Department for Farm Animals and Veterinary Public Health
University of Veterinary Medicine Vienna
Institute of Animal Husbandry and Animal Welfare
(Head: Univ.-Prof. PhD Jean-Loup Rault)

Tickled Pink? The effect of positive human contact on pigs' judgement biases

Master Thesis
Interdisciplinary Master in Human-Animal Interactions

submitted by
Katharine Creagh, BA

Vienna, March 2021

Supervisor 1:

Univ.-Prof. Jean-Loup Rault, PhD

Institute of Animal Husbandry and Animal Welfare

Department for Farm Animals and Veterinary Public Health

University of Veterinary Medicine

Veterinärplatz 1

1210 Vienna, Austria

Supervisor 2:

Dr.med.vet. Sara Hintze MSc. Ph.D

Division of Livestock Sciences

Department of Sustainable Agricultural Systems

University of Natural Resources and Life Sciences

Gregor-Mendel-Strasse 33, 1180, Vienna, Austria

Plagiarism disclaimer

I declare that this thesis was written by me and in my own words, using no sources other than those indicated or aids other than those permissible. The sources of all incorporated material, literally or logically, or a paraphrase of such material are exactly cited in text and in the reference list. The same applies to all web sources, additionally including the URL and date of access. I am conscious that any case of plagiarism leads to non-evaluation of the whole thesis and has to be reported to the coordinator of the study program. I further certify that I have not already submitted this work somewhere else.

Vienna, March 2021

Place / Date / Signature

Vienna / 22.03.2021 / Katharine Creagh 

Abstract

Previous research shows that positive human-animal interactions can improve animals' affective states, but the mechanisms underlying this change remain poorly understood. This study aimed to disentangle the effect of putative mood- and emotion-inducing treatments on pigs' responses in a judgement bias task. We studied 30 weaner pigs by administering the same five-minute positive contact treatment, either repeatedly over the course of three weeks (long-term positive contact: LTPC; intended to influence mood, n = 11) or immediately before a judgement bias test (short-term positive contact: STPC; intended to influence emotion, n = 10), while a control group (CON, n = 9) received no positive contact sessions. In addition to their daily positive contact sessions, LTPC pigs received STPC treatment sessions before 50% of test sessions to elucidate any interaction between emotion and mood. Pigs were trained to perform a spatial Go/No-go judgement bias test (JBT) with active trial initiation and the percentage of Go responses to ambiguous cues in the JBT was compared across treatments. STPC contact sessions were video recorded and coded for contact latency and duration, and saliva was collected pre- and post-testing in a sub-set of animals to measure cortisol concentration as an indicator of arousal. Data were analysed with generalised linear mixed-effect models. In the JBT, pigs went to the ambiguous positive and middle cues on average in over 90% of the trials and to the ambiguous negative cue in over 60% of the trials. Thus, the expected monotonically graded curve in responses was not present. This is surprising since other studies using a comparable task design with active trial initiation have found such graduated response patterns. We did not find any statistically significant differences between the treatment groups with respect to the pigs' behaviour during STPC treatment sessions, irrespective of their long-term treatment (LTPC vs. STPC or control), pigs' performance in the JBT, nor any differences in cortisol concentrations pre- and post-testing. The regular human handling for training and testing may have overwritten effects of the human contact treatments, rendering their effects undetectable. Further research is warranted to disentangle the effect of potential interactions between the training procedure and treatments including human-animal interactions.

Acknowledgements

Throughout the duration of this master's project I have received a great deal of support and assistance. I would first like to thank my supervisors, Dr. Sara Hintze and Prof. Jean-Loup Rault, who designed the study. Their expertise was invaluable during the planning, data collection and writing of my thesis. I thank them particularly for their patient support and help with running the statistical analysis of the data, and their helpful edits and suggestions when writing the paper.

I would also like to thank Christian Haberl, for building the apparatus and providing technical support, Maximilian Baldy, who executed the pilot experiment that this thesis was based on, Hana Volkmann, for help with collecting saliva samples and who conducted the lab analyses of the samples, Marie Teresa Martinez Navarrete for her help and advice for writing of the results section, Dr.med.vet. Doris Verhovsek and all of the VetFarm Medau staff, particularly Nikolic Sladjan, Sylvia Posseth and Tamara Strini, for their help and support during data collection. Thank you to Denise Kubala for providing the German translation for the summary.

Finally I would like to thank my family, particularly my husband, Mustafa, for their unending emotional support during the writing of my thesis.

Table of Contents

1. Introduction

1.1. Background to the study

In recent years, animal welfare research has moved its aims beyond alleviating suffering to incorporating opportunities for positive experiences. Promoting positive emotions in animals under our care can be viewed as one end of a welfare continuum, moving away from negative states such as pain and distress (Yeates & Main, 2008). In order to empirically assess such concepts, much research has focused on the "core affective state" (Russell, 2003), which is made up of the individual's background mood, often coupled with any current emotions or emotional response towards an event or stimulus (Mendl et al., 2010).

Emotions typically are intense but of limited duration, following a specific stimulus or event, while moods are of lower intensity, last longer, and are more ambiguous (Schnall, 2010). Emotions and moods have a bi-directional relationship, and it is thought that the cumulative effect of short-term emotional experiences can affect the longer-term background mood, while the individual's mood may affect how emotional events are appraised (Mendl et al., 2010). Therefore, any attempt to promote positive welfare in animals must take into account both short-term ("emotion-inducing") measures, and the accumulation of such measures ("mood-inducing") across the longer-term.

Research aimed at assessing the affective states of animals has often relied on physiological measures such as heart rate variability (Désiré et al., 2004; Coulon et al., 2015; Zupan et al., 2016) or levels of cortisol (Pederson et al., 1998), behavioural measures such as the assessment of spontaneous behaviour (Wemelsfelder et al., 2000), or behaviour under experimental conditions such as open field tests, elevated plus maze tests, novel object tests, and approach tests (Hemsworth et al., 1996a; Kooij et al., 2002; Donald et al., 2011; Rutherford et al., 2012), or a combination of these measures (Rutherford et al., 2006; Ralph et al., 2018). However, such research is not without its limitations, and reviews of these measures in pigs have found much inconsistency and ambiguity in results (Forkman et al., 2007; Murphy et al., 2014). One of the possible explanations for such inconsistencies within the literature is the interpretation of the results. Physiological measures may be indicative only of arousal states, an example of this being cortisol, which increases similarly in

situations that could be perceived as either positive or negative (Hubert et al., 1993; Mendl et al., 2009). Behavioural tests, such as open field tests, have similar problems of interpretation, as a lot of movement around the arena is interpreted by some researchers as high levels of explorative activity, while others might interpret such results to be indicative of anxiety (Murphy et al., 2014).

Affective states exist within a two-dimensional spectrum of arousal and valence (Mendl et al., 2010; see Figure 1). Thus, in order to avoid problems of interpretation and ambiguity, any attempt to measure emotions and mood requires consideration not only of the subject's level of arousal, but also whether the affective state is positively or negatively valenced.

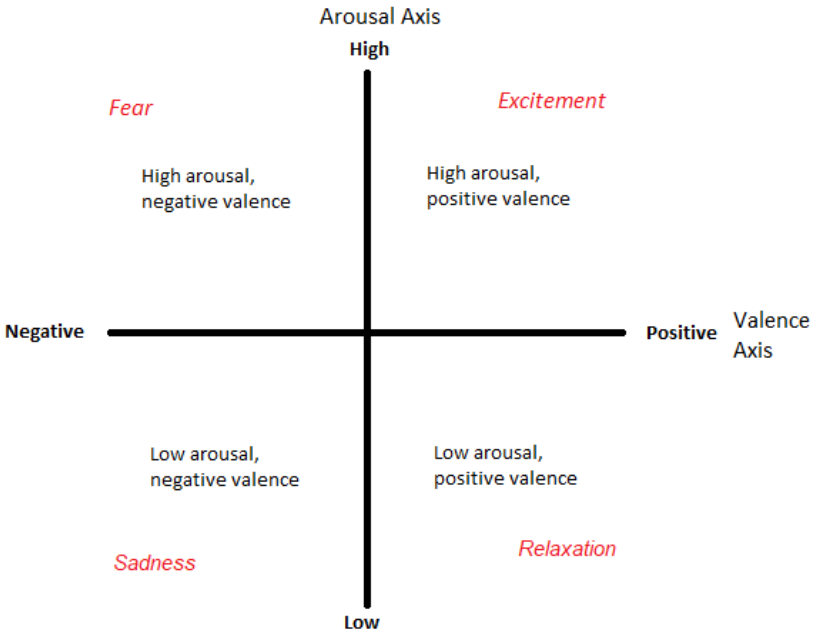

Figure 1: Dimensional aspects of affect, adapted from Mendl et al., 2010. The X axis represents the valence of the affective state, while the Y axis represents states of arousal. Examples of discrete emotional states are shown in red.

The valence of an individual's affective state influences several cognitive processes, including attention, memory and judgement (Mendl et al., 2009). With this knowledge, researchers have been able to develop a number of cognitive bias tests, which can act as a proxy for internal affective states, often inaccessible otherwise. Attention and memory bias

tests are common in psychological research on human mood and emotion. For example, humans in a positive affective state are more likely to pay more attention to positive stimuli (Tamir & Robinson, 2007), while sad moods can cause bias towards remembering negatively valenced words (Chepenik et al., 2007). Many studies on cognitive biases in animals have used judgement bias tests (JBTs) to measure the subject's valence through responses to ambiguous cues presented between a known "positive" outcome and a known "negative" outcome. It is thought that positive affective states will lead to "optimistic" choices when presented with ambiguous stimuli, while negative states will lead to "pessimistic" choices (Mendl et al., 2009). These assumptions are based on the idea of ecological fitness, i.e., when an animal is in an environment where threat is high (eliciting physiological stress responses), it is more ecologically advantageous to interpret ambiguous stimuli as potentially threatening, but in an environment where the threat is low (and therefore the animal is presumably in a relaxed state), the interpretation of ambiguous stimuli as potentially positive may lead to fitness-increasing outcomes, such as a new food source etc. (Mendl et al., 2009).

The JBT was first developed for non-human animals by Harding and colleagues (2004) who trained rats to discriminate between two tones (4 kHz or 2 kHz) and press a lever in response to one tone to receive a food reward or to refrain from pressing the lever in response to the other tone to avoid a punishment. Once they had learned this discrimination, the rats were exposed to novel tones (between the frequencies of the operantly trained tones, i.e. the "reference" cues) and their responses to these novel, ambiguous cues were recorded. Rats that had been housed in unpredictable housing conditions (inducing a depression-like state) showed a "pessimistic" judgement bias, in that they pressed the lever less often in response to the ambiguous tones, reflective of human studies that showed people in negative states such as depression or anxiety were more likely to judge ambiguous stimuli as negative.

As the JBT is made up of several components (cues, responses, reinforcers, affect manipulation), it allows researchers a large degree of flexibility to make species-appropriate modifications, to investigate a variety of research questions. Several types of JBT have been developed, including Active Choice Tasks (ACTs), where an animal is trained to discriminate between two levels of reinforcer (that correspond to a larger or smaller reward), and Go/No-go tasks, where the animal is trained to move towards a spatial cue that corresponds with a

reward (“Go”), or not to approach a cue that corresponds with either no reward or a punishment (“No-go”). Go/No-go tasks have been more commonly applied to animals due to the more cognitively challenging nature of the ACT where the animal is required to distinguish between two different cues (for example two tones), and pair these with the location of different sizes of reward (Murphy et al., 2013). This complexity however does make the ACT less susceptible to habitual responding, i.e. a response to any stimuli as opposed to a goal-directed action (Lagisz et al., 2020), as the animal always needs to actively respond by making a choice. The Go/No-go task, on the other hand, requires only that the animal learns the rewarded and unrewarded cues (for example which location is rewarded and which is not in a spatial Go/No-go task), but has the disadvantage that the animal may show a No-go response due to low arousal, distraction, confusion, or simply lack of motivation (Bethell, 2015), instead of due to an evaluation of a cue as potentially unrewarded or punished.

More recently, Hintze et al. (2018) developed a spatial Go/No-go task for use across multiple species that overcomes some of these problems by incorporating active trial initiations into the study design. They trained their subjects to initiate each trial by a signal in the experimental arena (such as nosing a plastic bottle for horses or the use of an infrared “nose poke” device for rodents), which the authors state considerably reduced the amount of training, in terms of length and number of sessions, compared to their earlier studies. Additionally, the trial initiators reduce ambiguity in responses as the animal is always required to show an active response (Go response or a trial re-initiation) and may even increase potential number of trials by reducing waiting times during the negative trials (Hintze et al., 2018). In order to perform the test, the animal is first operantly trained to use the trial initiator and go to a rewarded location, and later trained to discriminate between two spatially distinct locations, or “goals”, one baited with a food reward, the other not. During the testing phase, the animal initiates a trial (for example by nosing an object) and then is presented with either one of the non-ambiguous, or “reference”, goals, or a novel, ambiguous goal between the two reference goals. The animal should move towards the ambiguous goal if they expect it to be baited (the “optimistic” choice) but are less likely to do so if they anticipate no reward

(the “pessimistic” choice). The percentage of Go responses to the ambiguous goals is therefore used as an indication of the animal’s emotional valence.

JBTs have been used in the assessment of affective states in farm animals after a variety of events common to production systems such as restraint (Doyle et al., 2010a), shearing in sheep (Sanger et al., 2011) and disbudding in calves (Neave et al., 2013), or multiple aversive events common in animal husbandry (Doyle et al., 2011). They have also been used to assess different housing conditions, such as stocking density (Scollo et al., 2014), enriched housing (Asher et al., 2016; Douglas et al., 2012, Zidar et al., 2018), or isolation vs. pair housing (Bučková et al., 2019). An animal’s affective state may be influenced by previous experience with handling (Grandin & Shivley, 2015), however, only a few studies have explored the effects of different handling treatments on animals’ judgement biases. Brajon et al. (2015a) found that piglets who had experienced long-term gentle human contact were more likely to approach ambiguous cues during a Go/No-go JBT than piglets who had rough, or minimal handling. On the other hand, Carreras et al. (2017) did not find a significant difference in responses to a JBT between pigs who had received long-term positive handling and pigs who had received negative handling across the same time frame. Baciadonna et al. (2016) used a JBT to determine if a short-term positive human-animal interaction (grooming) induced a positive affective state in goats, but found no positive bias effect after the animals had been groomed.

In a recent review of 71 judgement bias studies, Lagisz et al. (2020) found 44 studies on long-term treatments and 29 studies that looked at treatments immediately before or during testing (i.e. short-term treatments). This meta-analysis found that timing of affect manipulation (short- vs. long-term) did not appear to significantly influence the magnitude of judgement bias effects between the different studies analysed. However to date, no study has compared the same treatment across both long- and short-term time frames.

Long-term positive interactions can result in a positive human-animal relationship (HAR; Waiblinger et al., 2006), defined as “the degree of relatedness or distance between the animal and the human, i.e., the mutual perception, which develops and expresses itself in their mutual behaviour” (Estep & Hetts, 1992, p. 6). The importance of the HAR has been illustrated in studies by researchers such as Coulon et al. (2015), who found evidence that

gentle physical contact with a familiar human is perceived as positive by lambs, and Waiblinger et al. (2004), who found that dairy cows who had previously experienced gentle interactions showed calmer behaviour and reduced heart rate during a veterinary procedure. Daily handling appears to be effective for improving the HAR in agricultural animals. Schmied et al. (2008) found that daily gentle stroking reduced avoidance of and increased approach reactions to humans in cattle, while Hemsworth et al. (1996a) observed that pigs subjected to a positive handling treatment for five minutes, five days per week over four weeks, were quicker to approach and interact with a human than those who did not have previous handling experience. Additionally, gentle handling of farm animals by caretakers is associated with reduced physiological indicators of stress such as cortisol concentration (Pedersen et al., 1998).

Short-term positive interactions can also have a powerful effect on an animal's behaviour and affective state. Muns et al. (2015) found that short-term positive human contacts early in life can affect the behavioural responses of piglets to stressful events later in life, and Lürzel et al. (2020) found that short, positive interactions with a human increased salivary oxytocin levels in pigs and cattle. Rault et al. (2019) found evidence that at least a short-term positive state (measured by behavioural responses and EEG) could be induced in pigs by short bouts of opportunistic belly rubbing by a human. During this study, a familiar human encouraged the experimental subject to interact with them, through verbal and hand gestures. If the pig approached, the experimenter applied gentle touch, such as petting, scratching and rubbing, to the pig, in order to encourage the pig to lie down for a belly rub. Belly rubbing typically elicited behaviours indicative of a positive affective state in the pigs, such as limb stretching, frequent short-lasting grunts and closing their eyes. This short-lived response might be indicative of a positive emotional state, but it is yet unclear if such a treatment performed over the long-term would result in an accumulative positive affect in the pigs, and thus a positive mood state, as suggested by Mendl et al. (2010).

1.2. Aims and overview of the study design and methods

In order to investigate further, this study aimed to assess the effects of the same positive handling treatment on pigs long- and short-term positive handling interventions and any interaction between the two. To do this, we trained 36 pigs in a spatial Go/No-go judgement bias test in the format developed by Hintze et al. (2018) and divided the pigs into three treatment groups: Long-term positive contact (LTPC), Short-term positive contact (STPC) and a control group (CON). Each pig in the LTPC group spent five minutes in a treatment area at the front of their home pen with a familiar experimenter at the end of each training and testing day, while the experimenter applied a positive handling treatment. Additionally, they received the same five-minute positive handling treatment before half of their six test sessions, to test for any interaction between the long- and short-term positive contact treatments. The STPC group on the other hand received only the five-minute positive contact treatment before half of their six test sessions, while the control group received no positive contact treatment at all. Therefore, the treatment design was as follows:

1) LTPC: *Long-term positive contact +/- short-term positive contact (3 x contact, "LT+", and 3 x No-contact, "LT-", random order)*
2) STPC: *No long-term positive contact +/- short-term positive contact (3 x contact, "ST+", and 3 x No-contact "ST-", random order)*
3) CON: *No long-term positive contact + no short-term positive contact (6 x No-contact)*

As the cumulative experience of emotions influences longer-term mood states (Mendl et al., 2010), repeated, long-term positive contact treatments were hypothesized to induce a positive mood in pigs, which would translate to more "optimistic" scores in the JBT for pigs in the LTPC treatment group than pigs in the STPC or CON groups.

