Spontaneous helping in pigs is mediated by helper's social attention and distress signals of individuals in need

Liza R. Moscovice[1*], Anja Eggert[2], Christian Manteuffel[1] and Jean-Loup Rault[3]

Affiliations:

[1] Institute of Behavioural Physiology, Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany

[2] Institute of Genetics and Biometry, Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany

[3] Institute of Animal Welfare Science, University of Veterinary Medicine, Vienna, Austria

*** Correspondence:**
Liza R. Moscovice
moscovice@fbn-dummerstorf.de

Word count:

Abstract: **200**
Full text + references + table and figure text = **10,148**
Figures: 5
Tables: 2

Running title: Spontaneous helping in pigs

Abstract

Helping behaviour is of special interest for the study of prosociality because it appears to be motivated by the needs of others. We developed a novel paradigm to investigate helping behaviour in pigs (*Sus scrofa domesticus*) and tested n = 75 individuals in eight groups in their home pens. During familiarization, two identical compartments were attached to the home pen, equipped with a mesh window and a door that could be opened from the outside by lifting a handle. During testing, a pig was briefly isolated from its group and then placed in one of the compartments. Each pig was trapped once, during two daily test sessions per group. Pigs spontaneously learned to open doors, and in 85% of cases, pigs released a trapped group member within 20 minutes (median latency = 2.2 minutes). Pigs who were more proficient at door-opening during familiarization, and more attentive to the trapped pig were more likely to help. Trapped pigs exhibited behavioural signals of distress and increases in cortisol. Distress signals by the trapped pig increased its chances of being helped. Results are consistent with several criteria for identifying targeted helping in animals, but we also discuss alternative explanations based on selfish motivations.

Key words: rescue, empathy, prosocial, *Sus scrofa*, targeted helping, cortisol

1. Background

Helping behaviour, in which an individual assists another to reach an otherwise unachievable goal, is of special interest for the study of prosociality. Helping can be costly (1), and often does not provide immediate benefits to the helper, but rather appears to be motivated by an understanding of the needs of others, also referred to as targeted helping (2). Examples of helping are wide-spread across animal taxa in the wild, and include intervening on behalf of group members against predators (ants: (3); primates: (4); whales: (5)), ii) releasing individuals caught in snares, traps or sticky seeds (ants: (6); wild boars (7); birds(1)) and defending group members from conspecific attacks (elephants: (8); primates: (9)).

Animal helping is also the subject of experimental research to identify common biological mechanisms underlying empathy, broadly defined as the ability to recognize the emotional states of others and generate appropriate responses (2,10). Animal helping and human empathic responses share several trait features, including that both are: i) Directed preferentially at in-group members (rodents: (11); primates: (12), humans: (13); ii) Enhanced by having previous experience with the same situation as the individual in need (Rodents: (14,15); humans: (16)); and iii) Mediated by the processing of social affective cues in similar brain regions (rodents: (17,18), humans: (19)).

Long-standing criticisms maintain that the available evidence is not sufficient to support empathy-like motivations underlying animal helping paradigms, and that more parsimonious explanations are possible (20–22). One major point of contention is about whether helping is truly goal-oriented, rather than being an inflexible or learnt response. Proving that helping is goal-oriented would require showing not only that animals help in response to individuals in need, but also that observers modify helping appropriately when the target's needs change or when they no longer require help (23). In animal models of helping across diverse taxa, individuals in need may use a range of acoustic, behavioural, physiological and/or pheromonal cues as communicative signals to

influence helper's behaviour. Several studies have emphasized the role of distress signals in attracting helpers (ants: (3,24); birds: (1)), but there has been little research examining how variation in the use of distress signalling, either between individuals in need if they react differently to the situation, or within individuals if their needs change, influences helping. There has also been little work exploring if and how animals may use other social signals to request help (but see (25,26)).

Another criticism results directly from the fact that many animal helping experiments are designed to elicit robust distress responses from individuals in need (e.g. (14,27)). Evidence for selective helping in contexts that elicit distress signals, but not in otherwise similar contexts when the source of distress is removed (e.g. 15), has been interpreted as consistent with empathic responses. However, others have pointed out the difficulty in discerning between helping responses that are motivated by concern for others, or by the selfish need to end aversive distress signals, which potential helpers have limited means to avoid other than by helping (reviewed in (25)).

We developed a novel helping paradigm that gives potential helpers and individuals in need more behavioural flexibility to express a range of responses, in an attempt to address some of these criticisms. Our paradigm is inspired by the work of Ben-Ami Bartal and colleagues (28), in which rats could open a door to release a partner trapped inside a restraint tube. However, our approach differs in several important aspects. Firstly, subjects are tested in social groups in their home environment (e.g. (29,30)), which improves ecological validity by giving animals the choice about whether, when and whom to help, while allowing for competing motivations (e.g. exploring, feeding, resting, social interactions with other group members). Secondly, subjects can simultaneously choose to perform the same behaviour in a helping context (opening the door of the compartment with a trapped group member inside) or in a non-helping context (opening the door of an identical but empty compartment). This allows us to assess prosocial as well as selfish motivations for door-opening. Finally, the trapped individual is able to move around and explore their compartment and can also maintain visual contact and interact with potential helpers through a mesh window. Giving

individuals in need more options to respond to being trapped allows us to investigate how different expressions of need, including social solicitations as well as distress signals, may influence helping (26,31).

