program ‘The Value of Animal Welfare’ of the Netherlands Organization for Scientific Research (NWO) and the Dutch Ministry of Economic Affairs. The results of this PhD project have been presented in this thesis and at 12 (inter)national conferences and seminars. Her presentation on pig emotions and emotional contagion at the 46th International Congress of the International Society for Applied Ethology in Vienna attracted a lot of attention and that led to an invitation to present these results also at the Joint Meeting of the 33rd International Ethological Conference & the Association for the Study of Animal Behaviour in Newcastle-Gateshead. She was also nominated for the award for best poster during the WIAS Science Day in 2012. Inonge has written six first author papers of which four have been published in and two submitted to a scientific journal, and she will be a co-author of nine other scientific papers. Her work also received attention in (inter)national popular media, with, for instance, an article in the French newspaper *Le Monde Science et Techno* and in the German magazine *SUS* (Magazin für Schweinezucht und Schweinemast). Apart from these scientific activities, Inonge was also involved in educational activities by supervising MSc students and giving lectures. Furthermore, Inonge has been an active member of the WIAS Associated PhD (WAPS) Council for two years and she took part in the organization of three symposia: the WIAS seminar ‘Scientific Research in Animal Welfare: Do we make a difference?’ in 2011, the symposium ‘Vreedzame Varkens’ in 2012, and a master class ‘Zicht op staartbijten’ in 2013. Inonge is currently appointed as a Postdoctoral researcher at ADP to continue studying the behavior and physiology of pigs. In addition, she was granted a WIAS fellowship to write a Postdoc proposal about facial (emotional) expressions in pigs. Therefore she will remain working at ADP until at least the end of 2014. Inonge can be reached at: inonge.reimert@wur.nl.

Publications



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Camerlink I, Reimert I, Duijvesteijn N, Benard M, Ursinus WW, Bijma P, Bolhuis JE, Kemp B, Van Arendonk JAM. 2011. Improving pig welfare and production through social genetic effects (poster). Symposium ‘The Value of Animal Welfare’, 30 November, Utrecht, The Netherlands.

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Zes tips voor langere staarten. 2013. Varkens.nl, 17 May.

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Barthémèly P. 2013. Comment reconnaître un cochon heureux? Le Monde Science et Techno, 2 February.

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Rotgers G. 2012. Varkenshouder en onderzoeker wantrouwen elkaar. V-focus, 9:38-39.

Project Vreedzame varkens gaat testen of staartbijten is aan te pakken met fokkerij. Fokken tegen staartbijtgedrag. 2012. Boerderij, 27:43-43.

Varken wil socializen. 2012. Varkens, 76:4-4.