We expected the STPC sessions to induce a positive emotional state in the pigs, and therefore see more optimistic responses when a pig received the STPC treatment (LT+, ST+) compared to when they did not (LT-, ST-, CON). However, as the long-term positive contact sessions were intended to build a positive HAR between the experimenter and pig, pigs in the LTPC treatment group should judge the short-term positive contact sessions as a more positive event than pigs in the STPC group, therefore we also expected to find an interaction

between the long-term treatment and the short-term treatment, with treatment condition "LT+" yielding the most optimistic responses in the JBT, as the putatively positive mood induced in the pigs in the LTPC group should affect their appraisal of the STPC treatment prior to the test. Pigs in the CON group were expected to show the least optimistic responses in the JBT as they received no positive contact treatments for the duration of the study.

Therefore, in terms of optimistic scores in the JBT our hypotheses were as follows:

1) LTPC > STPC > CON
2) LT+ > ST+ > CON

Additionally, we were interested in whether the long-term, "mood-inducing" positive contact treatment or the short-term "emotion-inducing" positive contact treatment would yield more optimistic responses in the JBT. In this case, we had two potential outcomes:

1) If the JBT is more sensitive to "mood", we expected to see more "optimistic" responses in the LTPC treatment group, regardless of whether or not they received a short-term contact before their test:
   LT+, LT- > ST+, ST-, CON
2) If the JBT is more sensitive to "emotion", we expected to see more optimistic responses when a pig received a short-term contact before their test:

   LT+, ST+ > LT-, ST-, CON

Behaviour during the short-term positive contact treatment sessions was also analysed. It was expected that pigs in the LTPC treatment group would show a stronger HAR than pigs in the STPC treatment group, evident by a shorter latency to approach and more time spent in contact with the human. These behavioural outputs were then measured against scores in the JBT to assess if any differences in optimism between the groups could be explained by the HAR.

In order to get a more holistic picture of the pigs' internal states, physiological measures were included in the study to complement the behavioural indicators. It is generally accepted that multiple indicators are essential to get make an adequate assessment of animal

welfare (Broom, 1991). In order to assess if the gentle handling experienced in the positive contact treatment reduced physiological indicators of stress in the pigs, salivary cortisol concentration was measured in all three treatment groups (LTPC, STPC and CON), before and after testing when the animal had received a short-term positive contact treatment (LT+ and ST+), and when they had not (LT-, ST-, CON). Salivary cortisol concentration is considered a good measurement of unbound cortisol, i.e. the cortisol that reaches the target tissue and elicits glucocorticoid effects, accurately reflecting the unbound cortisol circulating in the blood (Kirschbaum & Hellhammer, 1994). It was expected that the pigs in the LTPC treatment group should have significantly lower "baseline" cortisol concentration levels (i.e. in the "before" measure) than the STPC or CON treatment groups due to the cumulative effects of regular positive contact. It was also expected that cortisol concentration would be reduced when a pig received a short-term positive contact session immediately before their test (LT+, ST+), compared to when the pig did not receive such a treatment (LT-, ST-, CON). Additionally, lower levels of cortisol concentration were expected to be associated with more optimistic JBT responses.

2. Methods

2.1. Animals and housing conditions

This experiment was carried out at the Vetmeduni Vetfarm Medau, a research farm in Lower Austria, between May 2020 and September 2020. 36 piglets (18 male, 18 female) were selected from 12 litters (4 litters per replicate), across three time replicates, the first replicate beginning in May, the second in June and the third in August. At age four weeks (weaning) the pigs were separated from the litters and sorted equally into two adjacent home pens in the experimental unit (see Figure 2). In replicates one and three the two pens were balanced for sex, while litter was organised as per the table below (Table 1). In replicate two, the home pens were balanced for sex, but with a different litter organisation, with litter one and two in one home pen and litter three and four in the other, due to a mistake at sorting.

Table 1: Organisation of home pens. Number refers to litter and letter refers to sex, M= male; F= female. E.g. 2F refers to a female from litter 2.

Pen 1		Pen 2	
1F	1M	1F	2M
2F	2M	3F	3M
3F	4M	4F	4M

The home pens measured 9m by 2.5m, however an area of 1m before the main entrance had been separated from the rest of the home pen by a wooden partition approximately 1m in height. The partition included a hinged door with a locking mechanism so that the pigs could not access this front area, which was used in the experiment as the LTPC treatment area (referred to as “treatment area” henceforth).

The floor of the home pen was made of partially slatted concrete, and the treatment area was fully slatted. The pigs were provided with sawdust and straw for bedding, and a wooden log on a chain attached to the wall separating the two home pens was provided as enrichment in each home pen. A water trough was provided for water as the piglets were

initially too small to reach the drinking nipples on the wall of the pen. A feeding trough was fixed to the wall between the two home pens however this was not used for feeding the pigs during this study.

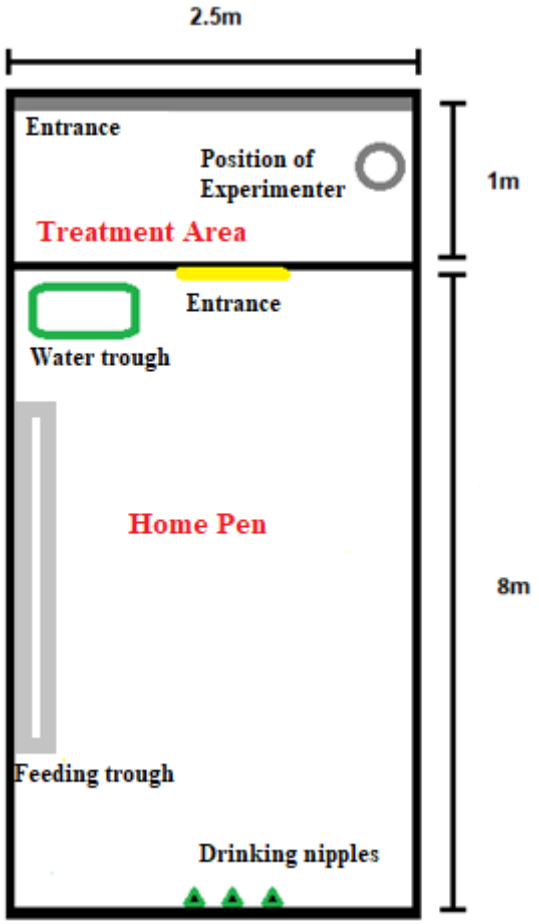

Figure 2: Schematic representation of a home pen and treatment area where LTPC sessions took place. Position of experimenter when engaging with the pig during the LTPC treatment sessions in the treatment area is marked by a grey circle. A grey line marks the entrance to the treatment area and a yellow line marks the location of the door on the wooden separator wall, leading from the treatment area to the home pen. Water trough and nipples are marked in green. Feeding trough went unused throughout the duration of this study.

Initially, (for all replicates) the pigs were fed two large scoops of commercial pig feed per pen, scattered on the floor of the home pen in the morning at approximately 08.00 and two more large scoops scattered per pen in the afternoon at approximately 15.00, however towards the later stages of shaping it was necessary to restrict feeding times to after training (i.e. afternoon only) to improve motivation for food rewards. In the adjusted feeding regime the pigs received the full amount of daily feed (four scoops per pen) in the afternoon only. The

adjusted regime was continued through the testing phase. A veterinarian was on site to treat any health problems that arose during the experiment.

2.2. Experimental treatments

2.2.1. Human Approach and Avoidance Tests

As this study would measure approach latencies and duration of contact with a familiar human, a human approach test and a human avoidance test were deemed necessary to ensure the treatment groups were balanced as far as possible for levels of fear of humans at the start of the study. The tests were carried out before the beginning of shaping stage of training, and again at the end of the study to check for the effects of the treatment on these measures (see Figure 4 for timeline). The human approach test involved the experimenter standing stationary in the home pen for up to five minutes and recording the latency of each pig to approach her and make contact (snout touching boot). For the human avoidance test, the experimenter would choose a focal pig, and start at 3m away from them and move towards them in steps of 0.5m, continually, until the pig moved to "avoid" the experimenter. The distance from the pig the experimenter could approach before the pig moved was recorded. If the pig did not move the experimenter would touch them on the forehead and the distance was recorded as 0.

2.2.2. Treatment Groups

The pigs were sorted into three groups: Long-term positive contact (LTPC), Short-term positive contact (STPC) and a control group (CON), balanced across sex, litter, home pen, and as far as possible, human approach and avoidance scores.

LTPC treatment: Pigs in the LTPC treatment group were given an individual five-minute positive contact treatment, from the first day of shaping until the final day of testing, at the end of each day, for a minimum of five days per week (usually Monday-Friday). As the number of training and testing days varied between replicates, this meant that pigs in the LTPC group in replicate one and three received 20 positive contact sessions, while replicate two received 18 positive contact treatments. The LTPC took place in the treatment area (L: 2.5m x W: 1m) separated from the home pen (see Figure 2) by a wooden partition with a hinged door with a locking mechanism. The floor of the treatment area was fully slatted concrete.

The pig would be gently separated from his or her pen-mates in the home pen and brought into the treatment area, and the door closed and locked so that no pig on either side could open it. Five minutes were set on a timer and the experimenter sat down, cross-legged, at one end of the treatment area, away from the door. The experimenter encouraged the pig to approach using gentle vocalization and hand signals. If the pig approached within reach, the experimenter would stroke and scratch the pig on the back, neck, head, sides and rear. After five minutes, the pig would be returned to their home pen by opening the door and allowing the pig to make their way back in. If a pig showed signs of distress the treatment was ended prematurely, and the pig was allowed to return to their home pen. The criteria for distress were continuous, escalating vocalizing for more than two minutes, or two or more escape attempts (jumping at the door).

STPC treatment: Pigs in the SPTC group received positive contact treatment sessions immediately before half of the six judgement bias test sessions. Pigs in the LTPC group also received the same positive contact treatment sessions immediately before half of the six test sessions, in addition to their long-term positive contact treatment sessions. This resulted in five treatment conditions during testing: LTPC with or without the STPC treatment session before the test (LT+ and LT-, respectively), STPC with or without treatment session before the test (ST+ and ST-, respectively) and the control group that received no additional human contact (CON).

STPC treatment sessions were identical in procedure to the LTPC treatment sessions, however they took place in the waiting area at the front of the experimental arena (see Figure 3, experimenter position during treatment session marked by grey circle), located opposite the home pens in the same experimental unit. The waiting area was separated from the experimental arena by a wooden partition with a hinged door with a locking mechanism, identical to the structure separating the home pen from the LTPC treatment area. The waiting area had the same dimensions as the treatment area adjacent to the home pen (L: 2.5m x W: 1m), and also had a fully slatted concrete floor. The pig would be gently separated from his or her pen-mates in the home pen and brought into the waiting area at the front of the experimental arena, and the door closed and locked. The procedures for the human contact in the STPC treatment sessions were identical to the LTPC treatment sessions. After five

minutes, the pig was allowed into the experimental arena by opening the door and allowing the pig to make their way inside to await their JBT, which was started within one minute of the end of the STPC treatment session. If a pig showed signs of distress (using the same criteria as above) the treatment session was ended prematurely, and the pig was allowed into the experimental arena. This happened on two occasions (with two different pigs in the STPC group), where the STPC treatment session was cut short by one minute, and on one occasion where the treatment session was ended and the pig (also from the STPC group) returned to her home pen as she had injured herself in an escape attempt. This pig was thereafter dropped from the study due to this injury (see Table 7 for attrition details).

For both groups, the order of test treatment condition (LT+, LT- or ST+, ST-) across the six test sessions was pseudorandomised, to ensure no more than two consecutive treatment conditions of the same type took place. When a pig did not receive a treatment session before their test (LT-, ST-, CON) they went straight into the testing arena. They were not required to stay for any length of time in the waiting area, to avoid potential isolation stress before the test. If a pig did not complete their JBT or failed the test (see Table 3 in methods section for detailed criteria), it was necessary to repeat the test on a different day, including test treatment condition. Therefore, three pigs received more than three STPC treatment sessions during the experiment (two pigs received four STPC treatment sessions, and one pig received five STPC treatment sessions).

2.3. Positive Contact

The STPC treatment sessions prior to the test were video recorded and analysed with the BORIS software (Behavioural Observation Research Interactive Software, Friard & Gamba, 2016) by a single observer (who was not blind to the treatment groups) using a behavioural ethogram (see Table 2). Inter- and intra-observer reliability was calculated and found to be equal or superior to 90%. The duration of types of physical contacts were recorded and coded as follows: “Contact by pig” (pig-initiated contact: pig would make contact with human by touching human’s body with snout), “Contact by Human” (human-initiated contact: could take the form of petting, scratching, rubbing pig’s back, head or neck), “Reciprocal contact” (both human and pig exchanged physical contacts), and “Belly rubbing” (pig lying on side, exposing belly to experimenter, leg stretching, eye closure and grunting in response to human

touching belly). The duration of each type of contact was measured and calculated as a percentage of total time of session. Latencies to initiate contact (by human, pig, or reciprocally initiated) were also recorded, along with other behavioural details such as defecation, urination or escape attempts.

Table 2: Ethogram of behaviours analyzed during STPC sessions. Behaviours recorded as state events were mutually exclusive, and behaviours recorded as point events that co-occurred with those recorded as states were scored simultaneously

Behaviour type	Behaviour code	Definition
State event	Contact by pig	Pig initiates contact. Any type of physical touch (snout to hand, sniffing, biting, mouthing) toward human
	Latency to contact by pig	The time from beginning of STPC treatment session until pig made first contact with human. Contact defined as snout touching any body part of experimenter
State event	Contact by human	Human initiates contact. Human scratches or strokes body (back, neck, head) of pig, except belly
	Latency to contact by human	The time from beginning of STPC treatment session until human made first contact with pig. Contact defined as hand touching any body part of pig
State event	Reciprocal contact	Pig interacts with human (snout/mouth to hand) whilst human gives physical contact (petting, scratching, rubbing)
	Latency to reciprocal contact	The time from beginning of STPC treatment session until pig and human made contact with each other simultaneously. Defined as snout touching any body part of human at the same time as

		human touching any body part of pig
State event	Belly rubbing	Human rubs belly of pig, pig is lying on its side
Point event	Defecating	Pig eliminates faeces
Point event	Urinating	Pig excretes urine
Point event	Escape attempts	Standing on hind legs and fore legs on the wall, or attempt to jump with the hind legs leaving the ground
State event	Other	Any other behaviour not listed above (e.g. exploration behaviour, standing still away from human, etc)

2.4. Judgement bias test

2.4.1. Experimental arena and apparatus

The pigs were trained and tested in a rectangular experimental arena (Length: 5.4m, Width: 2.5m; see Figure 3) located opposite the home pens. The experimental arena had a concrete floor (non-slatted, and kept free of any bedding materials), and a concrete trough was provided at the back of the arena so that the pigs had access to fresh drinking water. The experimental arena was modified to include a wooden partition with a swinging door between the arena and a waiting area used for the STPC treatment sessions (see Figure 3). A large wooden wall was installed at the back of the experimental arena (Height: 1.7m) with five rectangular goal-boxes (W: 20cm, H: 40cm), located 10cm above the floor and spaced equidistantly at intervals of 15cm across the wall. Each goal-box could be opened by the experimenter from behind the wall using a string and pulley system. A metal feeding bowl was secured behind each goal-box door (Radius: 6.5cm, Diameter: 5cm) at a height of 20cm above the ground.

A large metal bell (1.5kg, W: 15cm, H: 22.5cm, Depth: 7.5cm) serving as trial initiator was hung from a wooden beam using string in the middle of the experimental arena. The length of string was adjustable via a pulley system so that the experimenter could lift and

lower the bell as required. The bell was hung approximately 3.5m from the goal-box apparatus.

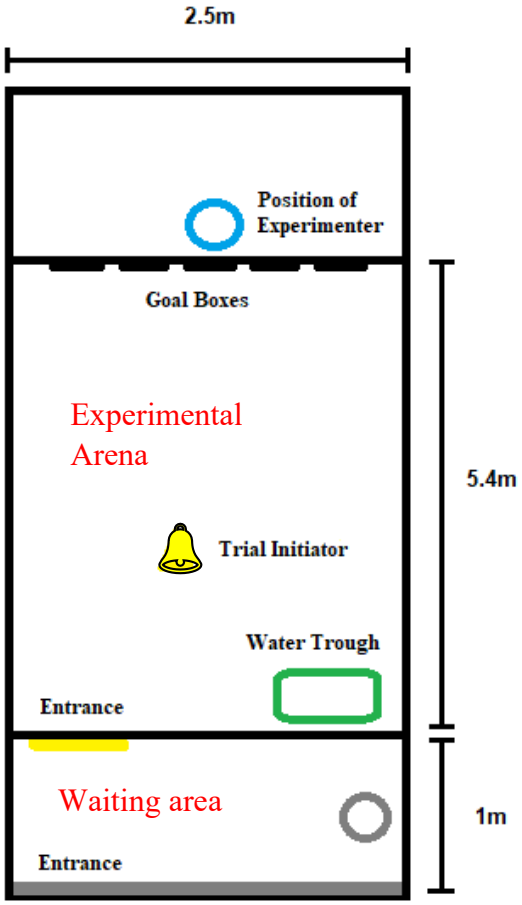

Figure 3: Schematic overview of the experimental arena. Overview of the experimental arena with waiting area at the front of the pen (where the STPC sessions took place). The position of the trial-initiator is marked in yellow and the position of the experimenter behind the goal-box apparatus (during JBT) is marked with a blue circle towards the rear of the pen. (Position of the experimenter during STPC sessions is marked in the waiting area by a grey circle).