We tested the paradigm in pigs, which are a promising model species for studying the mechanisms mediating helping behaviour. Pigs show evidence of emotional contagion when observing group members in stressful situations (32,33), but without opportunities to assist them. In addition, a case of spontaneous rescue behaviour was recently documented in wild boars (*Sus Scrofa*), the ancestors of domestic pigs (7). However, helping has not been systematically studied in pigs.

Our objectives were to determine whether pigs would show spontaneous helping behaviour, to identify characteristics of helpers and to determine whether the helpers' responses were consistent with emotional and cognitive components of targeted helping. We followed the three criteria suggested by Pérez-Manrique and Gomila (34) for determining whether animals demonstrate "empathic targeted helping": 1) Helpers should show an other-oriented reaction, including cognitive appraisal of the situation and a moderate level of arousal, consistent with emotional regulation; 2) Helpers should show a flexible helping response that is appropriate to the situation and 3) Helping should lead to an improvement in the situation for the individual in need.

We predicted that pigs would selectively and consistently open doors to release trapped group members, but not to access empty compartments. We further predicted that potential helpers would be more likely and quicker to help individuals: 1) if they were more proficient in the novel door-opening behaviour, 2) if they were more attentive to the trapped individual and 3) if the trapped individual expressed greater need to be released, either by producing distress signals, or by making social solicitations. We also measured changes in salivary cortisol (sCORT) in trapped pigs, to test whether specific behavioural responses to being trapped were associated with greater activation of the hypothalamic-pituitary-adrenal axis, consistent with a physical or psychological stress response (reviewed in (35)). Whenever the identity of helpers could be quickly

confirmed without requiring video analysis, we also measured their sCORT concentrations soon after helping, to test whether helpers showed evidence for increases in cortisol, consistent with emotional contagion of distress from trapped pigs, or for more moderate levels of arousal, consistent with emotional regulation that can facilitate targeted helping (34,36).

2. Methods

2.1 Animals and husbandry

All research procedures were approved under the German Animal Welfare Act (German Animal Protection Law, §8 TierSchG) by the Committee for Animal Use and Care of the Agricultural Department of Mecklenburg-Western Pomerania, Germany (permit LALLF 7221.3-1-036/20).

Subjects were German Landrace pigs born and housed at the Experimental Pig Facility at the Research Institute for Farm Animal Biology (FBN) in Dummerstorf, Germany. After birth, piglets were housed with their mothers in 6 m^2 farrowing pens. Piglets had *ad libitum* access to water and were offered dry food (HAKRA-Immuno-G; Una Hakra, Hamburg, Germany) beginning from 14 days of age in addition to milk from their mothers. Piglets received tattoos for identification at 1 day of age and ear tags with unique numbers at 27 days of age. Piglets were not subjected to tail docking, teeth clipping, castration or other invasive procedures, and none of the study subjects were cross-fostered to other litters, which can occur in farm practice. At 28 days of age, subjects were weaned and mixed into new social groups, by randomly selecting 3-5 pigs from each of two to three different litters to form groups of 9-10 individuals. Groups were sex-balanced and contained equal numbers of littermates from each contributing litter, such that each pig had either two (22% of group members) or four (44% of group members) relatives in their group. Pigs were tested in four temporally-separated cohorts, with each cohort consisting of two social groups, for a total of n = 78 subjects. Groups within a cohort were housed in identical, neighbouring 2.8 × 2.8 m pens in the

same room, with partial slatted flooring and a sleeping mat. Pigs in neighbouring pens did not have direct visual or physical contact but could exchange auditory and olfactory cues. Indoor lighting in the room was controlled on a timer, with lights on from 7:00-15:00 hr. Pigs also received natural light through a window. Food (Porcistart, Trede und von Pein GmbH, Damfleth, Germany) and water were provided *ad libitum*, and straw and an enrichment object (Easyfix® Luna 68, Ballinasloe, Ireland) were provided daily. Room temperature was maintained between 20-22°C. Pigs were regularly given back numbers using livestock spray paint to facilitate individual identification. The room was equipped with multiple video cameras in order to cover the entire home pen and two additional compartments that were attached to the home pen for the experiment (see section 2.2.). Pigs were tested between 46 – 55 days of age. Prior to testing, pigs were habituated to saliva sampling and had participated in one social play experiment within their social group (37) but had not been involved in other experiments. Of the 78 subjects, one died of unknown causes, and two subjects from the same group were removed from the study to be treated for illnesses before testing began. The remaining n = 75 subjects completed the study.

2.2 Familiarization, Separation and Testing phases

Two weeks after weaning, pigs went through the familiarization phase, during which they were presented as a group in their home pen with two identical compartments (90 × 90 × 80 cm in height) that were temporarily attached to opposite sides of the home pen (**Fig. 1**). Compartments were open on the top to facilitate video recording of the trapped pig. Each compartment had a mesh window and a door with a metal handle, placed at 20 cm height (**Fig. S1**). Opening the door required the use of 1.3 kg of force to lift the handle with the snout high enough to release an inner latch, causing the door of the compartment to swing open into the home pen. This design is suitable for pigs, who can exert strong force with their snout to dig or lift (38). Each group went through five consecutive days of one-hour familiarization sessions applied in a counter-balanced order between the two groups within a day. During each familiarization session, pigs were presented simultaneously with the compartments, with both doors closed. If a pig

spontaneously opened a door, the door was left open for two minutes so that pigs could freely explore the inside of the compartment. At that point, any pigs still in the compartment were gently led out and a researcher closed the door in a standardized way from outside the pen, using a hook to insert the door handle back into its slot. If no pig had opened the door of a compartment for five minutes, a researcher opened that door using the hook and left the door open for two minutes, before removing any pigs inside the compartment and closing the door.