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Education Certificate

WIAS Training and Supervision Plan

Description	Year
The Basic Package (4.2 ECTS[1])	
WIAS Introduction Course, Wageningen, The Netherlands	2010
EZ-NWO course Sustainable Animal Production, Lunteren, The Netherlands	2010
EZ-NWO course Ethics & Animal welfare, Lunteren, The Netherlands	2011
EZ-NWO course Market & Animal Welfare, Spaarnwoude, The Netherlands	2012
EZ-NWO course Governance & Policy Advice, Den Haag, The Netherlands	2013
International Conferences (4.3 ECTS)	
44th International Congress of the International Society for Applied Ethology (ISAE), Uppsala, Sweden	2010
46th International ISAE Congress, Vienna, Austria	2012
47th International ISAE Congress, Florianopolis, Brazil	2013
33rd International Ethological Conference (IEC), Newcastle-Gateshead, UK	2013
Benelux ISAE Congress, Sterksel, The Netherlands	2013
Seminars and Workshops (4.0 ECTS)	
WIAS Science Day, Wageningen, The Netherlands	2010 - 2012
WUR Symposium 'Pigs: The missing link?', Lelystad, The Netherlands	2010
PhD Workshop of the Annual Meeting of the Netherlands Society for Behavioural Biology (NVG), Soesterberg, The Netherlands	2010
Annual Meeting NVG, Soesterberg, The Netherlands	2011 - 2012
NWO Symposium 'Waardering voor Dierenwelzijn', Utrecht, The Netherlands	2011
Vreedzame Varkens Symposium, Sterksel, The Netherlands	2012
Connecting Conspecifics Colloquium, Edinburgh, UK	2012
WUR Symposium 'Genetics of social life: Agriculture meets evolutionary biology', Wageningen, The Netherlands	2013
Interactieve Masterclass: Zicht op staartbijten, Sterksel, The Netherlands	2013
NWO Symposium 'Waardering voor Dierenwelzijn', Den Haag, The Netherlands	2013
Presentations (12 ECTS)	
Oral presentation, PhD Workshop of the NVG, Soesterberg, The Netherlands	2010
Oral presentation, international pig meeting, Bristol, UK	2011
Oral presentation, NVG, Soesterberg, The Netherlands	2011
Poster presentation, NWO symposium, Utrecht, The Netherlands	2011
Poster presentation, WIAS Science Day, Wageningen, The Netherlands	2012
Oral presentation, Vreedzame Varkens Symposium, Sterksel, The Netherlands	2012
Oral presentation, Connecting Conspecifics Colloquium, Edinburgh, UK	2012
Oral presentation, 46th ISAE, Vienna, Austria	2012
Poster presentation, NVG, Soesterberg, The Netherlands	2012
Oral presentation, 47th ISAE, Florianopolis, Brazil	2013
Oral presentation, 33rd IEC, Newcastle-Gateshead, UK	2013
Oral presentation, ISAE Benelux, Sterksel, The Netherlands	2013

In Depth Studies (9.3 ECTS)

WIAS Advanced Statistics Course: Design of Experiments, Wageningen, The Netherlands	2010
Trends in Stress Biology: Interpretation of Animal Stress Responses, Viborg, Denmark	2011
WIAS/VLAG course Epigenesis & Epigenetics, Wageningen, The Netherlands	2011
WIAS course Statistics for the Life Sciences, Wageningen, The Netherlands	2012
WIAS Animal Welfare Discussion Group, Wageningen, The Netherlands	2010 - 2012

Statutory Courses (5.5 ECTS)

Use of Laboratory Animals, Wageningen, The Netherlands	2010
Laboratory Use of Isotypes, Wageningen, The Netherlands	2011

Professional Skills Support Courses (3.6 ECTS)

WGS course Techniques for Writing and Presenting a Scientific Paper, Wageningen, The Netherlands	2010
WGS course Information Literacy, including Introduction Endnote, Wageningen, The Netherlands	2010
ESD course Afstudeervak organiseren en begeleiden, Wageningen, The Netherlands	2011
WGS course Effective behaviour in your professional surroundings, Wageningen, The Netherlands	2011
WGS course Career assessment, Wageningen, The Netherlands	2013

Didactic Skills Training (15.4 ECTS)

Assisting practical of MSc course ADP-2	2010 - 2011
Reviewing papers and research proposals of Research Master Cluster students	2011
Lecture MSc course Health, Welfare and Management	2012 - 2013
Lecture BSc course Immunology and Thermoregulation	2013
Supervising 8 MSc students	2010 - 2014

Management Skills Training (7.0 ECTS)

Member of WIAS Associated PhD Council	2011 - 2012
Organization WIAS Seminar 'Scientific Research in Animal Welfare: Do we make a difference?'	2011
Organization Vreedzame Varkens Symposium	2012
Organization Interactieve Masterclass: Zicht op staartbijten	2013

Education and Training total	**65 ECTS**

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[1] 1 ECTS credit equals a study load of approximately 28 hours

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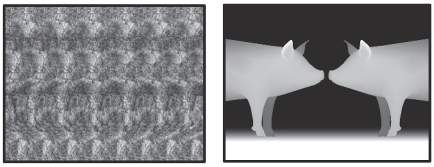

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