2.4.2. Habituation and Training

Pigs were habituated to the experimental arena, the chocolate rewards and the experimenter, before being trained individually once per day for five days per week (normally Monday-Friday) by the same experimenter (for overview of timeline, see Figure 4). Training consisted of three stages: the shaping stage, in which the pigs' behaviour was shaped through operant conditioning methods to initiate trials by ringing the bell before moving towards the goal-box,

and the Left/Right discrimination stage, in which they were trained to discriminate the left from right goal-box, and finally the Go/No-go discrimination stage, where they were trained to discriminate between a positive (rewarded) and a negative (unrewarded) goal-box. If the pig was successful at each stage of training, they moved on to the judgement bias test, which took place in six test sessions. For an overview of the criteria to pass each training stage, see Table 3.

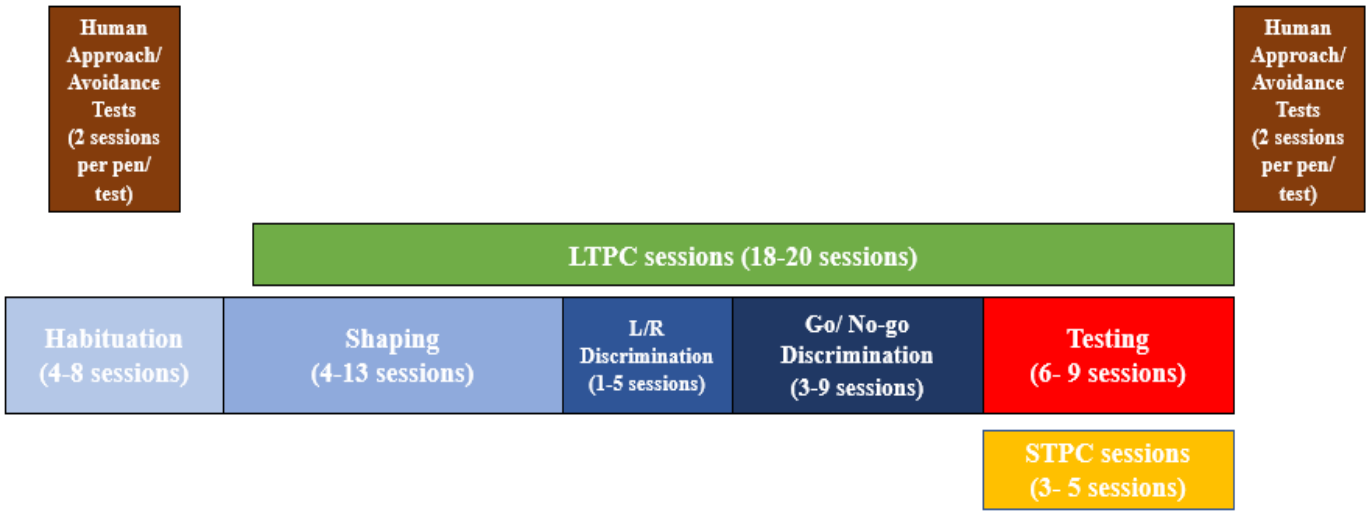

Figure 4: Timeline of habituation, training and long- and short-term positive contact sessions. Range of number of sessions required to reach stage criteria (see Table 3) across the three time replicates is included.

2.4.2.1. Habituation

Before training, pigs went through a period of habituation to the experimental arena, the reward (chocolate M&M®s) and to the experimenter. This was done in a stepwise manner, at first allowing all six pigs from one or other home pen to enter and explore the experimental arena, then again in groups of three, then pairs and finally singly, for ten minutes per session. During these ten-minute habituation sessions, rewards were scattered around the experimental arena, and all goal-boxes were opened and closed by the experimenter from behind the wooden apparatus wall a minimum of three times each. The experimenter would also enter the experimental arena and hand-feed each pig during the habituation sessions, in order to facilitate the later shaping stages. Habituation sessions were carried out twice per day, with at least one hour between sessions for each pig, so that each pig had 20 minutes per day to

habituate to the experimental arena for at least two days, with some rest periods in between. A pig was considered habituated if they stayed in the experimental arena for ten minutes without signs of distress (consistent and escalating vocalizing, two or more escape attempts, such as jumping at the exit door) and ate from the experimenter's hand 15 times. If a pig was having difficulty habituating at one step they moved back a step (for example from single to pair habituation) and were paired with a pen-mate who had already habituated and therefore was not showing any signs of distress. This meant the total number of habituation sessions varied between pigs and ranged from four to eight sessions (see Figure 4).

2.4.2.2. Shaping for trial initiation

Pigs received one shaping session per day. In a stepwise process, pigs were trained to initiate a trial by ringing the bell with the snout and to subsequently approach the positive location (Pos) to receive one M&M® as a reward.

First, pigs were trained to touch the trial initiator and immediately given a reward from the hand of the experimenter. If they did this successfully and consistently for 20 trials the session was complete, and they moved on to the next shaping session the following training day. In the next shaping session, the experimenter would move away from the trial initiator, towards the positive goal-box, thus increasing the distance the pig had to move to collect the reward. If the pig consistently rang the trial initiator and retrieved the reward from the experimenter 20 times, the session would be complete. This same protocol was followed, with increasing distances between the trial initiator and the experimenter's position, until the pig would ring the trial initiator and move to the open positive goal-box, where the experimenter would place the reward in the metal feeding bowl located inside the goal-box. After this stage, the experimenter would move behind the wooden apparatus wall to operate the opening and closing of the goal-boxes, and the final shaping session involved the pig ringing the trial initiator and waiting for the positive goal-box to be opened, before approaching and consuming the reward from the bowl. Each shaping session lasted no more than 45 minutes, after which the pig would be returned to their home pen if they had not successfully learned the task, and the same shaping session would be repeated the following training day.

2.4.2.3. Left/Right discrimination

Once the pig was consistently ringing the trial initiator and consuming the reward from the positive goal-box they moved on to the Left/Right (L/R) discrimination stage. At this stage, the experimenter would open either the positive or negative goal-box, each time the pig rang the trial initiator, for 40 trials. The order of goal-box opening was pseudo-randomized and balanced for number of times each goal-box would open. Movement towards the open goal-box with snout inserted through the opening was considered a Go response. At this stage, a Go response towards either goal-box was rewarded to teach the pig to pay attention to any open goal-box. To pass this stage, the pig needed to show a Go response to the open goal-box for 40 trials without escape attempts or refusal to participate. The maximum time allowed for a session was 45 minutes, after which if the above criteria had not been met, the session was considered failed, and the pig would have to repeat this stage the following training day.

2.4.2.4. Go/No-go discrimination

If a pig passed the L/R discrimination stage they proceeded to the Go/No-go discrimination stage. During this stage, both the left and right goal-boxes were opened upon trial initiation by the pig, however only Go responses to the positive goal-box were rewarded to teach the pig to discriminate between the positive and negative (unrewarded) goal-box locations. The locations (L/R) of the positive goal-boxes were balanced across each replicate of pigs, so that half of the group learned the right side was always rewarded, while the other half learned the left side was always rewarded. Each training session consisted of 40 trials split equally into two segments, within which the positive or negative goal-boxes were opened in a pseudo-randomized order. In order to pass this stage the pig had to learn to make a No-go response to the negative goal-box, defined as either no movement towards the open goal-box within 5 seconds after trial initiation, moving towards but not putting their snout into the goal-box (goal-box would be closed by experimenter after 10 seconds), or initiating a new trial by ringing the bell. The pig needed to complete four consecutive segments with two or fewer mistakes per goal-box (i.e., two or fewer Go responses towards a negative goal-box and two or fewer No-go responses towards a positive goal-box) per segment. When a pig had passed this stage they were considered ready for testing.

2.4.3. Judgement Bias Test

2.4.3.1. Judgement bias test procedure

Once a pig had reached the test criteria (see Table 3 for an overview of criteria to reach testing), they were tested in six test sessions across six days. Test days were not always consecutive to allow a rest day for the experimenter, but there was never more than one non-testing day across a period of seven days for any pig. Each test session consisted of 43 trials, with 20 positive and 20 negative trials presented in a pseudorandomised order, and the three ambiguous trials, represented by three goal-boxes between the positive and negative goal-boxes (Ambiguous Positive– "AP", Ambiguous Middle– "AM", Ambiguous Negative– "AN") each being presented only once per test session. The ambiguous goal-boxes were opened at trials 11, 26 and 41, three times after a positive and three times after a negative trial across the six test sessions, and in the same manner for all pigs. Go responses in positive trials were rewarded, while Go responses in negative trials were not rewarded and marked as a mistake. Tests were divided into segments at the midway point between the first and the second 20 non-ambiguous, or reference trials. If a pig made more than three Go responses in a negative trial per segment, the test was considered failed. Similarly, if the pig made more than three No-go responses to a positive trial per segment, this also was considered a fail. Go responses in ambiguous trials were always rewarded, to reduce the possibility of an extinction of response due to the pigs' learning the ambiguous cues were never rewarded (Doyle et al., 2010a).

If a pig failed the test, did not complete the test within 45 minutes, or if they showed signs of severe distress (defined below), the test was terminated, and the pig re-tested on a different day. Occasionally, tests would need to be terminated due to other reasons, such as loud, distracting noises in adjacent units (e.g., power hosing/cleaning) that were outside of the experimenter's control. In this case the test was terminated and restarted from the beginning once the distraction had ceased.

Table 3: Overview of necessary steps and criteria to pass each stage and reach testing.

Stage	Trials per session	Goal-boxes used	Goal-box rewarded	Criteria
Habituation	15	N/A	N/A	1. Takes reward from experimenter's hand 2. 10 minutes in experimental arena with two pen-mates without signs of distress (consistent and escalating vocalising, two or more escape attempts) 3. 10 minutes in experimental arena with one pen-mate without signs of distress 4. Alone in experimental arena for 10 mins without signs of distress
Shaping	20	Pos	Pos	1. Touches bell with snout ("rings"), retrieves reward from experimenter's hand 2. Rings bell, moves towards goal-box to retrieve reward from experimenter's hand 3. Rings bell, retrieves reward from bowl in open goal-box 4. Rings bell, waits for goal-box to open to retrieve reward from bowl
Left/Right Discrimination	40 trials within 2 segments	Pos/Neg	Pos/Neg	1. Rings bell, goes to open goal-box (side presentation pseudorandomised)

				2. Must show Go response to any open goal-box for 40 trials within 45 minutes
Go/No-go Discrimination	40 trials within 2 segments	Pos/Neg	Pos	1. Rings bell and shows Go response only to rewarded goal-box (L/R-balanced across group) 2. Must show less than three Go responses to unrewarded goal-box in each 20 trial segment 3. Must show a No-go response to unrewarded goal-box, and less than three No-go responses to rewarded goal-box 4. Must meet criteria 2. and 3., for four consecutive segments to move to testing
Judgement Bias Testing	43	Pos, AP, AM, AN, Neg	Pos, AP, AM, AN	1. As per Go/No-go discrimination criteria, however less than four Go and four No-go mistakes across each 20 non-ambiguous trial segment. 2. If the pig failed criteria 1., the test would be repeated on a separate day, after the other tests for this pig had been completed.

If at any stage during habituation, training or testing a pig showed signs of severe distress (defined as two or more escape attempts or consistent and escalating high-pitched vocalizing) the session was stopped, and the pig returned to their home pen. The same session were

attempted again at a later time, or the following day (depending on the time of day incomplete session took place). No pig was kept in the experimental arena for more than 45 minutes at any stage of training or testing.

2.4.3.2. Exclusion criteria

During training and testing, pigs were excluded for various reasons. Two pigs (one pig from replicate one, one pig from replicate three) were excluded because of injury. Five pigs (one pig in replicate one, one pig in replicate two and three pigs in replicate three) showed 'Low Motivation' as they did not complete a training session within 45 minutes across five consecutive training days. See Table 7 for overview of attrition rate.

2.5. Salivary Cortisol

In order to assess if the gentle handling experienced in the positive contact treatment reduced physiological indicators of stress in the pigs, salivary cortisol concentration was measured in all three treatment groups. Saliva samples were collected from the pigs in replicates two and three, before and after testing. Replicate one was not included in the sampling as it was necessary to gage the behavioural reactions of the pigs to training and testing before introducing another potentially stressful element into the study. As the pigs in replicate one habituated to the training and testing well, saliva sampling was included in the subsequent two time replicates.

Saliva sampling is considered non-invasive and less stressful compared to blood sampling (Hillman et al., 2008) and therefore better suited to the purposes of our study. For the LTPC and STPC treatment groups, saliva samples were collected on one occasion where the pig did not receive a treatment prior to testing (sampling performed before and after testing) and on two occasions where the treatment was administered prior to testing (sampling performed before treatment and after testing). CON pigs were sampled on two days (before and after testing). Sampling was done by an unfamiliar human and the experimenter (replicate two) or the experimenter only (replicate three).

Basal concentrations of cortisol in pigs' blood and saliva samples have been shown to be typically higher in the morning than in the afternoon and evening (Hillman et al., 2008), therefore care was taken to reduce variation of the timing of samples. Sampling always took

place in the morning between 08.00 and 12.00. The pigs were presented with a saliva swab attached to a plastic zip tie which was held on one end by the human. They were encouraged to chew on the swab for up to 30 seconds, and the swab was then retrieved and stored in a plastic tube in a polystyrene box containing dry ice. Samples were then stored in a -20°C freezer on site and later transported to the lab for analysis.

Analyses to determine cortisol concentrations of the saliva samples were conducted by using the Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay kit (No 1-3002, Salimetrics, State College, PA, USA), running each sample in duplicate for validity, with intra-assay CVs ≤ 5.8% and inter-assay CVs of 3.4% and 5.3% for the high- and low-control samples.

2.6. Ethical Considerations

This study was discussed and approved by the institutional ethics and animal welfare committee in accordance with GSP guidelines and national legislation (ETK-108/06/2019).

2.7. Statistical Analyses

We used RStudio version 3.6.1 for the general linear mixed-effects models (function Lme), generalised linear mixed-effects models (function Glmer), and Minitab version 17 for the general linear models and Spearman's Rho correlations. An overview of the statistical analyses including software used, fixed and random effects is presented in table form in Appendix A.

2.7.1. Human approach and human avoidance tests

For each replicate, two human approach tests were carried out per pen, and two human avoidance tests were carried out per pig, both before shaping began and after testing was completed (see Figure 4 for timeline). An average of the two values was calculated and recorded, and results are displayed in Appendix B. No statistical analyses were run on the results of the human approach and human avoidance tests, however for each pig a comparison was made between the results of these tests when measured before shaping began and after testing was completed.

2.7.2. Positive Contact

To analyse if treatment group affected the durations of human-initiated and reciprocally initiated contact between pig and human we used a general linear mixed-effects model with "duration of contact" (as a percentage of total time) of each type of contact as the outcome measure, "treatment group" (LTPC, STPC) as fixed effect and "ID" nested in "pen" nested in "replicate" included as random effects.

In order to analyse whether treatment group affected the total duration of overall contact between the pigs and the experimenter, the durations of human-initiated and reciprocal contact were combined and analysed using a general linear mixed-effects model. Fixed effect was "treatment group" (STPC, LTPC), while "ID" (nested in "replicate") and "treatment session" (i.e. whether it was the first, second or third positive contact treatment session across the six test sessions) were included as random effects.

A general linear mixed-effects model was run to analyse the effect of treatment group on latency to contact by pig. "Latency to contact" was taken as the outcome measure while fixed effect was "treatment group" (STPC, LTPC), and "ID" (nested in "replicate") and "treatment session" included as random effects.

2.7.3. Learning Performance

Number of training sessions needed to learn each stage of the task are presented descriptively by replicate and by treatment group (mean ± SD). The number of animals excluded at each training stage is also presented descriptively. To investigate the effect of the LTPC treatment on learning performance, a general linear mixed-effects models were run, with whether or not the pig received a long-term treatment (i.e. LTPC, STPC or CON) as a fixed effect, and number of sessions required to learn the Go/No-go discrimination task (i.e. training sessions in stage four only) as the outcome measure. Random effects were "test session" (i.e. the numerical order of the test, 1-6) nested in "ID" nested in "pen" nested in "replicate".

2.7.4. Judgement bias test

According to the principles of the judgement bias task, Go responses in ambiguous trials were interpreted as "optimistic" responses, whereas No-go responses were interpreted as

"pessimistic" responses. In order to make a valid interpretation of responses to the ambiguous cues, test sessions where the pig made more than three mistakes in either positive or negative trials (i.e. a Go response to a negative trial or a No-go response to a positive trial) within a block of 20 trials were excluded from the analysis (as per Hintze et al., 2018). All pigs included in the analysis completed six test sessions except for one pig (from the STPC group) in replicate three, who successfully completed three test sessions before being excluded due to injury.

As the monotonically graded slope to responses in the JBT is an important indicator of internal validity, a generalised linear mixed-effects model was run to ensure this was present. The outcome measure was the pigs' decisions in the ambiguous trials in the JBT (Go response = 1, No-go response = 0), with "trial type" (NP, M, NN) as a fixed effect, and "test session" nested in "ID" nested in "pen" nested in "replicate" were included as random effects.

The interaction between the short- and long-term positive contact was analysed using a generalised linear mixed-effects model, with "Treatment group (CON+STPC versus LTPC)" and "Test treatment condition (LT+, LT-, ST+, ST-)" as interaction term and "Trial type" as fixed effects. The outcome measure was the pigs' decisions in the ambiguous trials in the JBT (Go response = 1, No-go response = 0) and random effects were "test session" nested in "ID" nested in "pen" nested in "replicate".