Pigs then experienced the testing phase, which was initially similar to the familiarization phase in that on each testing day the compartments were attached to the home pen and pigs could freely explore and open the doors. Researchers monitored the pigs from a different room using a live video stream. Every ten minutes a researcher entered the room and, if the doors were opened, led out any pigs inside the compartments and closed the doors. Once the pigs had not touched or opened any door for ten minutes (this typically occurred within 30 min of initial exposure on each test day), a researcher entered the home pen and removed the designated trapped pig, using a pre-determined pseudo-random order, so that each pig was trapped once over the course of the testing phase. The designated trapped pig was placed in a separate room in a 2.8 m^2 empty arena without any contact with other pigs. During this separation period, researchers monitored the test group remotely. If no doors were opened during the first five minutes of the separation, the designated trapped pig was then returned to the room and placed in one of the two compartments, which then became the test compartment. In the event that a pig opened a door during the initial 5-minute separation period, a researcher entered the room, closed the door and left the room. We then waited an additional five minutes before returning the designated trapped pig and placing it in the test compartment. As a result, the total isolation time for each pig ranged from 5–11 minutes (median isolation time = 5.8 minutes).

The trial began once the trapped pig was placed in the test compartment. The researcher left the room and monitored the pigs remotely using live video. During the trial, the trapped pig had olfactory and auditory contact with their group members (the

potential helpers) at all times. In addition, the trapped pig could maintain visual and limited physical contact with group members through a mesh window located on one side of the compartment. The trapped pig remained in the compartment until either: 1) A helper pig opened the door, releasing the trapped pig, or 2) Twenty minutes elapsed without helping, at which point a researcher entered the room and opened the door to release the trapped pig. If the trapped pig showed signs of distress (screaming, squeals and/or escape attempts) continuously for three minutes and was not let out by a pig, a researcher opened the door, thus ending the trial for ethical reasons. This happened only once. Once the door of the test compartment was opened (either by a pig or by a researcher), the pigs were left undisturbed for an additional 15 minutes to allow for subsequent hormone sampling (see section 2.4). The compartments were then removed and cleaned. On each of five test days, we alternated trials between the two groups until two pigs per group have been tested as trapped pigs, each in a different compartment, with the location of the first test compartment (at the front or side of the pen) alternated across days. At the completion of the testing phase, each pig had been trapped once, and potential helpers had between 6-9 opportunities (depending on their group size) to release different members of their social group from the test compartment.

2.3 Behavioural measures

Behavioural data were coded from video recordings by two trained assistants using the Observer software (version 15, Noldus, The Netherlands), with an inter-observer reliability of 91.2%. During the familiarization phase, data were recorded on each pig's daily frequency of contacts with the handle, reflecting curiosity and their frequency of door-opening, reflecting task proficiency. The daily latency for a door to first be opened within each group was also recorded as a measure of group task performance, to test for learning effects over the course of the familiarization phase.

During the testing phase, we recorded all occurrences of squeals or screams by the trapped pig, which were identified as prolonged (> 2 sec) vocalizations with high-

frequency components and amplitude modulation (39). Squeals or screams are commonly associated with negative affect in pigs (39,40). As further validation that we were recording vocalizations related to negative affect, and to test whether parts of the data analysis can be automated in future studies, we compared our manual coding of screams and squeals with analysis of vocalizations using the Stremodo software, which was developed for the automated detection of distress in pigs (41). Stremodo detects distress signals in 100 ms time windows of audio recordings without considering the context. To facilitate comparison with human classifications of vocalizations, a post-processing routine automatically detected the beginning and end of a vocalisation based on the loudness of the audio data. Then this vocalisation as a whole was marked as a stress event regardless of its duration, if Stremodo identified one or more of its segments as stressful. We also recorded each pig's rate of escape attempts (attempting to climb or jump against the side of the compartment) and their investigations of either the window or the door, with investigations counted whenever the snout was within 5 cm of the object. For two pigs, the compartment was not entirely visible in the video recording while they were trapped, so behavioural data were analysed for the remaining n = 73 trapped pigs. For each potential helper (referring to all other pigs in the group besides the trapped pig), we recorded the amount of time that they spent looking in the window, defined as being within 10 cm of the window with their head oriented towards the window. When a potential helper released the trapped pig from the test compartment within the 20-minute trial, we recorded the identity of the helper, and their latency to release the trapped pig, based on the time at which the latch of the door was successfully lifted, leading to the door opening. In the event that the control compartment was opened before the trapped pig was released, we also recorded the identity of the pig who opened the control compartment.

2.4. Hormonal measures

We assessed the physiological responses of trapped pigs by measuring changes in the concentration of sCORT, which increases in response to physical or psychosocial challenges in mammals, including pigs (37). Saliva samples were collected by one of

the authors (LRM) and two assistants using SalivaBio® Infant Swabs (Salimetrics, CA, USA). Subjects were familiar with the sample collectors and had been previously habituated to sample collection, which occurred voluntarily and took one to two minutes per pig, thus minimizing the possibility of any stress associated with sampling. We collected saliva samples from the designated trapped pig in their home pen before they were removed from the group (pre-test), and 15 minutes after the door of the test compartment was open and the trapped pig was released (post-test). During the post-test sampling, we also attempted to collect saliva samples from the pig that helped when this was already confirmed, to examine physiological responses of helpers. Due to the time delay between exposure to a stimulus and peak cortisol responses in pig saliva (42), the post-test sample should reflect free cortisol concentrations around the time that pigs were released. Saturated swabs were inspected and discarded if there were any signs of blood on the swabs. Swabs were placed in polypropylene tubes on ice immediately after collection and were centrifuged within 30 minutes of collection at 4° C (15 min, 2,000 × g). The eluent was stored at -80°C until hormone analyses. Cortisol analyses were conducted at the FBN, using an ELISA kit for salivary cortisol (Demeditec Diagnostics®, GmbH, Kiel, Germany) that our lab has previously validated in pigs (37). The inter-assay CVs of low- and high-concentration pooled controls were 9.3% and 8.3% respectively (n = 16 samples). Intra-assay CVs of low- and high-concentration pooled samples were 10.7% and 8.6% respectively.

2.5 Statistical analyses

Data were analysed using the R statistical software (version 4.2.1, (43)). For continuous predictors, we fit linear models and linear mixed models (LMM) using the R package `lme4` (44). For binomial data, we fit generalized linear mixed models (GLMM) using the R package `glmmTMB` (45). When necessary, continuous response and predictor variables were square-root or log-transformed to approximate a normal distribution and all continuous predictors were z-transformed to facilitate interpretability of the results. We assessed model performance by checking for normality of residuals, over-dispersion and collinearity of predictors using the `performance` package (46). We used

likelihood ratio tests to compare the full model to a reduced model excluding the test predictors, and present results when the model differed significantly from the reduced model. To test for fixed effects of predictors, we calculated deviance tables and Chisq-statistics using the `car` package (47). If applicable, pairwise post-hoc comparisons on estimated marginal means were performed and p-values were adjusted for multiple comparisons using Tukey's HSD tests in the `emmeans` package (48). Unless otherwise indicated, results are presented as Mean ± SD

To test for evidence of learning how to open doors, we fit a linear model comparing the latency for a door to first be opened within a group across familiarization days, with familiarization day as the test predictor and group as a control predictor. We also determined the proportion of pigs that successfully opened a door at least once, on each familiarization day and cumulatively across that day and previous days.

To test whether helping was influenced by potential helpers' previous experience and their appraisal of the situation, we fit a GLMM with a zero-inflated negative binomial error distribution. For each potential helper (n = 7-9 pigs) in each of the 75 trials, we indicated whether that group member helped the trapped pig or not. We then tested how each pig's likelihood of helping was influenced by their mean daily curiosity and proficiency during familiarization and their proportion of time spent near the window with their head oriented towards the window while a pig was trapped, as a measure of social attentiveness. We included control predictors for each potential helper's sex, their relatedness to the trapped pig (sibling or non-sibling), the test order within each group and whether the pig opened a door or not while the designated trapped pig was separated from the group before the trial began. We included random effects for potential helper identity. We found no evidence for collinearity of the predictors (VIFs < 1.06), or for over-dispersion (dispersion parameter = 0.65).

We also fit a second GLMM to test two unplanned predictions that we became interested in after determining that helpers were consistently releasing unrelated pigs as well as relatives. In this model, we asked whether potential helpers were more likely to

help: i) after they had been trapped and released themselves, consistent with generalized reciprocity (49), or ii) if the trapped individual had previously helped them, consistent with direct reciprocity (50). Both processes can help to explain cooperation among non-kin in humans and rodents (reviewed in (51)). We included the same control predictors and random effects as described for the previous GLMM. There was no evidence for collinearity of predictors (VIFs < 1.74) or for over-dispersion (dispersion parameter= 0.64).

We tested for correlations between different behavioural responses of trapped pigs using Pearsons correlation coefficients with Bonferroni corrections for multiple testing. Rates of distress vocalizations (screams or squeals) and escape attempts were correlated (see results), so we summed these two measures to obtain a combined rate of distress signals. We fit a LMM to test whether changes in sCORT concentrations in trapped pigs from pre- to post-test samples were related to their behavioural responses while trapped. We included in this model n = 70 pigs for which we had complete data on their physiological and behavioural responses to being trapped. The response was each pig's salivary cortisol concentrations. As test predictors we included each pig's rate of investigations to the window, as a measure of social solicitations, and their rate of distress signals (the sum of their screams, squeals and escape attempts). We also included the duration that each pig was separated from the group before being trapped and the duration that each pig was trapped. To model changes in sCORT in trapped pigs, we tested for two-way interactions between sampling context (pre- or post-trapped, representing relative changes in sCORT), and the various test predictors. As control predictors, we included each pig's sex, the order in which they were tested within their group and the time that each sample was taken. We included subject and date of sampling as random effects. We also compared sCORT concentrations of trapped pigs and their helpers shortly after pigs had been released, using paired t-tests and Pearson correlation coefficients.