To investigate whether variation in the pigs' behaviour during the STPC treatment had an effect on the pigs' responses on the JBT ambiguous cues, two generalised linear mixed-effects models were run, with the outcome measure as the pigs' decisions in the ambiguous trials in the JBT (Go response = 1, No-go response = 0), and "duration of time" (as a percentage of total duration) of human contact or reciprocal contact included as a fixed effect, while "ID" nested in "pen" nested in "replicate" were included as random effects.

To check for interactions between sex or replicate and treatment, two generalised linear mixed-effects models were run with the outcome measure as the pigs' decisions in the ambiguous trials in the JBT (Go response = 1, No-go response = 0), and "trial type" and the interaction of "sex" and "treatment group" as fixed effects, while "test session" nested in "ID" nested in "pen" nested in "replicate" were included as random effects in each analysis.

Additionally, to preclude the possibility that pigs were learning that the ambiguous cues were always rewarded and thus affecting responses to ambiguous cues in later test

sessions, the number of Go responses over the course of the six test sessions was analysed using a generalised linear mixed-effects model. The outcome measure was the pigs' decisions in the ambiguous trials in the JBT (Go response = 1, No-go response = 0), with "trial type" "test session" as fixed effects, while "test session" nested in "ID" nested in "pen" nested in "replicate" were included as random effects. To check for an interaction between treatment group and learning across trials, the generalised linear mixed-effects model was run with the outcome measure as the pigs' decisions in the ambiguous trials in the JBT (Go response = 1, No-go response = 0), and "trial type" and the interaction between "test session" and "treatment" as fixed effects, while "test session" nested in "ID" nested in "pen" nested in "replicate" were included as random effects.

2.7.5. Salivary Cortisol

During replicates two and three, salivary cortisol concentration was measured on one occasion where the pig did not receive a treatment prior to testing (sampling performed before and after testing) and on two occasions where the treatment was administered prior to testing (sampling performed before treatment and after testing). Pigs in the Control group were sampled before and after testing on two days only.

Due to the small sample size, a Spearman's Rho correlation coefficient was computed to assess the relationship between the duration of contact (human contact, reciprocal contact) and change in salivary cortisol concentration (post-test value minus pre-test value) as well as latency to contact (human, pig) and change in salivary cortisol concentration. A general linear mixed-effects model was run to see if there were significant differences in cortisol change between whether the animal received a positive contact treatment before their test or not, and whether the amount of reciprocal or human initiated contact during treatment had an effect on the cortisol concentration change. For this, the "cortisol concentration difference" (post-test value minus pre-test value) was used as the outcome measure, with "test treatment condition" as the fixed effect and "ID" (nested in "treatment group") and "treatment session" entered as random effects.

Additionally, a generalised linear mixed-effects model was run to investigate if any interaction effect could be observed between the treatment groups and the change in salivary cortisol and pigs' responses to the JBT, with the fixed effects as the interaction between

"treatment group" and "cortisol difference" and "trial type". The outcome measure was the pigs' decisions in the ambiguous trials in the JBT (Go response = 1, No-go response = 0) with "test session" nested in "ID" nested in "pen" nested in "replicate" included as random effects.

3. Results

3.1. Human Approach and Avoidance Tests

As this study would measure approach latencies, human approach and avoidance tests were carried out to help balance the treatment groups with respect to the pigs' fear of humans. Results of the human approach and avoidance tests are presented descriptively in Appendix B. Approach times were reduced for all but one pig, while avoidance tests showed that only one pig avoided contact with the experimenter at the end of the study (at 0.5m compared to 2m at the beginning of the study).

3.2. Positive Contact

3.2.1. Overview of behavioural observations during STPC sessions

During the STPC treatment sessions, the pigs almost always stayed in the waiting area with the experimenter for the full duration of the five-minute session, with the exception of three instances across three pigs, two of whom were allowed out early due to mild distress (one pig from the LTPC group and one pig from the STPC group), and one who reacted with severe distress (from the STPC group), who was later dropped from the study.

A comparison of the mean duration of time that pigs in each treatment group spent performing each type of behaviour is displayed as a percentage of the total duration of time spent in the STPC treatment session in Figure 5. In terms of this behavioural time budget, pigs in both groups spent the majority of their time engaging in "Reciprocal Contact" (LTPC: 32%; STPC: 38%), followed by "Other" behaviours (e.g. exploring the area, standing still, etc., LTPC: 30%; STPC: 32%). The least amount of time was spent engaging in pig-initiated contact, i.e. "Contact by Pig" (1% for both groups) however this was due to the fact that once a pig had initiated contact the experimenter would reciprocate, at which point the behavioural coding was changed to "Reciprocal Contact". The amount of time the pig allowed the human to touch them (without reciprocating), coded as "Human Contact", was slightly higher for the LTPC treatment group (19%) than the STPC treatment group (15%).

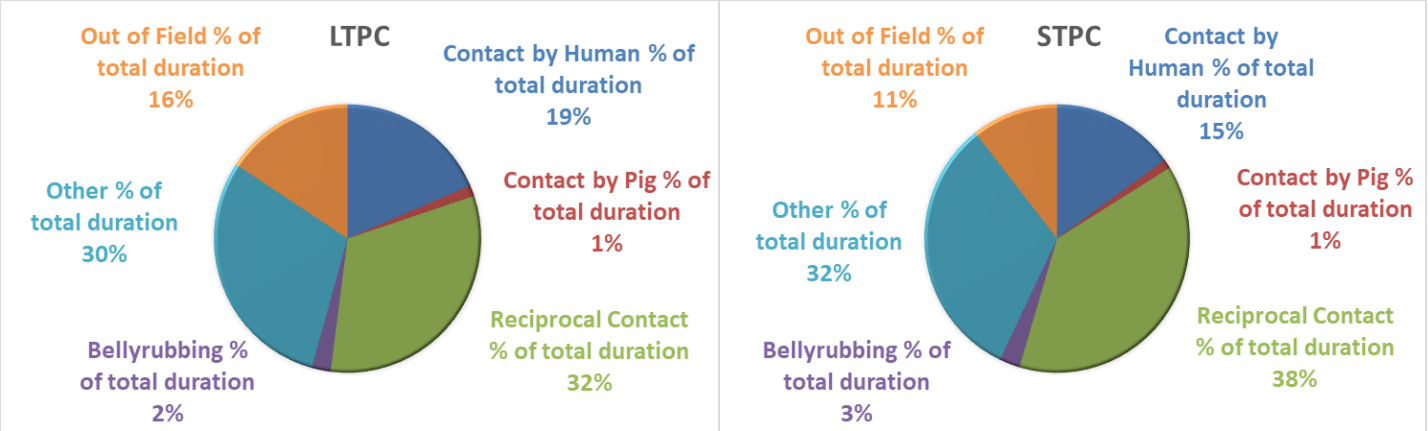

Figure 5: Mean time budget of the LTPC and STPC treatment groups during the STPC treatment. Each behaviour is presented as a percentage of the total time spent in the STPC pen with the experimenter.

3.2.2. Duration of contact between human and pig

As discussed above, once a pig had made contact, the experimenter would reciprocate contact immediately, and so the duration of contact initiated by pigs was usually very short (< 4 seconds on average) before reciprocal contact began (see Figure 6), therefore the duration of pig-initiated contact ("Pig Duration") was not included in the analysis. In terms of the duration of contact initiated by the experimenter, pigs in the LTPC treatment group allowed slightly more contact from the experimenter (results are expressed in seconds, mean ± standard deviation: 54.8 ± 36.3) than pigs in the STPC treatment group (41.8 ± 34.1). However the standard deviations were high, which suggests a lot of individual variance within the groups. The opposite was observed for "Reciprocal Contact" between the pigs and the experimenter, as pigs in the STPC group engaged in reciprocal contact slightly more (117.9 ± 78.4) than pigs in the LTPC group (90.0 ± 37.9). However, again the standard deviations were high, suggesting high levels of individual variation within the groups. There was no significant difference between the groups in terms of the amount of "Human Contact" ($F_{1,19} = 0.25$, $p = 0.62$), nor "Reciprocal Contact" ($F_{1,19} = 1.70$, $p = 0.21$).

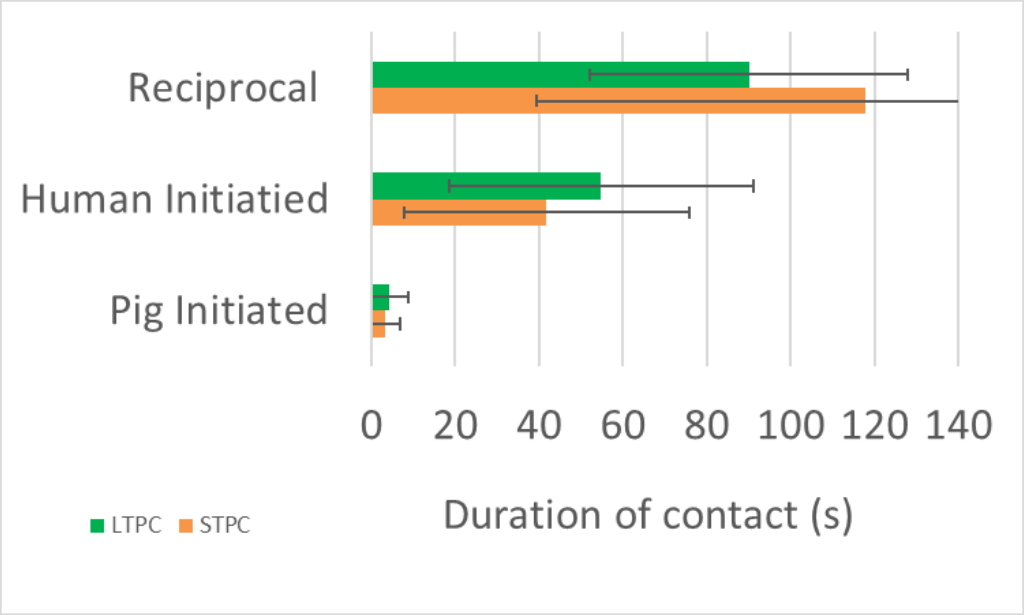

Figure 6: Mean durations and standard deviations of contact (in seconds) between human and pig for the LTPC and STPC treatment groups. LTPC scores are shown on the left, in green, and STPC scores are shown on the right, in orange.

When the durations of human and reciprocal contacts were combined to assess overall contact differences between the groups, still no significant effect of treatment group was observed ($F_{1,19} = 0.4$, $p = 0.51$).

3.2.3. Latency to contact between human and pig

In terms of latency to contact, the latency for the pig to make contact with the experimenter ("Pig Contact") was lower for pigs in the LTPC treatment group (results are expressed in seconds, mean ± standard deviation: 34.9 ± 40.6) than pigs in the STPC treatment group (46.5 ± 41.0, see Figure 7). Similarly, the latency to "Human Contact" was lower for the LTPC group (56.2 ± 44.2) compared to the STPC group (62.5 ± 24.9). The shortest latencies were recorded for "Reciprocal Contact" for both groups, however the LTPC group showed a longer latency to contact and a much higher variance within the group (20.2 ± 40.0) when compared to the STPC group (10.5 ± 2.5). Data for the pig latencies to approach were log transformed, after which they met the criteria of normality and homogeneity of variance. No significant effect of treatment group was found ($F_{1,19} = 0.6$, $P = 0.47$).

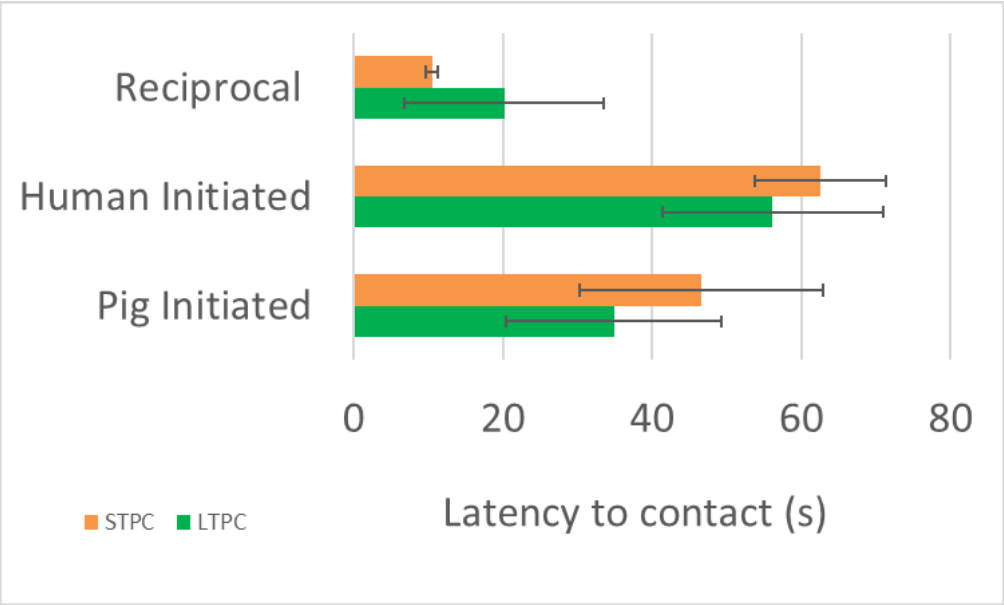

Figure 7: Average latency to contact (in seconds) by type of contact, per treatment group. LTPC scores are shown in green, and STPC scores are shown in orange.

3.3. Learning performance

Overall, the pigs needed between 8 and 20 training sessions (results are expressed in number of sessions, mean ± standard deviation: 14.2 ± 3.0) to reach testing criteria. Pigs in the STPC and CON groups needed between 9 and 18 training sessions (13.9 ± 2.7), while pigs in the LTPC needed between 8 and 20 training sessions (14.7 ± 3.5). In order to learn the Go/No-go discrimination task pigs needed between 3 and 9 training sessions (5.0 ± 1.7). Pigs in the STPC and CON groups needed between 3 and 8 training sessions (5.0 ± 1.6), while pigs in the LTPC needed between 3 and 9 training sessions (5.3 ± 2.0) to complete this stage and reach testing criteria with no significant differences between the groups ($F_{1,23} = 0.61$, $p = 0.44$).

Training duration and attrition rate per replicate and training stage are presented descriptively. Data are expressed in sessions (mean ± standard deviation) for the training duration by replicate (Table 4) by treatment group (Table 5) and as number of animals at each stage for attrition rate (Table 6).

Table 4: Mean number of sessions necessary to pass each stage by time replicate (TR), mean ± SD

Replicate	Habituation	Shaping for Trial Initiator	Left/Right Discrimination	Go/No-go Discrimination	Testing
TR 1	8.0±0.0	9.2±1.5	1.6±1.3	5.8±1.6	6.8±1.1
TR 2	5.5±0.9	6.9±0.8	1.3±0.7	5.5±2.0	6.5±0.7
TR 3	4.5±1.2	8.0±2.9	1.3±0.5	3.8±0.7	6.8±1.0

Table 5: Mean number of sessions necessary to pass each stage by Treatment group, mean ± SD

Treatment	Habituation	Shaping for Trial Initiator	Left/Right Discrimination	Go/No-go Discrimination	Testing
CON	5.9±1.8	8.2±1.6	1.3±0.5	4.6±1.3	6.6±1.2
Short-Term	5.9±1.8	7.4±1.9	1.5±1.2	5.3±1.9	6.7±0.9
Long-Term	5.8±1.8	8.4±2.5	1.4±0.8	5.3±2	6.7±0.8

Table 6: Attrition during each stage and time replicate (TR) with reason (LM= Low Motivation; I= Injury)

Replicate	Habituation	Shaping for Trial Initiator	Left/Right Discrimination	Go/No-go Discrimination	Testing
TR 1	0	1 (I)	0	1 (LM)	0
TR 2	0	0	0	1 (LM)	1 (I)
TR 3	1 (LM)	0	1 (LM)	1 (LM)	0

3.4. Judgement Bias Test

There was no statistically significant interaction between "Treatment group" (CON+STPC versus LTPC) and "Test treatment condition" (LT+, LT-, ST+, ST-) with regards to the pigs' decisions in the ambiguous trials in the JBT ($\chi^2_1 = 0.50, p = 0.48$; Figure 8). When looking at the main effects only, there was no significant difference in pigs' responses between "Treatment groups" (CON+STPC versus LTPC; $\chi^2_1 = 0.53, p = 0.46$) nor "Test treatment condition" (LT+, LT-, ST+, ST-; $\chi^2_1 = 0.03, p = 0.86$). The overall effect of "Treatment group" including the different "Test treatment conditions" (i.e. LTPC, STPC, CON) was not statistically significant ($\chi^2_2 = 2.11, p = 0.35$; Figure 9).

Figure 8: Mean percentage of Go responses ± standard errors of the mean (SEM) are shown for each test treatment condition: Control, ST- (Short-term positive contact group without a treatment before test), ST+ (Short-term positive contact group with a treatment before test), LT- (Long-term positive contact without a treatment before test) and LT+ (Long-term positive contact with a treatment before test) at each trial type: Pos (Positive), AP (Ambiguous Positive), AM (Ambiguous Middle), AN (Ambiguous Negative) and Neg (Negative).

Figure 9: Mean percentage of Go responses ± standard errors of the mean (SEM) are shown for each treatment group: CON (Control), STPC (Short-term positive contact), LTPC (Long-term positive contact), at each trial type: Pos (Positive), AP (Ambiguous Positive), AM (Ambiguous Middle), AN (Ambiguous Negative) and Neg (Negative).