To test whether helpers' responses were appropriate to the expressions of need by the trapped pig, we fit a Cox proportional hazards regression model using the Survival

package (v 3.2-7; (52)). We tested whether the occurrence or frequencies of a trapped pig's behaviours predicted their latency and likelihood to be helped by other pigs. In survival analyses, latencies and likelihoods of occurrence of an event of interest (in this case being released from the test compartment by a pig) are combined into a hazard ratio (HR), which indicates the probability of the event happening. An HR above one indicates a covariate that is associated with a greater likelihood and shorter latency for the event to occur. Trials in which pigs were released by humans were right-censored, to indicate that the event of interest did not occur during that pig's observation period. To compare behavioural data that varied over time while ensuring that we did not violate the proportional hazards assumptions, we stratified each trapped pig's behavioural data at one-minute intervals and removed any intervals of less than 60-sec at the beginning of each pig's trapped period. We also excluded from analysis each pig's behaviour during the last 10-secs before they were released, to avoid committing "immortal biases" (53) by using information from the future to predict the outcome. Based on these criteria, we removed from the survival analysis n = 15 pigs who were released in under one minute, as well as n = 2 pigs whose behavioural data could not be analysed, resulting in a subset of n = 58 trapped pigs. We included each trapped pig's number of investigations to the window per minute interval as a measure of social solicitations. The number of distress signals (screams, squeals or escape attempts) per minute could not be transformed to approximate normality. Therefore, we included the presence or absence of distress signals (screams, squeals or escape attempts) at one-minute intervals as a binomial predictor in the model. We included as control predictors the sex and group identity for each trapped pig, the counter-balanced location of the test compartment (either located at the front or the side of the pen), the pseudo-randomly assigned order in which each pig was trapped within the group and the time when the trapped pig was first placed in the compartment. Diagnostic tools indicated that the proportional hazards assumptions were not violated (global hazard ratio, $\chi 2 = 22.07$, df = 21, $p = 0.40$). The Concordance Index (CI) was 0.69, indicating good predictive discrimination of the covariates. We used the ANOVA function in the car package (47) to test for effects of the predictors on helping outcomes. Data and scripts related to this

study can be found on-line at: https://github.com/AnjaEggert/spontaneous-helping-in-pigs.

3. Results

3.1 Spontaneous learning of door opening

Despite having no exposure or training in the task beforehand, the majority of pigs in every group spontaneously opened a door on the first day of the familiarization phase, with the first successful door-opening occurring within Mean ± SD = 41.6 (± 21.7) sec. Latencies to first open doors differed across familiarization days ($F = 3.95$, $df = 4$, $p = 0.012$). Groups were quicker to first open the door on days 3 and 4, compared to day 1 (Tukey's p-values < 0.04, **Fig. S2**). Within groups, 82.3 (± 15.7)% of pigs opened doors on each familiarization day. By the end of the familiarization phase all but 1 of the 75 pigs opened a door at least once **(Fig. S3**). However, there was also variation between pigs in their curiosity (15.5 ± 9.2 daily contacts with the handle), and in their proficiency (3.3 ± 2.3 daily door openings) during familiarization.

3.2 Comparison of door opening in helping and non-helping contexts

Across all trials, 85.3% (n = 64) of the trapped pigs were helped by group members, with a median latency of 2.2 min (range= 0.2–17 min) to be released, **Fig. 2**). Of the additional 11 pigs, ten were released by a researcher after the 20-minute trial duration ended and one was released for ethical reasons after exhibiting repeated distress signals for three minutes without being helped.

In total, n = 33 pigs (44% of potential helpers) helped a trapped pig, and each helper helped a median of two trapped pigs (range= 1–4 pigs, or 11-57% of their group members). In 69% of cases (n = 44 out of 64) in which helping occurred, the helper was an unrelated pig from a different litter as the trapped pig. In 19% of helping trials (n = 12), the helper did not enter the test compartment within a minute of helping. In the

remaining n = 52 cases when helpers did enter the test compartment soon after helping, in 54% (n = 28) of those cases, the trapped pig had already left the compartment, so entering the compartment did not result in gaining social contact with the trapped pig.

During each trial, potential helpers also had opportunities to perform the same door-opening behaviour in non-helping contexts, either by opening a compartment during the separation period before the designated trapped pig was placed in the test compartment, or by opening the empty compartment during the testing phase. In total 37 pigs (49% of potential helpers) opened doors during the separation period, during 36 trials (48% of trials). During each separation period, doors were opened 0.2 (± 0.3) times per minute, accounting for the variable duration of isolation time (from 5 to 11 minutes) required to meet the criteria that no door had been opened for five minutes before a test trial began. Of the pigs who opened doors of empty boxes during the isolation period, only nine of them (23%), went on to open the door for the trapped pig during the subsequent test. In 16 test trials (21%), a potential helper (n = 13) opened the door of the control compartment before the trapped pig had been released from the test compartment. In only two of these trials (12.5%) did the same pig proceed to open the door for the trapped pig.

3.3 Role of previous experience, attentiveness to trapped pigs and physiological responses in helping behaviour.

The full GLMM predicting the likelihood of helping differed significantly from a reduced model that excluded the test predictors (likelihood ratio test, $\chi 2 = 44.91$, df = 2, $p < 0.001$). The strongest predictor of helping was the proportion of time spent looking in the window. Pigs who spent a greater proportion of time looking in the window of the test compartment while a pig was trapped inside were more likely to help the trapped pig (est ± SE = 0.57 ± 0.09, $p< 0.001$, **Fig. 3**). Pigs who were more proficient with door-opening across familiarization days were also more likely to help their group members (est ± SE= 0.38 ± 0.16, p= 0.017). The other predictors, including the potential helper's curiosity score during familiarization, whether they opened a door during the pre-test

separation, their sex and their relatedness to the trapped pig did not influence the likelihood of helping a trapped group member (**Table 1**).