Neither the duration of human contact nor duration of reciprocal contact during these treatment sessions had a significant effect on the responses to the ambiguous trials in the JBT (contact by human: $\chi^2_1 = 0.76, p = 0.38$; reciprocal contact: $\chi^2_1 = 0.18, p = 0.67$).

No effect was found for the interaction between sex and treatment group ($\chi^2_2 = 2.34, p = 0.31$), nor was any significant difference effect found for the interaction in terms of the percentage of Go responses to ambiguous trials in the JBT ($\chi^2_1 = 0.16, p = 0.69$). A modest effect was found for the interaction between replicate and treatment group on the responses to the ambiguous trials ($\chi^2_4 = 11.78, p = 0.05$). Inspection of the graphs revealed the strongest interaction effect when pigs were exposed to the AN cue: LTPC pigs showed least Go responses in replicates one and two, but most Go responses in replicate three (compared to STPC and CON treatments), whereas STPC pigs showed most Go responses in

replicates one and two and an intermediate number of Go responses (i.e. between LTPC and CON pigs) in replicate three.

There was no evidence that pigs were learning that ambiguous cues were always rewarded and this affecting their responses, indicated by the non-significant interaction between treatment group and test session ($\chi^2_{10} = 10.24, p = 0.42$), and in general no evidence of learning across test sessions ($\chi^2_5 = 8.16, p = 0.15$).

3.5. Salivary Cortisol

Of the 30 pigs who made it to the testing stage, a third of these were sampled via saliva swabs to measure cortisol concentrations (from replicates two and three only), before and after two (CON pigs) and three (LTPC, STPC pigs) tests. However, small amounts of saliva recovered from the swabs meant that there was often not enough saliva to run the cortisol tests (in duplicate) to analyse both the before and after samples of the same pig, on the same testing day. Thus, the pre-/post-testing cortisol concentration measurement was only successfully performed on 25 occasions in total. Three LTPC pigs and two STPC pigs supplied enough saliva for pre- and post-testing across the three tests days (two treatment days, one non-treatment day) as planned, while two LTPC pigs and one STPC pig supplied enough saliva to be sampled on two days (for the LTPC pigs both successful samples happened on treatment days, while the STPC pig gave a successful sample on one treatment day and one non-treatment day). Two control pigs supplied enough saliva measure for successful cortisol concentration measurement pre- and post-testing on two days. Over the 25 successful measurements, 12 showed a decrease in post-test salivary cortisol concentration after testing compared to before testing (results are expressed in µg/dL, mean ± standard deviation: -0.5 ± 0.04), while 13 showed an increase in salivary cortisol concentration after testing compared to before testing (0.07 ± 0.06). Results between and within individual pigs and within treatments were highly variable.

Cortisol concentration decreased in two pigs when they received a STPC treatment session (mean ± standard deviation: -0.06 ± 0.05), while two pigs showed an increased cortisol concentration after the STPC treatment session (0.08 ± 0.05). For three pigs, cortisol concentrations decreased on one day of STPC treatment, but increased on the other.

In terms of treatment conditions (i.e. whether or not a pig received the STPC treatment before their test), the average decrease of salivary cortisol when a pig received the STPC treatment was –0.05 (SD = 0.04), while the average increase after testing on a treatment day was 0.7 (SD = 0.06). Results are presented by treatment condition (LT+, ST+, LT-, ST- and CON) in Figure 10.

The average pre-test measurement of salivary cortisol concentration was calculated for the three treatment groups (LTPC= 0.39 µg/dL, STPC= 0.40 µg/dL, CON= 0.39 µg/dL). There were no significant differences in terms of cortisol concentration change between whether the pig received a STPC treatment or not ($F_{1,8} = 2.35$, $p = 0.15$). No significant differences were observed in terms of the change in cortisol concentration based on the duration of human initiated contact ($F_{1,8} = 0.04$, $p = 0.85$) or duration of reciprocal contact ($F_{1,8} = 0.15$, $p = 0.71$) during the STPC treatment. The pigs' responses to the JBT were not affected by the change salivary cortisol concentration ($\chi^2_1 = 0.97$, $p = 0.32$).

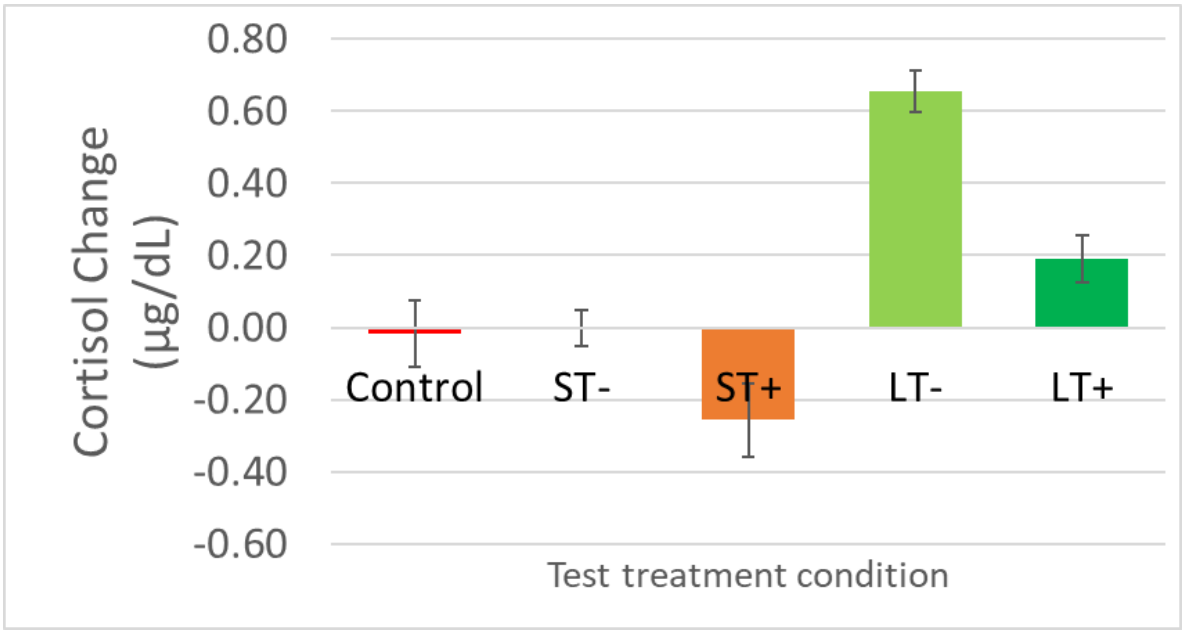

Figure 10: Mean change in cortisol concentration *(µg/dL)* between before testing and after testing samples ± standard errors of the mean (SEM) are shown for each test treatment condition: Control, ST- (Short-term positive contact group without a treatment before test), ST+ (Short-term positive contact group with a treatment before test), LT- (Long-term positive contact without a treatment before test) and LT+ (Long-term positive contact with a treatment before test).

4. Discussion

Evaluating positive affective states in farm animals may improve welfare assessment beyond the traditional focus on the absence of negative states such as disease or distress (Boissy et al., 2007). This study aimed to investigate the effects of long-term and short-term positive contact on affective states in pigs by measuring their responses in a JBT. No significant difference was observed between the LTPC or STPC treatment groups in terms of the amount or type of pig-human contact during the STPC treatment sessions. No significant differences were observed between the groups in their responses to the ambiguous cues in the JBT, nor any interaction found for the LTPC and STPC treatments. No significant differences were recorded in salivary cortisol concentrations pre- and post-test, regardless of treatment group (LTPC, STPC, CON), or treatment condition (LT+, ST+, LT-, ST-, CON), nor was cortisol concentration associated with responses in the JBT.

4.1. Positive Contact Treatments

The behavioural analysis of the pigs during the STPC treatment sessions, which measured frequency of contact, duration of contact, and latency to contact between pig and human, showed no significant differences between the LTPC treatment group, who had been receiving regular positive contact with the experimenter for five minutes at the end of each training day, and the STPC treatment group, who had not. Most pigs interacted with the experimenter during these sessions for at least a few minutes, with only one pig (from the STPC group) showing signs of distress severe enough for the sessions to be stopped altogether. Three pigs (one from the LTPC group, two from the STPC group) even lay on their side to elicit a belly rub from the experimenter during their treatment sessions, thought to be a sign of a positive, relaxed state (Rault et al., 2019). Therefore, it was assumed that these contact treatments prior to testing were appraised by the pigs as a positive event.

The results were surprising in that pigs in the LTPC treatment group did not have a shorter latency to contact the human, nor did they interact for longer, than pigs in the STPC treatment group. This is in contrast to research by Tallet et al. (2014) and Wang et al. (2020), who found that gently handled pigs spent more time in contact with a researcher, than non-

handled pigs. Hemsworth et al. (1996a) found that pigs given a positive handling treatment for five minutes, five days per week for four weeks, a very similar time frame to this study, were significantly quicker to approach the experimenter, and interacted with them for longer, than pigs who had minimal human contact. However, in these studies, the pigs in the control groups generally only interacted with humans during routine feeding and pen maintenance. This differs from the human interactions experienced by the pigs in our study, as all pigs were trained by the experimenter to perform the JBT. Therefore, the level of human contact for the pigs in the STPC and CON groups was significantly more than for pigs who received minimal handling in other studies.

It may be the case that even minimal positive handling, or even time spent in close proximity with a human, is enough for pigs to elicit a positive reaction to a human handler. Such conditions, necessary during this study to train the pigs to perform the JBT, might have created a ceiling effect, after which point additional positive handling treatments were no longer detected through behavioural observations or the JBT. Evidence from the human approach and human avoidance tests at the beginning and end of the experiment (see Appendix B) shows that almost all pigs, including those in the control group who had never received any positive contact, approached the experimenter quickly and showed no avoidance reactions at the end of the experiment. These results are in line with Gonyou et al. (1986), who found positively, minimally and even negatively handled pigs did not differ in terms of approach times in a human approach test, differing only from pigs subjected to an aversive treatment. Habituation to a familiar human alone may be enough to encourage approach behaviour in pigs, regardless of handling treatments (as long as they are not aversive). Brajon et al. (2015b) found that piglets who received gentle handling or food rewards from a researcher did not significantly differ in terms of contact rate, latency to first contact or percentage of time spent in contact with that researcher, to piglets who had spent time in the pen with a passive researcher. The piglets from these groups however did show increased contact to the experimenter compared with the control piglets (no contact at all), or negatively handled piglets, suggesting habituation to the presence of a benign individual may be enough to increase contact behaviours in young pigs. In our study, all pigs, including the control pigs, spent considerable time alone with the experimenter during training, which involved food rewards, which might account for the lack of difference between the two treatment groups

during the STPC treatment sessions and all of the groups during the human approach and avoidance tests.

If indeed the time spent with the experimenter during training creates such a salient effect in pigs, it is possible that in order for the effects of additional positive handling to be detected, more time spent in positive contact with the experimenter is necessary. Certain studies found a difference between handled and non-handled pigs, but the amount of handling experienced by the handled pigs was significantly more than what the pigs in this study experienced during LTPC treatments. For example, Tallet et al. (2014) found gently handled pigs investigated the handler sooner and spent more time investigating her than control pigs in both the home pen and during arena tests, but their handling treatment consisted of ten minutes twice per day. Using a similar handling technique, Wang et al. (2020) found gently handled pigs approached the experimenter more and avoided them less in human approach and avoidance tests than non-handled pigs, after four hours per day of gentle handling (08.00-10.00 and 14.00-16.00) within the home pen. Moreover, in a cognitive judgement bias test similar to ours, Brajon et al. (2015a) found pigs subjected to a gentle handling treatment approached an ambiguous cue in a JBT more than roughly or minimally handled pigs, however their handling treatments were also significantly more intensive (four sessions of five min/day, five d/week) than our study. As our study only involved five minutes of positive contact per day, five (and occasionally six) days per week, it may be the case that increasing the amount of LTPC treatment sessions to several per day or increasing the amount of time the pig spent in these sessions, might elicit additional positive effects.

Still, other influences, such as the environment within which the behaviour was observed, may account for the results documented in this study. Villain et al. (2020) found no significant differences between minimally handled pigs and pigs given additional human contact in terms of anticipation behaviour and vocalisations. To investigate these results further, isolation/reunion tests were performed on the pigs after the main anticipatory testing was completed. They found a similar attraction towards the human for both minimally handled and additionally handled pigs, however the latter group showed more exploratory behaviour to the test room, which the authors suggested may have been indicative of a loss of interest in the human (unpublished data). In our study, the pigs spent a significant amount of

their time budget “Out of field” (LTPC: 16%; STPC: 11%) at the far end of the pen (away from the experimenter) where the camera could not access, and engaging in “Other” behaviours (LTPC: 30%; STPC: 32%). These results might be interpreted as a fear of the experimenter; however the latency data refute this conclusion as almost all of the pigs approached and made contact with the experimenter of their own accord within one minute. Nevertheless, the total amount of time spent not engaging with the experimenter is still quite high, accounting for almost half of the time budget (LTPC: 46%; STPC: 43%). One possible explanation is the pigs’ motivation to explore. Pigs are known to be highly neophilic (Wood-Gush & Vestergaard, 1991), and so the new environment offered by the waiting area where the STPC treatment session took place may have conflicted with any attraction to the familiar experimenter.

However, as (presumed) exploratory behaviours (as measured by the percentage of time the pigs spent engaging in “other” behaviours and “out of field” in the behavioural observations) did not differ significantly between the two treatment groups, the question of why no significant difference was observed between the groups in terms of contact with the experimenter still stands. Luna et al. (2021) found that pigs who had observed a conspecific receiving ten minutes of positive contact for five weeks were quicker to approach a stockperson and interact with them more in an open field test. As the LTPC treatment sessions took place in a compartment separated from the home pen, it was possible for the pigs who were not receiving treatment to observe the pig and experimenter in the treatment pen through small gaps between the wooden partition and its door, however this evidence is at present purely anecdotal. Video recording and analysis of the non-treated pigs during the LTPC treatments in future studies could shed more light on this speculation.

Alternatively, a simple explanation for the homogeneity of the groups might be the effects of associative learning, where the experimenter had, over the course of training, been associated with food rewards. Hemsworth et al. (1996b) found that pigs who had received human contact while feeding were quicker to approach the experimenter and interacted more with them than pigs who had not received human contact while feeding. Future research might consider using one human to conduct training and testing, while a different human would conduct the positive contact sessions, to avoid confounding the positive experience of

the training/rewards with the HAR, although care must also be taken here. Pigs are known to generalise positive experiences with a human to other humans (Hemsworth et al., 1996b; Rault et al., 2020a), although this may depend on the level of the relationship between the human and the pig, shown by studies involving miniature pigs, who, as family pets, presumably have a much higher affinity to their caregivers than farmed pigs. For example, Tanida and Nagano (1998) found that eight-week-old Göttingen line miniature pigs preferred their handler to a stranger after five weeks of interacting with the handler, which included feeding of rewards. Similarly, Fraga et al. (2020) found family-living miniature pigs did not avoid a stranger completely, but (in contrast to dogs) they were more avoidant of social contact in general when a stranger was present, avoiding both the stranger and the caregiver. In this context, the experimenter in our study could be considered as a caregiver, or primary handler, as they were in close proximity to the pigs for the duration of the study (approximately five weeks) and provided not only food rewards but also regular feed and water. A positive HAR can be intrinsically rewarding for domestic animals, and the development of a HAR can be accelerated by associative learning through for example food rewards (Rault et al., 2020b), however in this study the effects of the provision of food rewards during interactions with the experimenter during training cannot currently be disentangled from the effects of either single or repeated positive handling treatments with the same person. In a study with rams Chaumont et al. (2021) found that their subjects showed a preference (in terms of latency to contact and duration of time spent in physical contact) for both a person who had brushed them and a person who had fed them compared to another known person, therefore it is possible that both these elements are important for domesticated animals, and it is unclear in the present study which of these elements the pig may have been responding to. Future studies in this area may be improved by employing different researchers for different aspects of the study, to reduce confounding the effects of food rewards during training with any HAR developed through positive handling.

4.2. Judgement Bias Test

The outcome of the different levels of positive human-animal interactions in this study was measured by a JBT, which, to perform the test and measure accurate results, required a

number of weeks of training. First, the pigs were trained in a stepwise manner with operant training methods to use an active trial initiator (a bell), and then to go to an open goal-box to collect the food reward. After mastering this task, they were later trained to discriminate between a rewarded and an unrewarded goal-box. There is some evidence to suggest that positive mood positively affects learning performance (Vögeli et al., 2014), however in this study the LTPC treatment (intended to improve mood) did not affect learning, with no significant difference observed between the LTPC group and the STPC or CON groups in terms of the number of training sessions needed in the Go/No-go discrimination stage to reach the testing criterion.

In general, the pigs in this study needed an average of 8 shaping sessions to learn the operant task to use the trial initiator. This was significantly longer than the horses trained by Hintze et al. (2018) who needed 4.7 sessions for this stage, despite a similar training protocol. In a study with calves using a similar training protocol Bučková et al. (2019) found their subjects needed on average 11.1 sessions to learn the trial initiation task, but all calves did eventually learn the task. Similarly, all but two of the pigs in our study learned the task, however one of these was excluded due to an injury, while the other was excluded due to low motivation and lack of interaction with the trainer. Hintze et al. (2018) found some horses (five individuals across two time replicates) did not learn the task, while rodents in the same study did not have any problems learning to use the trial initiator. It must be noted however that the rodent training involved moving away from the goal-box, towards the initiator, instead of towards it, as per our study. These results support the ease of integration of a trial-initiator into Go/No-go judgement bias tasks for pigs, and may even improve their compliance rates in JBTs. It is thought that trial initiation will increase motivation and attention (Hintze et al., 2018) and our results indicated a very large compliance with this training method, in that over 85% of the pigs successfully learned the task and made it to testing (excluding one pig for injury at beginning of training), compared to only 59% of pigs in a similar JBT task in a similar study that did not use a trial initiator (Brajon et al., 2015a).

This difference in learning success between our study and the study by Brajon et al. (2015a) might also be due to the nature of the cue used in the study. While Brajon and colleagues used an audio cue, our study used spatial location, which may be more

ecologically relevant to a foraging species such as pigs. Other studies in pigs that have used a spatial design to our study have had similar learning success (Düpjan et al., 2013; 2017). Even in studies where most or all pigs learn the task, it appears that it still takes pigs longer to learn JBTs when they need to respond correctly to audio cues. For example, pigs in the study by Douglas et al. (2012) needed an average of 19 training sessions to reach testing criteria in an auditory Go/No-go JBT. The pigs in our study needed an average of 6.4 sessions to learn the discrimination task based on spatial locations (1.4 sessions to learn to differentiate between the left and right goal-boxes, and 5 sessions to reach training criteria for the Go/No-go task).

This rate of learning was also remarkably fast when compared to similar spatial Go/No-go experiments with other species, for example calves who needed around 11.2 sessions (Bučková et al., 2019), or horses who needed 12.8 sessions (Hintze et al., 2018). This rapid learning might have been influenced by the higher number of trials per session (40 trials) compared to the study with calves (16 trials), although the trial number per session was less than the horses received for training in the study by Hintze et al. (2018; 50 trials).

In terms of the responses to the ambiguous cues in the JBT, pigs from all treatment groups overwhelmingly showed a Go response to the ambiguous positive and ambiguous middle goal-boxes (93.5% and 93.4%, respectively), and a Go response to the ambiguous negative goal-box in over half of the ambiguous negative trials (68%), with no significant differences between treatment groups. Overall, the pigs showed Go responses in the ambiguous trials 84.6% of the time, compared to 38% in calves (Bučková et al., 2019), and 63% in horses (Hintze et al., 2017). Nawroth et al. (2013) found poorer performance in a choice task when pigs respond impulsively, however, the relatively low amount of Go responses to the negative goal-box in our study (10.5%) indicates that the pigs understood and were attentive to the task, so the high number of Go responses in the ambiguous trials was not simply due to difficulty with impulse suppression. This conclusion is sustained by the high standard deviations among responses to the ambiguous cues (up to 20.8% variance) with relatively low deviations to reference cues (Pos and Neg cues, 1.4% and 3% respectively).

There is some evidence to suggest that pigs can learn about the outcomes of ambiguous cues (Murphy et al., 2013; Scollo et al., 2014) which would result in more Go responses to ambiguous cues if ambiguous cues are rewarded as the testing progressed,

however we found no evidence of this effect. The pigs in this study did not show increased Go responses to ambiguous cues in later tests compared to earlier tests, thus ruling a learning effect out as an explanation for the high overall levels of Go responses to the ambiguous cues.

At least three ambiguous cues are considered necessary for robust and comprehensive JBTs (Lagisz et al., 2020) and an important measure of the internal validity of the JBT is the monotonic graded response across the different cues (Hintze et al., 2018). In a JBT, this graded slope indicates that the animal is assessing the ambiguous cues with reference to the position of the positive and negative cues learned through training, as opposed to simply responding to a novel cue. Although analysis on the slope found to be significant, the expected shape of the slope was not observed in this study. Instead, a similarly high number of Go responses to the ambiguous positive (AP) and ambiguous middle (AM) cues were recorded, while Go responses to the ambiguous negative (AN) cue were also surprisingly common. However, a high degree of variation, as evidenced by the large standard deviations, was noted for the ambiguous cues, particularly at the ambiguous negative cue (AP: 10.1%, AM: 10.5%, AN: 20.8%). Differences in the design of this study compared to previous studies might account for such results.

The original Go/No-go task for animals was developed in a way that rats had to press a lever in order to gain a reward in response to a “positive” cue, and refrain from pressing a lever in order to avoid a negative event in response to the “negative” cue (Harding et al., 2004). Later studies have often included an aversive event at the negative cue, which the animal learns to avoid out of fear of punishment. For example, during a spatial JBT with calves, Lecorps et al. (2018) used an air puff to the face of the calf at the negative cue and found the animals showed a graded response in terms of latency to approach the cues (gradually higher latencies towards the negative cue across five spatially distinct cues). Düpjan et al. (2017), found that in their pilot study (2013), pigs did not consider the unrewarded cue as something to avoid, and so the researchers increased the putative “cost” of a response to their negative cue by introducing a negative event. The result was an improved gradient in response latencies. Although it is important to note that both these studies used latency-based (i.e. continuous) outcome measures, while our study used a choice-based (i.e. binomial) outcome measure, and so care must be taken when making comparisons, it may

nonetheless be significant that in our study no aversive events were included at the negative goal-box. Therefore, it may be the case that the high level of Go responses recorded in our study might reflect an imbalance between the pigs' reward seeking and punishment avoidance systems (Mendl et al., 2009; 2010). As no punishment was included, the pigs may simply have evaluated that there was little risk in approaching any ambiguous cue, or that the potential "reward" from approaching the ambiguous cue (chocolate M&M®) outweighed the possible "punishment" (no reward and longer wait time until next potential reward) for going to the ambiguous cue instead of a reinitiation, weighting their responses towards Go. This asymmetry in perceived payoff of the positive and negative response outcomes, and its subsequent effects on responses in the JBT, has been noted by Lagisz et al. (2020), who found Reward-Punishment tasks to have the largest effect size, and therefore may be more sensitive to manipulations of affective state.

Furthermore, in studies that included a negative treatment, such as Brajon et al. (2015a), the differences in the emotional states of the treatment groups may have been more pronounced (e.g. "negative" vs. "positive" conditions). As the experience of negative events for animals in the negative treatment group may have induced a state of anxiety, this would mean more cautious responses in the JBT, translating to fewer Go responses to ambiguous cues. Our study, on the other hand, did not include any negative treatment, but only a control and putatively positive treatments ("neutral" vs. "positive" conditions), therefore the differences in background affect of the animals in the different treatment groups in our study would not have been as polarized as in studies that included positive and negative conditions. Other studies which investigated the differences between presumably positive and negative treatments found similarly significant differences between their treatment groups (Bučková et al., 2019; Zidar et al., 2014). However, studies that have only studied negative conditions compared to controls ("neutral" vs. "negative") have still found significantly different results between the groups (Harding et al., 2004; Doyle et al., 2011). Perhaps the cautious outlook brought about by the negative conditions elicits a stronger effect than a similar amount of positive treatment. This makes sense on an ecological level, as a low level of pessimism (and therefore caution) may have a potentially more disastrous effects (e.g. succumbing to predation) than a low level of optimism. The tendency of potential costs being more heavily weighted than potential gains (if each element is of equal intensity) when making a decision

under risk is well documented in psychological literature (Kanouse & Hanson, 1987; Peeters & Czapinski, 1990). Future studies might use these suggestions as a starting point to optimise study design, incorporating a mild aversive event (e.g. a spray of water) into the negative goal to increase perceived "risk" of going to an ambiguous cue.

However, it is important to note that this study's design was based on the spatial Go/No-go task developed by Hintze et al. (2018), that also did not include a punishment at the negative goal-box, yet still observed monotonically graded responses to the task in horses, rats, and two strains of mice (although not in all individuals). In our study, the inverse was true. A graded response was only present in four individuals, while a number of pigs (n =7; 23%) always showed a go response, regardless of the ambiguous cue presented. A further twelve (40%) showed a No-go response to 3 or fewer ambiguous cues across 18 presentations (3 ambiguous presentations per test, 6 tests). Hintze and colleagues did not investigate different treatment effects on their animals, as the goal of the study was to develop the spatial Go/No-go task with an active trial initiation on different species. It is possible that by applying positive handling treatments to selected pigs, the background affect of all of the pigs was positively influenced, possibly via emotional contagion.

Emotional contagion comes about when a particular emotion is aroused in an individual after witnessing it in another (Hatfield et al., 1993). Pigs housed together adopt similar emotional behaviours in response to certain stimuli, even when only a small number of them have been conditioned to that stimuli. Reimert et al. (2013) found evidence for emotional contagion when they measured the behaviour and salivary cortisol response of naive pigs during anticipation and experience of a rewarding or aversive event by their conditioned pen-mates. Furthermore, a later study found that naive pigs seemed to be both positively and negatively affected by the treated pigs after they returned from a positive or negative treatment respectively (Reimert et al., 2017). The current study had a similar treatment set-up to the cited study (six pigs per home pen, two pigs in treatment per pen) therefore it is possible that the mood induced by the positive contact condition was carried across to the other pigs in the home pen, which led to similar results in the JBT.

It is crucial to note that in studies that used the JBT as a measure and found significant effects of treatments such as housing (Düpjan et al., 2013; Bučková et al., 2019), enrichment

(Douglas et al., 2012) or unpredictable stressors (Doyle et al., 2011) the animals in each condition were housed together, eliminating any potential for a transfer of effects from animals in one treatment group to other treatment groups through emotional contagion. This contrasts with our study, where pigs of different treatment groups were housed together. This explanation may also account for both the behavioural observations during the STPC treatment sessions and the results of the human approach and avoidance tests, which showed greatly reduced latencies to approach and reduced avoidance for all pigs in all treatment groups at the end of the experiment. Wang et al. (2020) found greater levels of pig-human contact during human approach and avoidance tests for positively handled piglets compared to controls, but the positive handling took place within the home pen, again meaning all pigs that received the same treatment were housed together. Future research could investigate whether housing pigs in the same treatment groups together might lead to significantly different scores in a JBT. If keeping pigs in the same treatment groups together does lead to significantly different results, this would lend support to the emotional contagion theory, meaning that inducing a positive mood in only a few pigs may generate a positive affective state in their larger group, thus pointing towards what may be a novel approach to improving efficiency of HAR-related welfare measures on farms aiming to improve affective states at group level.

It is also possible that the responses recorded in this study across the treatment groups and test treatment conditions were affected by factors other than those we set out to measure. Neville et al. (2020) have suggested that affective states within the context of a JBT and decision-making towards its ambiguous cues are not always straightforward and in line with the general hypothesis that positive and negative affective states generate ‘optimistic’ and ‘pessimistic’ responses, respectively. They posit that environments in which the rewards and punishers are experienced and the environment of the test situation itself may generate contrasting effects that could influence the subject’s affect during the JBT. In this respect, some aspects of our study’s design may have influenced the pigs’ affect in such a way that could have shaped the results of the JBT.

For example, in order to increase motivation during training and testing, the pigs’ feeding times were restricted to the afternoons, when the sessions had finished. Brajon et al.

(2015a) also restricted feeding for their experimental subjects. In their study, they allowed 1/3 of the normal food allotment in the morning, however they reported that this rationing may have motivated the pigs in their study to take more risks (i.e. more Go responses to negative cues). The pigs in this study were not fed at all in the morning, which may have increased their motivation to find food. Although, unlike the study by Brajon and colleagues, this did not translate into a high number of Go responses in the negative trials (as evidenced by the relatively low percentage of Go responses to the negative goal-box), it may still have had an effect on their responses in the ambiguous trials, of which they were not certain of the outcome. Nevertheless, there does not appear to be considerable support for this explanation in the available literature, as in their recent meta-analysis, Lagisz et al. (2020) found that food deprivation during JBTs did not significantly influence results.

Alternatively, a training effect might account for the high levels of optimism-like responses across this sample. It has been suggested that operant training can be considered as cognitive enrichment. Cognitive enrichment for farm animals is defined as "the ability to elicit perceptive processes for operant learning of discriminatory cues which lead eventually to better active control of the environment" (Manteuffel et al., 2009; p. 88). The extrinsic nature of the reinforcement in this study (the chocolate reward) may have led to long lasting effects (Tarou & Bashaw, 2007). Westlund (2014) has also suggested that training might have long-term effects that spill over into non-training days. It may be possible that the weeks of training leading up to testing affected the mood of all pigs, to a ceiling level meaning any effect of the positive handling conditions would be no longer detectable in the JBT. In a similar thread, previous studies have shown that environmental enrichment produces an optimistic bias in pigs (Douglas et al., 2012), and therefore the enrichment that the pigs in this study experienced through training may have encouraged the pigs to be more exploratory (Ralph et al., 2018). A higher propensity for exploratory behaviour, coupled with lack of experience with negative events, may have translated into more Go responses to the novel, ambiguous cues. However, a high number of training sessions are often required in order for the animal to participate in a JBT, and other studies with a similar (or larger) amount of training sessions than our study have not observed similar results. Therefore, the enriching effect of training may not fully account for the high levels of optimistic-like responses observed in this study.

There has been some progress by ways of automating JBTs, which may eliminate the need for manual training, therefore removing the potential for training and handling effects altogether from the study design. Jones et al. (2018) recently developed an automated and self-initiated JBT for rats, using the animal's natural investigative behaviour. The rat inserts its nose into a device to start the trial (active initiation) and learns to stay for a reward or move away quickly, depending on a tone played. The researchers noted high success rates in engagement with the task, without food restriction during training, removing the possibility of this potential stressor. Such a self-initiated trial could be adapted for pigs, allowing them to engage with automated equipment to learn and perform the JBT, eliminating any need for a manual training period, which could be very useful for studies that aim to investigate the effects of handling or HAR on pigs' responses in a JBT.

Furthermore, cognitive bias tests that measure naturally occurring behaviour based on ecologically relevant stimuli have the potential to reduce the amount of associative training prior to conventional cognitive bias tests (Brilot et al., 2009; Salmeto et al., 2011; Bethell et al., 2016). For pigs, for example, it may be possible to harness their strong motivation to explore, and in place of goal-boxes there could be objects to manipulate and learn about the outcomes (whether or not manipulating a given object is rewarded), before presenting them with ambiguous objects (ranging, for example, in size, relative to the reference objects). Both automated and ecologically relevant versions of the JBT have been found to be learned successfully by animals and produce internally valid results (Krakenberg et al., 2019), and a combination of these two elements may represent a promising advancement of judgement bias testing.

4.3. Salivary Cortisol

This study measured salivary cortisol concentrations; however it was only possible to get sufficient amounts of saliva to analyse both samples from the same day, pre- and post-testing, from ten pigs. Of these, half were in the LTPC group, while only two were in the control group. This distribution may have been due to the sampling procedure, as the experimenter would hold a swab to the pig, who was required to chew on it for up to 30 seconds to allow the swab to absorb the necessary amount of saliva for adequate sampling. Pigs in the LTPC

group were more accustomed to close contact with a human (the experimenter) and therefore this may be why more samples were gathered successfully from this group than the others. Pigs that were not included in the salivary analysis either refused to accept the saliva swab at the pre- or post-test measurement (or both measurements) or would not continue to chew on the swab for the necessary length of time. Therefore, the possibility of a sampling bias toward pigs who accepted close human proximity cannot be ruled out.

Pedersen et al. (1998) found cortisol concentration levels were significantly lower in pigs in a positive handling treatment than those in a minimal or negative handling treatment. No such difference was found in our study, which found no significantly different levels of cortisol concentrations between the pre- and post-test measurement whether a STPC treatment session had been performed or not. Coulon et al. (2013) similarly found, rather unexpectedly, no significant differences in levels of blood cortisol concentrations in lambs during an isolation and reunion test with a familiar caregiver. They surmised that this may have been due to a release of oxytocin, which is thought to regulate the HPA axis (Cook et al., 1997). Unfortunately due to the small amount of saliva that was collected from the pigs in this study, we were not able to measure oxytocin levels in order to examine if there was any increase in this hormone after the STPC treatment sessions that may have affected our subjects' cortisol concentrations.

However, the concentrations of salivary cortisol recorded in this study were quite high compared to studies that used similar sampling techniques on pigs of a similar age, for example Düpjan et al. (2013), who recorded average salivary cortisol concentrations between 1.71 ng/ml and 2.18 ng/ml during their study. The pre-test measurement of salivary cortisol concentration in our study was surprisingly high across all treatment groups (values when converted to ng/ml for comparison: LTPC= 3.92 ng/ml, STPC= 4.03 ng/ml, CON= 3.85 ng/ml). This may have been due to the fact that the sampling was done between 08.00 and 12.00, when a pig's cortisol level is naturally at the highest point of its circadian pattern (Ekkel et al., 1996), or perhaps due to their young age. Ruis (2001) observed that cortisol concentration levels generally decrease as pigs age, however the youngest pigs in his study were twelve weeks old (the average Midline Estimating Statistic Of Rhythm, or MESOR, recorded for the twelve-week-old pigs was 1.19 ng/ml). The pigs in this study were

approximately nine weeks old at time of sampling. However, Düpjan et al. (2013) observed lower overall levels of salivary cortisol concentrations in a very similar study, with similar collection methods on a similar age group, so it is unlikely that age alone explains our results. Düpjan and colleagues recorded the average basal levels of salivary cortisol concentrations in pigs subjected to an isolation treatment (2.18 ng/ml) and a control group (1.83 ng/ml). Similar to our study, they found no differences in average cortisol concentration levels before and after judgement bias testing.

High concentrations of cortisol are normally associated with stress. Rutherford et al. (2006) found elevated cortisol concentrations in pigs who had been subjected to a social stressor compared to controls (Test = 1.45 ng/ml, Control = 1.05 ng/ml: pigs approximately sixteen weeks old at time of testing), while Parrott & Misson (1989) recorded increased salivary cortisol concentrations in pigs who had been food and water deprived. In a study on recently weaned piglets, Rault et al. (2015) found levels of plasma cortisol concentrations were significantly reduced at 28 hours after weaning, compared to 4 hours after weaning. The results of the saliva sampling in the present study might be explained by timing, as the studies cited here allowed a longer time between pre-treatment sampling, treatment, and post-treatment sampling. It may also be possible that the separation and short isolation period required for the saliva sampling in this study might have initiated a stress response which could explain the high cortisol concentrations detected in the pre-treatment samples. Düpjan et al. (2013) did not find effects of isolation on levels of salivary cortisol concentrations in their pigs, however they point out that this may have been due to the fact that because they imposed the isolation treatment on the pigs at the same time each day, the pigs may have habituated to this predictable treatment, rendering it less stressful than initially assumed. The pigs in this study would have been similarly used to the separation and short isolation periods before and during their trainings, so the effects of separation and isolation alone are an unlikely reason for the high levels of cortisol observed.