The model in which we tested for evidence of direct or generalized reciprocity in influencing helping decisions tended to differ from a reduced model that excluded the test predictors ($\chi 2 = 5.84$, df = 2, $p = 0.054$). We therefore interpreted the results with caution. Results indicate that pigs were more likely to help trapped pigs who had previously helped them (est ± SE = 1.11 ± 0.42, p= 0.008, **Table S1 and Fig. S4**). However, there was no effect of generalized reciprocity on subsequent helping (see Table S1). Of the eight cases of direct reciprocity, in two cases, helpers opened the door for the trapped pig on the subsequent trial after being helped by that pig, and in four cases, helpers opened the door for the trapped pig within two trials of being helped by the trapped pig.

For a subset of n = 50 trials where we were able to identify the helper without requiring additional video analysis, we obtained post-helping saliva samples from the helper and the recipient fifteen minutes after helping had occurred. Helpers had lower sCORT concentrations in comparison with time-matched samples from the released pig (3.9 (± 1.9) ng/mL vs 7.3 (± 2.4) ng/mL, t = 8.14, df = 49, $p < 0.001$, **Fig. 4**), and there was no correlation between the sCORT concentrations of released pigs and their helpers shortly after the release (Pearson, r = 0.07, t = 0.49, df = 48, p = 0.63).

3.4 Behavioural and physiological responses of trapped pigs and influences on being helped

Of the 73 pigs for whom behavioural data could be analysed from video recordings, 72 investigated the window and 71 investigated the door. Per minute trapped, pigs investigated the window 4.8 (± 3.2) times and they investigated the door 2.4 (± 2.5) times. Rates of investigations to the window and door were positively correlated (r = 0.80, t = 11.3, df = 71, adj. p-value < 0.001). The majority of trapped pigs (74%, n = 54) attempted to escape and 44% of trapped pigs (n = 32) emitted screams or squeals while

trapped. Measures of distress vocalizations based on manual coding of screams or squeals and the automated Stremodo analysis were positively correlated ($r = 0.54$, $t = 5.52$, $df = 71$, $p < 0.001$). The lack of a stronger correlation is likely related to interference from additional noises in the room, which the Stremodo algorithm may have sometimes falsely classified as screams or squeals. We used data from manually-coded distress vocalizations in subsequent analyses. Rates of distress vocalizations and escape attempts were positively correlated with each other ($r = 0.34$, $t = 3.04$, $df = 71$, adj. p-value= 0.02), but were not correlated with either of the investigatory behaviours ($r < 0.22$, adj. p values > 0.36). We therefore summed rates of distress vocalizations and escape attempts to create a combined measure of distress signals. Trapped pigs exhibited decreases in investigatory behaviours as well as distress signals with longer time spent in the compartment (**Fig S5).**

For the n = 72 pigs with sufficient sample volumes to measure sCORT before and after being trapped, most pigs (87.5%, n= 63) exhibited increases in sCORT in their post-trapped samples (7.3 (± 2.7) ng/mL), compared to their samples before being trapped (4.7 (± 2.0) ng/mL, $t = 7.97$, $df = 71$, $p < 0.001$). The LMM predicting changes in cortisol in the n = 70 trapped pigs with both hormonal and behavioural data available differed from a reduced model with the test predictors excluded ($\chi2 = 51.03$, $df = 9$, $p < 0.001$). Pigs who exhibited higher rates of distress signals while trapped tended to have greater changes in sCORT concentrations ($\chi2 = 3.83$, $df = 1$, $p = 0.050$, **Table S2** and **Fig. S6**). Changes in sCORT concentrations while trapped were not related to the other predictors (see Table S2).

The Cox proportional hazards model indicated that trapped pigs who exhibited distress signals in a given interval were 2.4 times more likely to be subsequently released, compared with pigs who did not exhibit any distress signals during that interval ($\chi^2 = 4.65$, $df = 1$, $p = 0.031$, **Table 2**, **Fig. 5 and Supplementary videos**). The number of window investigations by trapped pigs did not influence responses of potential helpers (see Table 2). Interestingly, there were significant differences among groups in the likelihood that pigs would be helped by a group member ($\chi^2 = 16.26$, $df = 7$, $p = 0.023$,

see Table 2). The other control predictors did not influence the probability of being helped.

4. Discussion

Pigs spontaneously learned to open doors, and subsequently used this ability to help a trapped group member, while rarely opening identical doors leading to an empty compartment, ruling out a simple curiosity or exploration effect to explain door opening during testing. Pigs who were more attentive to the trapped pig were more likely to help, and distress signals from trapped pigs increased their chances and decreased their latency to be helped. Our results support anecdotal evidence for spontaneous rescue behaviour in wild boars (*Sus scrofa*), the ancestor of domestic pigs (7). We avoid using the term ‘rescue’ here, since not all trapped pigs exhibited signs of distress, and helping did not necessarily require a great cost or risk, which are two of the criteria suggested by Hollis and Nowbahari (54) to define rescue behaviour.