Perhaps a more plausible explanation would be the anticipation of the food rewards in the test, which may have triggered an excitatory response in the pigs. Increases in cortisol concentrations during dog-human interactions have been recorded (Handlin et al., 2011), which has been interpreted by some authors to reflect excitement (Rault et al., 2020b). As

discussed above, the presence of the experimenter could have been associated with food (Hemsworth et al., 1996b), and the food-restricted pigs may have therefore anticipated the rewards when the experimenter began the familiar procedure of separating the pig and taking it out of the home pen. It is also possible that the anticipation and effects of the exercise and challenge involved in the JBT led to elevated cortisol concentrations before and after collection. Exercise such as running on a wheel in rodents has been suggested to be "voluntary, controllable stress", which activates the HPA axis in a similar way to stressful events, but with rewarding as opposed to aversive consequences in the brain (Stranahan et al., 2008). The majority of the pigs during testing would enter the experimental arena voluntarily to participate in the JBT, and due to the active trial initiation, this activity was somewhat controllable, therefore anticipation of and participation in the JBT might account for the elevated cortisol concentrations observed.

Angle et al. (2009) emphasised the importance of timing when collecting measurements of cortisol, as their subjects (sled dogs) showed increased cortisol concentrations pre-exercise, suggesting an anticipatory effect. The act of separating the pigs from their home pen, or simply even the presence of the experimenter may have stimulated an anticipatory effect on the pigs that led to increased cortisol concentrations in the minutes before collection. Thus, the pre-test measure, assumed in this study to reflect a "baseline" level of cortisol concentration, may actually have reflected an already elevated state, leading to a ceiling effect where no further increase could be detected post-test. Future studies should attempt to find a true baseline, with care taken to control for circadian rhythms, perhaps by sampling the pigs several times throughout the day in order to establish a midline estimated statistic of rhythm (MESOR), several times across the stages of training and testing, to better compare the effects of the treatments.

As noted in the introduction, a high cortisol concentration may only be an indicator of high arousal (Hubert et al., 1993; Mendl et al., 2009), and this type of bodily stress response may actually at times be evidence of a positive experience (Seyle, 1958; Ralph & Tilbrook, 2016). The results of this JBT indicate positive valence, which may support to the suggestion that the observed high cortisol concentrations were more likely a consequence of an excitatory response as opposed to distress. The combination of the results of the physiological and

cognitive measures might indicate a state of "eustress", a type of stress associated with positive feelings and a healthy physical state (Lazarus, 1993). Eustress is a concept that has been notoriously difficult to study, due to the problem of differentiation from (negative) states of distress (Kupriyanov & Zhdanov, 2014). Combining physiological measures such as cortisol concentrations with cognitive measures such as the JBT might harbour some potential as a step towards the study and validation of measures of eustress. Because eustress is strongly associated with adaptive function and, following from that, positive emotions (Villalba & Manteca, 2019), this could be an important factor to consider when studying the impact of interventions aimed at improving the emotions and moods of animals. Additional sampling to establish a baseline for cortisol concentrations in saliva may also be effective in disentangling eustress brought on by the anticipation of challenge and reward from other, more negative states such as chronic anxiety or depression.

4.4. Conclusion

This study provided evidence that a spatial Go/No-go judgement bias task with active trial initiation can be easily and quickly learned by weaner pigs in a farm-based setting. Our results indicate that the pigs in this study appeared, at least within the temporal parameters of the test, to have a positively valenced and a potentially highly aroused affective state. However, no discernible differences were found between the long-term, short-term or control groups in this study in terms of their responses to the JBT, nor were any differences observed between whether or not a pig received positive handling immediately prior to their test. Therefore, whether the positive valence detected in the JBT reflected mood or emotion is yet to be elucidated. Furthermore, the possibility that the affective state was induced by a variable other than the positive handling treatments, which overshadowed any effect of either the long- or short-term treatments, cannot be precluded as an explanation for the high levels of optimism-like scores recorded in the JBT.

Improvements in study design might yield more useful results. For example, different researchers performing the training and the positive contact treatments may reduce or eliminate the confounding effects of handling and conditioning during the training phase. The inclusion of a mild punishment at the negative goal-box might lead to a better balance

between the reward-seeking and punishment-avoidance systems within the subjects, while housing animals in the same treatment groups together may reduce the possibility of emotional contagion as a confounding influence on JBT scores.

However, it is vital to note that because pigs are prone to generalising positive experiences with humans, perhaps the current format of JBT, which requires much hands-on training, is not the best indicator of the effects of positive human contact in pigs, as the positive reinforcement generated by the necessary training, in addition to the close and regular proximity of the researcher(s) may act as a confounding factor that could affect the responses of the animals within the test situation. Automated JBT arenas may present an opportunity to eliminate the effects of manual training and improve test validity.

5. Summary

Previous research shows that positive human-animal interactions can improve animals' affective states, but the mechanisms underlying this change remain poorly understood. This study aimed to disentangle the effect of mood- and emotion-inducing treatments on pigs' responses in a judgement bias task. We administered the same five-minute positive contact treatment, either repeatedly over the course of three weeks (long-term positive contact: LTPC; intended to influence mood, n = 11) or immediately before a judgement bias test (short-term positive contact: STPC; intended to influence emotion, n = 10), while a control group (CON, n = 9) received no positive contact sessions. In addition, LTPC pigs received STPC treatment sessions before 50% of test sessions to elucidate any interaction between emotion and mood. Pigs were trained to perform a spatial Go/No-go judgement bias test (JBT) and the percentage of Go responses to ambiguous cues in the JBT was compared across treatments. STPC contact sessions were coded for contact latency and duration, and saliva was collected pre- and post-testing in a sub-set of animals to measure cortisol concentration as an indicator of arousal. We did not find any statistically significant differences between the treatment groups with respect to the pigs' behaviour during STPC treatment sessions, their performance in the JBT, nor any differences in cortisol concentrations pre- and post-testing. There may be several reasons for these results. Pigs from different treatment groups were housed together, allowing for the possibility of emotional contagion from the LTPC pigs to pigs in the other treatment groups. The regular human handling for training and testing may have overwritten effects of the human contact treatments, rendering their effects undetectable. The results of human approach and avoidance tests carried out before and after the training and testing phases may support this conclusion. Further research is warranted to disentangle the effect of potential interactions between the training procedure and treatments including human-animal interactions.

6. Zusammenfassung

Bisherige Untersuchungen zeigen, dass positive Interaktionen zwischen Mensch und Tier den affektiven Zustand der Tiere verbessern können. Die Mechanismen, die diesem Zusammenhang zugrunde liegen sind jedoch weitgehend unbekannt. Ziel dieser Studie war es, den Effekt von Stimmungs- und Emotionsinduzierenden Behandlungen auf die Reaktion von Schweinen in einem Judgement Bias Test zu erforschen. Die Schweine erhielten dieselbe fünf-minütige Sequenz aus positiven Interaktionen entweder wiederholt über einen Zeitraum von drei Wochen (long-term positive contact: LTPC; mit dem Ziel die Stimmung zu beeinflussen, n = 11) oder einmalig, direkt vor dem Judgement Bias Test (short-term positive contact: STPC; mit dem Ziel die Emotionen zu beeinflussen, n = 10). Außerdem wurde eine Kontrollgruppe ohne jeglichen positiven Kontakt getestet (CON, n=9). Zusätzlich erhielten die Schweine der LTPC Gruppe vor 50 % der Testeinheiten die Behandlung der STPC Schweine, um für eine mögliche Interaktion zwischen Stimmung und Emotionen zu testen. Die Schweine wurden darauf trainiert, einen örtlichen Go/No-go Judgement Bias Test (JBT) zu absolvieren. Die Tiere wurden mit zwei Zielen (rechts und links) konfrontiert und lernten, dass eine der beiden Seiten immer mit einer Belohnung verbunden ist, die andere aber nie. Im JBT wurde der Prozentanteil der Go-Reaktionen auf Ziele, die sich irgendwo zwischen dem äußerst rechten und dem äußerst linken befanden, zwischen den Gruppen verglichen. Für die STPC Einheiten wurde die Dauer bis zur Kontaktaufnahme und die Dauer des Kontakts aufgezeichnet. Um die Cortisol-Konzentrationen als Indikator für Aufregung zu messen wurden Speichelproben von einigen Tieren erhoben. Die Gruppen zeigten keine statistisch signifikanten Unterschiede im Verhalten während der STPC Einheiten, in den JBT oder in den Cortisol-Konzentrationen vor und nach den Testeinheiten. Es gibt mehrere mögliche Gründe für diese Ergebnisse. Die Schweine aus den unterschiedlichen Gruppen wurden in derselben Unterkunft gehalten, was eine Übertragung der Emotionen der LTPC Schweine auf die anderen Tiere ermöglicht haben könnte. Der regelmäßige Umgang mit Menschen, der für Training und Tests notwendig war, könnte die Effekte der Unterschiedlichen Behandlungen ausgedünnt haben, wodurch sie nicht mehr erkennbar waren. Die Ergebnisse der Approach- und Avoidance-Tests, die vor und nach dem Experiment durchgeführt wurden, unterstützen

diese Vermutung. Um diesen Ausdünnungseffekt zu überprüfen und zu verstehen sind weitere Forschungen notwendig.

7. References

Angle, C. T., Wakshlag, J. J., Gillette, R. L., Stokol, T., Geske, S., Adkins, T. O., & Gregor, C. (2009). Hematologic, serum biochemical, and cortisol changes associated with anticipation of exercise and short duration high-intensity exercise in sled dogs. *Veterinary Clinical Pathology*, 38(3), 370-374.

Asher, L., Friel, M., Griffin, K., & Collins, L. M. (2016). Mood and personality interact to determine cognitive biases in pigs. *Biology letters, 12*(11), 20160402.

Baciadonna, L., Nawroth, C., & McElligott, A. G. (2016). Judgement bias in goats (Capra hircus): investigating the effects of human grooming. *PeerJ, 4,* e2485.

Bethell, E. J. (2015). A "how-to" guide for designing judgment bias studies to assess captive animal welfare. J*ournal of Applied Animal Welfare Science, 18*(1), S18-S42.

Bethell, E. J., Holmes, A., MacLarnon, A., & Semple, S. (2016). Emotion evaluation and response slowing in a non-human primate: new directions for cognitive bias measures of animal emotion?. *Behavioral Sciences, 6*(1), 2.

Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissiera, I. & Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology & behavior, 92*(3), 375-397.

Brajon, S., Laforest, J. P., Schmitt, O., & Devillers, N. (2015a). The way humans behave modulates the emotional state of piglets. *PLoS One, 10*(8), e0133408.

Brajon, S., Laforest, J. P., Bergeron, R., Tallet, C., Hötzel, M. J., & Devillers, N. (2015b). Persistency of the piglet's reactivity to the handler following a previous positive or negative experience. *Applied Animal Behaviour Science, 162*, 9-19.

Brilot, B. O., Normandale, C. L., Parkin, A., & Bateson, M. (2009). Can we use starlings' aversion to eyespots as the basis for a novel 'cognitive bias' task?. Applied Animal *Behaviour Science, 118*(3-4), 182-190.

Broom, D. M. (1991). Animal welfare: concepts and measurement. *Journal of Animal Science 69* (10), 4167-4175.

Bučková, K., Špinka, M., & Hintze, S. (2019). Pair housing makes calves more optimistic. *Scientific Reports, 9*(1), 1-9.

Carreras, R., Arroyo, L., Mainau, E., Valent, D., Bassols, A., Dalmau, A., Faucitano, L., Manteca, X., & Velarde, A. (2017). Can the way pigs are handled alter behavioural and physiological measures of affective state?. *Behavioural processes, 142*, 91-98.

Chaumont, S., Freitas-de-Melo, A., Pinto-Santini, L., Menant, O., Zambra, N., & Ungerfeld, R. (2021). Rams recognize and prefer the human who regularly brushed them. *Applied Animal Behaviour Science, 236*, 105250.

Chepenik, L. G., Cornew, L. A., & Farah, M. J. (2007). The influence of sad mood on cognition. *Emotion, 7*(4), 802.

Cook, C. J. (1997). Oxytocin and prolactin suppress cortisol responses to acute stress in both lactating and non-lactating sheep. *Journal of Dairy Research, 64*(3), 327-339.

Coulon, M., Nowak, R., Andanson, S., Ravel, C., Marnet, P. G., Boissy, A., & Boivin, X. (2013). Human–lamb bonding: oxytocin, cortisol and behavioural responses of lambs to human contacts and social separation. *Psychoneuroendocrinology, 38*(4), 499-508.

Coulon, M., Nowak, R., Peyrat, J., Chandèze, H., Boissy, A., & Boivin, X. (2015). Do lambs perceive regular human stroking as pleasant? Behavior and heart rate variability analyses. *PLoS One, 10*(2), e0118617.

Désiré, L., Veissier, I., Després, G., & Boissy, A. (2004). On the way to assess emotions in animals: do lambs (Ovis aries) evaluate an event through its suddenness, novelty, or unpredictability?. *Journal of Comparative Psychology, 118*(4), 363.

Donald, R. D., Healy, S. D., Lawrence, A. B., & Rutherford, K. M. (2011). Emotionality in growing pigs: is the open field a valid test?. *Physiology & behavior, 104*(5), 906-913.

Douglas, C., Bateson, M., Walsh, C., Bédué, A., & Edwards, S. A. (2012). Environmental enrichment induces optimistic cognitive biases in pigs. *Applied Animal Behaviour Science, 139*(1-2), 65-73.

Doyle, R. E., Fisher, A. D., Hinch, G. N., Boissy, A., & Lee, C. (2010a). Release from restraint generates a positive judgement bias in sheep. Applied Animal Behaviour Science, 122(1), 28-34.

Doyle, R. E., Vidal, S., Hinch, G. N., Fisher, A. D., Boissy, A., & Lee, C. (2010b). The effect of repeated testing on judgement biases in sheep. *Behavioural Processes, 83*(3), 349-352.

Doyle, R. E., Lee, C., Deiss, V., Fisher, A. D., Hinch, G. N., & Boissy, A. (2011). Measuring judgement bias and emotional reactivity in sheep following long-term exposure to unpredictable and aversive events. *Physiology & Behavior, 102*(5), 503-510.

Düpjan, S., Ramp, C., Kanitz, E., Tuchscherer, A., & Puppe, B. (2013). A design for studies on cognitive bias in the domestic pig. *Journal of Veterinary Behavior, 8*(6), 485-489.

Düpjan, S., Stracke, J., Tuchscherer, A., & Puppe, B. (2017). An improved design for the spatial judgement task in domestic pigs. *Applied Animal Behaviour Science, 187*, 23-30.

Ekkel, E. D., Dieleman, S. J., Schouten, W. G., Portela, A., Cornélissen, G., Tielen, M. J., & Halberg, F. (1996). The circadian rhythm of cortisol in the saliva of young pigs. *Physiology & behavior, 60*(3), 985-989.

Estep, D. Q., & Hetts, S. (1992). Interactions, relationships, and bonds: The conceptual basis for scientist–animal relations. In H. Davis & D. A. Balfour (Eds.), *The inevitable bond: Examining scientist–animal interactions* (p. 6–26). Cambridge University Press.

Forkman, B., Boissy, A., Meunier-Salaün, M. C., Canali, E., & Jones, R. B. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior, 92*(3), 340-374.

Fraga, P. P., Gerencsér, L., Lovas, M., Újváry, D., & Andics, A. (2020). Who turns to the human? Companion pigs' and dogs' behaviour in the unsolvable task paradigm. *Animal Cognition*, XX 1-8.

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution, 7*(11), 1325-1330.

Gonyou, H. W., Hemsworth, P. H., & Barnett, J. L. (1986). Effects of frequent interactions with humans on growing pigs. *Applied Animal Behaviour Science, 16*(3), 269-278.

Grandin, T., & Shivley, C. (2015). How farm animals react and perceive stressful situations such as handling, restraint, and transport. *Animals, 5*(4), 1233-1251.

Handlin, L., Hydbring-Sandberg, E., Nilsson, A., Ejdebäck, M., Jansson, A., & Uvnäs-Moberg, K. (2011). Short-term interaction between dogs and their owners: effects on oxytocin, cortisol, insulin and heart rate—an exploratory study. *Anthrozoös, 24*(3), 301-315.

Harding, E. J., Paul, E. S., & Mendl, M. (2004). Cognitive bias and affective state. *Nature, 427*(6972), 312-312.

Hatfield, E., Cacioppo, J. T., & Rapson, R. L. (1993). Emotional contagion. *Current directions in psychological science, 2*(3), 96-100.

Hemsworth, P. H., Price, E. O., & Borgwardt, R. (1996a). Behavioural responses of domestic pigs and cattle to humans and novel stimuli. *Applied animal behaviour science, 50*(1), 43-56.

Hemsworth, P. H., Verge, J., & Coleman, G. J. (1996b). Conditioned approach-avoidance responses to humans: the ability of pigs to associate feeding and aversive social experiences in the presence of humans with humans. *Applied Animal Behaviour Science, 50*(1), 71-82.

Hillmann, E., Schrader, L., Mayer, C., & Gygax, L. (2008). Effects of weight, temperature and behaviour on the circadian rhythm of salivary cortisol in growing pigs. *Animal, 2*(3), 405-409.

Hintze, S., Roth, E., Bachmann, I., & Würbel, H. (2017). Toward a choice-based judgment bias task for horses. *Journal of applied animal welfare science, 20*(2), 123-136.

Hintze, S., Melotti, L., Colosio, S., Bailoo, J. D., Boada-Saña, M., Würbel, H., & Murphy, E. (2018). A cross-species judgement bias task: integrating active trial initiation into a spatial Go/No-go task. *Scientific reports, 8*(1), 1-13.

Hubert, W., Möller, M., & de Jong-Meyer, R. (1993). Film-induced amusement changes in saliva cortisol levels. *Psychoneuroendocrinology, 18*(4), 265-272.

Jones, S., Neville, V., Higgs, L., Paul, E. S., Dayan, P., Robinson, E. S., & Mendl, M. (2018). Assessing animal affect: an automated and self-initiated judgement bias task based on natural investigative behaviour. *Scientific reports, 8*(1), 1-12.

Kanouse, D. E., & Hanson, L. R., Jr. (1987). *Negativity in evaluations*. In E. E. Jones, D. E. Kanouse, H. H. Kelley, R. E. Nisbett, S. Valins, & B. Weiner (Eds.), *Attribution: Perceiving the causes of behavior* (p. 47–62). Lawrence Erlbaum Associates, Inc.

Kirschbaum, C., & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology, 19*(4), 313-333.

Kooij, E. E. V., Kuijpers, A. H., Schrama, J. W., Van Eerdenburg, F. J. C. M., Schouten, W. G. P., & Tielen, M. J. M. (2002). Can we predict behaviour in pigs?: Searching for consistency in behaviour over time and across situations. *Applied Animal Behaviour Science, 75*(4), 293-305.

Krakenberg, V., Woigk, I., Rodriguez, L. G., Kästner, N., Kaiser, S., Sachser, N., & Richter, S. H. (2019). Technology or ecology? New tools to assess cognitive judgement bias in mice. *Behavioural brain research, 362*, 279-287.

Kupriyanov, R., & Zhdanov, R. (2014). The eustress concept: problems and outlooks. *World Journal of Medical Sciences, 11*(2), 179-185.

Lagisz, M., Zidar, J., Nakagawa, S., Neville, V., Sorato, E., Paul, E. S., Bateson, M., Mendl, M. & Løvlie, H. (2020). Optimism, pessimism and judgement bias in animals: A systematic review and meta-analysis. *Neuroscience & Biobehavioral Reviews*, *118*, 3-17

Lazarus, R. S. (1993). From psychological stress to the emotions: A history of changing outlooks. *Annual review of psychology, 44*(1), 1-22.

Lecorps, B., Weary, D. M., & von Keyserlingk, M. A. (2018). Pessimism and fearfulness in dairy calves. *Scientific reports, 8*(1), 1-9.

Luna, D., González, C., Byrd, C. J., Palomo, R., Huenul, E., & Figueroa, J. (2021). Do Domestic Pigs Acquire a Positive Perception of Humans through Observational Social Learning?. *Animals, 11*(1), 127.

Lürzel, S., Bückendorf, L., Waiblinger, S., & Rault, J. L. (2020). Salivary oxytocin in pigs, cattle, and goats during positive human-animal interactions. *Psychoneuroendocrinology, 115*, 104636.

Manteuffel, G., Langbein, J., & Puppe, B. (2009). From operant learning to cognitive enrichment in farm animal housing: bases and applicability. *Animal Welfare, 18*(1), 87-95.

Mendl, M., Burman, O. H., Parker, R. M., & Paul, E. S. (2009). Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Applied Animal Behaviour Science, 118*(3-4), 161-181.

Mendl, M., Burman, O. H., & Paul, E. S. (2010). An integrative and functional framework for the study of animal emotion and mood. *Proceedings of the Royal Society B: Biological Sciences, 277*(1696), 2895-2904.

Minitab 17 Statistical Software (2010). [Computer software]. State College, PA: Minitab, Inc. (www.minitab.com)

Muns, R., Rault, J. L., & Hemsworth, P. (2015). Positive human contact on the first day of life alters the piglet's behavioural response to humans and husbandry practices. *Physiology & behavior, 151*, 162-167.

Murphy, E., Nordquist, R. E., & van der Staay, F. J. (2013). Responses of conventional pigs and Göttingen miniature pigs in an active choice judgement bias task. Applied Animal *Behaviour Science, 148*(1-2), 64-76.

Murphy, E., Nordquist, R. E., & van der Staay, F. J. (2014). A review of behavioural methods to study emotion and mood in pigs, Sus scrofa. *Applied Animal Behaviour Science, 159*, 9-28.

Nawroth, C., Ebersbach, M., & von Borell, E. (2013). Are juvenile domestic pigs (Sus scrofa domestica) sensitive to the attentive states of humans?—The impact of impulsivity on choice behaviour. *Behavioural processes, 96*, 53-58.

Neave, H. W., Daros, R. R., Costa, J. H., von Keyserlingk, M. A., & Weary, D. M. (2013). Pain and pessimism: dairy calves exhibit negative judgement bias following hot-iron disbudding. *PloS one, 8*(12), e80556.

Neville, V., King, J., Gilchrist, I. D., Dayan, P., Paul, E. S., & Mendl, M. (2020). Reward and punisher experience alter rodent decision-making in a judgement bias task. *Scientific Reports, 10*(1), 1-14.

Parrott, R. F., & Misson, B. H. (1989). Changes in pig salivary cortisol in response to transport simulation, food and water deprivation, and mixing. *British veterinary journal, 145*(6), 501-505

Pedersen, V., Barnett, J. L., Hemsworth, P. H., Newman, E. A., & Schirmer, B. (1998). The effects of handling on behavioural and physiological responses to housing in tether-stalls among pregnant pigs. *Animal Welfare, 7*(2), 137-150.

Peeters, G., & Czapinski, J. (1990). Positive-negative asymmetry in evaluations: The distinction between affective and informational negativity effects. *European review of social psychology, 1*(1), 33-60.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. URL https://www.R-project.org/.

Ralph, C. R., & Tilbrook, A. J. (2016). Invited review: The usefulness of measuring glucocorticoids for assessing animal welfare. *Journal of animal science, 94*(2), 457-470.

Ralph, C., Hebart, M., & Cronin, G. M. (2018). Enrichment in the sucker and weaner phase altered the performance of pigs in three behavioural tests. *Animals, 8*(5), 74.

Rault, J. L., Dunshea, F. R., & Pluske, J. R. (2015). Effects of oxytocin administration on the response of piglets to weaning. *Animals, 5*(3), 545-560.

Rault, J. L., Truong, S., Hemsworth, L., Le Chevoir, M., Bauquier, S., & Lai, A. (2019). Gentle abdominal stroking ('belly rubbing') of pigs by a human reduces EEG total power and increases EEG frequencies. *Behavioural brain research, 374*, 111892.

Rault, J. L., Hintze, S., Camerlink, I., & Yee, J. R. (2020a). Positive welfare and the Like: distinct views and a proposed framework. *Frontiers in Veterinary Science, 7*, 370.

Rault, J. L., Waiblinger, S., Boivin, X., & Hemsworth, P. (2020b). The power of a positive human–animal relationship for animal welfare. *Frontiers in Veterinary Science, 7*.

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2013). Indicators of positive and negative emotions and emotional contagion in pigs. *Physiology & behavior, 109*, 42-50.

Reimert, I., Fong, S., Rodenburg, T. B., & Bolhuis, J. E. (2017). Emotional states and emotional contagion in pigs after exposure to a positive and negative treatment. *Applied Animal Behaviour Science, 193*, 37-42.

Ruis, M. A. W. (2001). Social stress as a source of reduced welfare in pigs. University Library Groningen][Host].

Russell, J. A. (2003). Core affect and the psychological construction of emotion. *Psychological review,* 110(1), 145.

Rutherford, K. M., Haskell, M. J., Glasbey, C., & Lawrence, A. B. (2006). The responses of growing pigs to a chronic-intermittent stress treatment. *Physiology & behavior, 89*(5), 670-680.

Rutherford, K. M., Donald, R. D., Lawrence, A. B., & Wemelsfelder, F. (2012). Qualitative Behavioural Assessment of emotionality in pigs. *Applied animal behaviour science, 139*(3-4), 218-224.

Salmeto, A. L., Hymel, K. A., Carpenter, E. C., Brilot, B. O., Bateson, M., & Sufka, K. J. (2011). Cognitive bias in the chick anxiety–depression model. *Brain research, 1373*, 124-130.

Sanger, M. E., Doyle, R. E., Hinch, G. N., & Lee, C. (2011). Sheep exhibit a positive judgement bias and stress-induced hyperthermia following shearing. Applied Animal *Behaviour Science, 131*(3-4), 94-103.

Schmied, C., Boivin, X., & Waiblinger, S. (2008). Stroking different body regions of dairy cows: effects on avoidance and approach behavior toward humans. *Journal of Dairy Science, 91*(2), 596-605.

Schnall, S. (2010). Affect, mood and emotions. In S. Järvelä (Ed.), *Social and emotional aspects of learning*, (pp. 59-64). Oxford: Academic Press.

Scollo, A., Gottardo, F., Contiero, B., & Edwards, S. A. (2014). Does stocking density modify affective state in pigs as assessed by cognitive bias, behavioural and physiological parameters?. *Applied Animal Behaviour Science, 153*, 26-35.

Seyle, H. (1958). *Stress: The stress of life*. Stockholm: Rydahls Boktryckeri AB.
Skinner, B. F. (1938) *The Behavior of Organisms: An Experimental Analysis*. Oxford: Appleton-Century.

Stranahan, A. M., Lee, K., & Mattson, M. P. (2008). Central mechanisms of HPA axis regulation by voluntary exercise. *Neuromolecular medicine*, *10*(2), 118-127.

Tallet, C., Sy, K., Prunier, A., Nowak, R., Boissy, A., & Boivin, X. (2014). Behavioural and physiological reactions of piglets to gentle tactile interactions vary according to their previous experience with humans. *Livestock Science, 167*, 331-341.

Tamir, M., & Robinson, M. D. (2007). The happy spotlight: Positive mood and selective attention to rewarding information. *Personality and Social Psychology Bulletin, 33*(8), 1124-1136.

Tanida, H., & Nagano, Y. (1998). The ability of miniature pigs to discriminate between a stranger and their familiar handler. *Applied Animal Behaviour Science*, *56*(2-4), 149-159.

Tarou, L. R., & Bashaw, M. J. (2007). Maximizing the effectiveness of environmental enrichment: Suggestions from the experimental analysis of behavior. *Applied Animal Behaviour Science, 102*(3-4), 189-204.

Villain, A. S., Hazard, A., Danglot, M., Guérin, C., Boissy, A., & Tallet, C. (2020). Piglets vocally express the anticipation of pseudo-social contexts in their grunts. *Scientific reports, 10*(1), 1-13.

Villalba, J. J., & Manteca, X. (2019). A case for eustress in grazing animals. *Frontiers in veterinary science, 6*, 303.

Vögeli, S., Lutz, J., Wolf, M., Wechsler, B., & Gygax, L. (2014). Valence of physical stimuli, not housing conditions, affects behaviour and frontal cortical brain activity in sheep. *Behavioural brain research, 267*, 144-155.

Waiblinger, S., Menke, C., Korff, J., & Bucher, A. (2004). Previous handling and gentle interactions affect behaviour and heart rate of dairy cows during a veterinary procedure. *Applied Animal Behaviour Science, 85*(1-2), 31-42.

Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M. V., Janczak, A. M., Visser, E. K., & Jones, R. B. (2006). Assessing the human–animal relationship in farmed species: a critical review. *Applied Animal Behaviour Science*, 101(3-4), 185-242.

Wang, C., Chen, Y., Bi, Y., Zhao, P., Sun, H., Li, J., ... & Bao, J. (2020). Effects of long-term gentle handling on behavioral responses, production performance, and meat quality of pigs. *Animals, 10*(2), 330.

Wemelsfelder, F., Hunter, E. A., Mendl, M. T., & Lawrence, A. B. (2000). The spontaneous qualitative assessment of behavioural expressions in pigs: first explorations of a novel methodology for integrative animal welfare measurement. *Applied Animal Behaviour Science, 67*(3), 193-215.

Westlund, K. (2014). Training is enrichment—and beyond. *Applied Animal Behaviour Science, 152*, 1-6.

Wood-Gush, D. G. M., & Vestergaard, K. (1991). The seeking of novelty and its relation to play. *Animal Behaviour, 42*(4), 599-606.

Yeates, J. W., & Main, D. C. (2008). Assessment of positive welfare: a review. *The Veterinary Journal, 175*(3), 293-300.

Zidar, J., Campderrich, I., Jansson, E., Wichman, A., Winberg, S., Keeling, L., & Løvlie, H. (2018). Environmental complexity buffers against stress-induced negative judgement bias in female chickens. *Scientific reports, 8*(1), 1-14.

Zupan, M., Buskas, J., Altimiras, J., & Keeling, L. J. (2016). Assessing positive emotional states in dogs using heart rate and heart rate variability. *Physiology & behavior, 155*, 102-111.

8. List of figures and tables

9. Appendices

Appendix A

Table detailing overview of statistical methods and software used for each research question.

Research Question	Model	Software	Outcome Measure	Fixed Effects	Random Effects
Effect of positive Contact- Behaviour	GLM	Minitab	Latency to pig contact	Treatment Group	Replicate: ID, Treatment session
Effect of positive Contact- Behaviour	GLM	Minitab	Latency to reciprocal contact	Treatment Group	Replicate: ID, Treatment session
Effect of positive Contact- Behaviour	LME	R	% Human Contact	Treatment Group	Replicate: Pen: ID
Effect of positive Contact- Behaviour	LME	R	% Reciprocal contact	Treatment Group	Replicate: Pen: ID
Effect of positive Contact- Behaviour	GLM	Minitab	% contact Human + Reciprocal combined	Treatment Group	Replicate: ID, Treatment session
Difference between treatment groups- JBT responses	GLMER	R	Go/No-go	Treatment Group Trial type (AP, AM, AN)	Replicate: Pen: ID: Test session
Effect of long-term positive Contact- JBT responses	GLMER	R	Go/No-go	Treatment Group (CON+STPC, LTPC)	Replicate: Pen: ID: Test session
Effect of short-term positive contact- JBT responses	GLMER	R	Go/No-go	Test Treatment Condition (LT++ST+, LT-+ST-+CON)	Replicate: Pen: ID: Test session
Interaction of short- and long-term positive contact- JBT	GLMER	R	Go/No-go	Treatment group (CON+STPC versus LTPC)" * Test treatment condition (LT+, LT-, ST+, ST-), Trial type	Replicate: Pen: ID: Test session
JBT (Learning effect)	GLMER	R	Go/No-go	Test session, Trial type	Replicate: Pen: ID: Test session

Interaction of sex/ replicate and treatment group- JBT responses	GLMER	R	Go/No-go	Sex * Treatment group, Trial Type/ Replicate * Treatment group, trial type	Replicate: Pen: ID: Test session
Correlation positive contact and change in salivary cortisol concentration	Spearman' s Rho	Minitab	Relationship	Duration/ latency of contact, change in cortisol concentration	N/A
Effect of positive contact- salivary cortisol concentration	GLM	Minitab	Cortisol pre-test measure	Treatment Group (CON, STPC, LTPC),	Replicate: ID: Treatment session
Effect of positive contact- salivary cortisol concentration	GLM	Minitab	Cortisol Difference pre-/post-test	Treatment Condition (LT+, ST+, LT-, ST-, CON)	Replicate: ID: Treatment session
Interaction treatment group and salivary cortisol concentration- JBT responses	GLMER	R	Go/No-go	Treatment group * Cortisol difference, Trial type	Replicate: Pen: ID: Treatment session

Appendix B

Average Human Approach (HAp) scores measured in seconds (s) and Human Avoidance (HAv) scores measured in meters (m) from tests carried out before shaping (beginning of study- 1) and after testing (end of study- 2). "N" indicates when a pig did not approach within 5 minutes, while "–" indicates absence of pig (one pig was euthanised before the end of the study due to lameness).

ID	Sex	Group	HAp 1 (s)	HAp 2 (s)	HAv 1 (m)	HAv 2 (m)
Mael	M	LTPC	49	3	2	0
Hedwig	F	LTPC	30	7	2	0
Angela	F	LTPC	N	7	1.5	0
Scaramucci	M	LTPC	N	24	2	0.5
Xena	F	STPC	41	7	2	0
Dennis	M	STPC	33	15	1.5	0
Jackie	F	STPC	N	7	2.5	0
Tagor	M	STPC	N	7	1.5	0
Patsy	F	CON	23	7	1.5	0
Chester	M	CON	39	7	2	0
Ima	F	CON	N	31	1	0
Loki	M	CON	N	-	2	-
Ivan	M	LTPC	1	1	0	0
Zeta	F	LTPC	1	1	0	0
Tina	F	LTPC	N	145	2	0
Leo	M	LTPC	200	1	1.5	0
Olive	F	STPC	1	1	0	0
Frodo	M	STPC	5	13	0	0
Yasmine	F	STPC	193	1	1.8	0
Conor	M	STPC	197	1	1	0
Pricilla	F	CON	1	1	0	0

Seamus	M	CON	20	1	0	0
Adele	F	CON	170	1	1	0
Jerome	M	CON	N	10	2	0
Tiger	M	LTPC	198	9	1.3	0
Nala	F	LTPC	200	14	1.5	0
Helga	F	LTPC	N	13	1.3	0
Drago	M	LTPC	N	8	1.5	0
Ivy	F	STPC	292	7	1.8	0
Archie	M	STPC	191	6	2	0
Gabby	F	STPC	N	17	1.5	0
Juan	M	STPC	N	11	1.8	0
Coco	F	CON	302	15	2	0
Simba	M	CON	208	10	1.5	0
Ella	F	CON	N	20	1.8	0
Wilson	M	CON	N	N	1.5	0