In the majority of cases, trapped pigs were released within a few minutes, and the latency to help decreased with increasing test trials, suggesting a learning or sensitization effect. This is also supported by the fact that nine of the ten cases in which pigs were not released within the allotted test period occurred within the first six trials for the group, demonstrating that helpers become more motivated or proficient in helping with increasing exposure to trapped pigs. In addition, pigs were more likely to help if they were more successful at opening the door during the preceding familiarization phase, suggesting that their ability to learn this novel task also played a role in their likelihood of helping. While all but one pig opened doors during the familiarization phase, roughly half (44%) of the pigs in each group helped others during the test phase. However, given that only one pig could be a helper per trial, our group-testing paradigm may also underestimate the potential for helping behaviour at the population level.

Pigs varied in their likelihood and latency to help trapped group members, which enabled us to investigate the influence of individual and contextual characteristics on

helping behaviour. The strongest predictor of helping was the proportion of time that potential helpers spent looking at the window, through which they could see the trapped pig. It is possible that pigs who spent more time oriented towards the window at close range were visually assessing the situation of the trapped pig, in order to acquire information relevant for helping. This interpretation is consistent with evidence from a targeted helping paradigm in chimpanzees, where individuals who made more efforts to visually assess the situation of their partners were more likely to provide appropriate help (55). As an alternative explanation, it is likely that the movements and vocalizations of the trapped pig acted as salient features to attract attention to the test compartment and window, from which potential helpers were then much closer to the door and handle, increasing the chances of an effect of stimulus enhancement on door-opening. However, under a stimulus enhancement explanation we would expect that within a given trial potential helpers would be similarly interested in and attentive to the cues from the trapped pig, which was not the case here (see Fig. 5 and supplementary video 2).

Distress signals given by the trapped pig, in the form of high-frequency vocalizations (screams or squeals) or escape attempts, increased the pig's subsequent chances of being released, by a 2.4 odds ratio. This suggests evidence for flexible responses that are appropriate to the situation of the trapped pig, which is one of the criteria for "empathic targeted helping" (34,56). An alternative social facilitation explanation is that distressed pigs produced more salient signals that attracted the attention of potential helpers, who then paid more attention to the window in order to gather information about the situation for themselves (20). Their subsequent helping could similarly result from a selfish motivation to enter the compartment to gather more information. This interpretation is consistent with the fact that in the majority of cases (81%), the helper entered the test compartment after helping, whether the trapped pig was still in the compartment or had already left, each of which occurred roughly half of the time. Interestingly, in rodent helping paradigms, similar proportions of helpers entered a tube where conspecifics had previously been restrained (28,57). In these contexts, it was proposed that helpers may be motivated to enter the tube as a form of defensive or

escape behaviour in an otherwise barren test arena (57). Given that the pigs were tested in their home environments and with social partners present, it is unlikely that opening the door and entering the compartment were due to a similar motivation to escape the test environment. It is also unlikely that helping was due to a selfish motivation for social contact more generally (the “social contact hypothesis”; (21)), although we cannot rule out that the helper may have had an interest in interacting with the specific trapped individual.

Wild boars reside in stable, cohesive social groups (58) and forced separations from their social group can induce behavioural and physiological signs of stress in young domestic pigs (37,42). The majority of trapped pigs had increases in sCORT concentrations, and pigs with greater increases in sCORT exhibited more behavioural responses that were consistent with distress. Interestingly, helpers did not show corresponding increases in sCORT concentrations along with trapped pigs, as expected if helpers experienced emotional contagion of distress. Although emotional contagion has been shown in pigs in non-helping contexts (32,33), it does not appear to explain helping behaviour in this case. It is possible that helpers were not stressed by witnessing the trapped pigs, which is consistent with a previous study in which juvenile pigs did not show emotional contagion of fear after hearing playbacks of distress vocalizations from unfamiliar pigs (59). Alternatively, it is possible that helpers were able to regulate their emotional responses in order to help, which would be consistent with theoretical and empirical evidence that high levels of emotional arousal can interfere with helping behaviour (36,60). For example, rats who exhibited stronger corticosterone responses to observing partners in need were less likely to help than rats who exhibited weaker physiological responses (36). Indeed, a moderate level of emotional arousal, consistent with emotional control regulation, is one of the criteria used to distinguish empathic targeted helping from selfish responses in order to alleviate one’s personal distress (34).

While distress signals enhanced the likelihood of being helped, they were neither necessary nor sufficient for helping to occur. Of the n = 15 pigs who were released in

under one minute, 67% (n = 10) did not produce measurable distress signals before being released. This evidence suggests that spontaneous helping was not always motivated by selfish needs to alleviate the source of aversive distress signals (25). Various non-aversive forms of social solicitation may also influence helping decisions, but are less investigated than distress signals (25,31). Trapped pigs investigated the window, where they could make visual contact with potential helpers, twice as often as they investigated the door, where no visual contact with group members was possible (see Fig. S5). We hypothesized that investigations to the window may indicate attempts to make social contact with group members, and possibly to solicit help. Frequencies of window investigations by trapped pigs were not correlated with their frequencies of distress signals, suggesting different underlying motivations for these behaviours. However there was no evidence that increases in window investigations influenced a pig's chances of being helped. Given the promising evidence that social attention to the individual in need can influence helping behaviour in pigs, follow-up research should test whether a broader range of communicative signals by trapped pigs may mediate helping. Two likely candidates are low-frequency vocalizations such as grunts, and nose-to-nose contacts, both of which are common socio-communicative behaviours in pigs (61). Grunts were emitted frequently during trials but could not be attributed to specific pigs with our recording equipment. Anecdotal observations suggest that potential helpers sometimes engaged in nose-to-nose contact with the trapped pig through the mesh window, although these situations could not be systematically recorded with our cameras. Helping paradigms in which individuals in need can express a broader range of behavioural responses will help to clarify the role of social solicitations in influencing helping.

More research is also needed to identify additional social dynamics, within groups and pairs, which influence helping decisions. In a follow-up analysis, we found that pigs were more likely to help a conspecific who helped them previously, consistent with direct reciprocity. Out of 29 possible chances, pigs exhibited direct reciprocity 8 times (27%). This is interesting, especially since the helper had to also be the quickest of the group members to open the door. The majority of the reciprocity occurred within a short

timeframe, either within one or two trials, consistent with a 'tit-for-tat' strategy. Rats also demonstrate reciprocity in helping that is based on their most recent interactions (50). Similar to evidence in rats (51), we found stronger support for direct reciprocity than for generalized reciprocity, given that the mere experience of having been trapped and helped did not increase a pig's likelihood of helping on subsequent trials. Further research with repeated opportunities to help in smaller groups and pairs is needed to confirm these findings, for example by manipulating the ability of individuals to help and then testing for direct and also indirect (*i.e.* helping a pig that helped another pig before) reciprocity.

5. Conclusion

This group-testing paradigm offers a novel experimental approach to systematically study the proximate factors mediating spontaneous helping in a broad range of social species, including farm animals. Applying the framework suggested by Pérez-Manrique & Gomila (34) for evaluating comparative evidence for empathy, our results are consistent with "empathic targeted helping" in that: 1) helpers exhibited visual assessment of the situation and weak to moderate physiological responses, consistent with an other-oriented reaction and emotional regulation, 2) pigs were more likely to help shortly after trapped pigs signalled distress, consistent with a flexible, other-oriented response and 3) helping improved the situation for trapped pigs by reuniting them with their social group. However, there are alternative, more parsimonious explanations for the results, which we also discussed. Thus, follow-up studies are needed to further explore the motivations underlying spontaneous helping in pigs.

Acknowledgements

We thank V. Hörsch, B. Sobczak and E. Normann for their assistance with running the experiments and collecting saliva samples. We thank P. Müntzel for conducting the laboratory analyses. We are grateful to V. Hörsch and M. Wagenknecht for their help with behavioural coding of videos. We thank M. Zenk for her help with the coordination

of this project and D. Ameling, H. Asmus, F. Hintze and H. Schumann for their daily care of the pigs at the Experimental Pig Facility of the FBN. We are especially grateful to T. Therneua, for his repeated advice that helped us to improve our survival analysis.

Funding

This research was funded in part by a Universities Federation of Animal Welfare grant (44-19/20) to L.R.M.

References

1. Hammers M, Brouwer L. Rescue behaviour in a social bird: Removal of sticky “bird-catcher tree” seeds by group members. Behaviour [Internet]. 2017 Jan 1 [cited 2022 Dec 19];154(4):403–11. Available from: https://brill.com/view/journals/beh/154/4/article-p403_2.xml
2. De Waal FBM, Preston SD. Mammalian empathy: Behavioural manifestations and neural basis [Internet]. Vol. 18, Nature Reviews Neuroscience. Nature Publishing Group; 2017 [cited 2022 Dec 19]. p. 498–509. Available from: https://www.nature.com/articles/nrn.2017.72
3. Hollis KL, Nowbahari E. A comparative analysis of precision rescue behaviour in sand-dwelling ants. Anim Behav. 2013 Mar;85(3):537–44.
4. Boesch C. The effects of leopard predation on grouping patterns in forest chimpanzees. Behaviour [Internet]. 1991 Jan 1 [cited 2022 Dec 19];117(3–4):220–42. Available from: https://brill.com/view/journals/beh/117/3-4/article-p220_5.xml
5. Pitman RL, Deecke VB, Gabriele CM, Srinivasan M, Black N, Denkinger J, et al. Humpback whales interfering when mammal-eating killer whales attack other species: Mobbing behavior and interspecific altruism? Mar Mammal Sci [Internet]. 2017 Jan 1 [cited 2022 Dec 19];33(1):7–58. Available from: https://onlinelibrary.wiley.com/doi/10.1111/mms.12343
6. Nowbahari E, Scohier A, Durand JL, Hollis KL. Ants, Cataglyphis cursor, use

precisely directed rescue behavior to free entrapped relatives. PLoS One. 2009 Aug 12;4(8).

7. Masilkova M, Ježek M, Silovský V, Faltusová M, Rohla J, Kušta T, et al. Observation of rescue behaviour in wild boar (Sus scrofa). Sci Rep [Internet]. 2021 Dec 1 [cited 2022 Jan 26];11(1):16217. Available from: https://doi.org/10.1038/s41598-021-95682-4

8. Bates LA, Lee PC, Njiraini N, Poole JH, Sayialel K, Sayialel S, et al. Do elephants show empathy? J Conscious Stud [Internet]. 2008 [cited 2023 Feb 2];15(10–11):204–25. Available from: https://philpapers.org/rec/RNEDES

9. Vogel ER, Fuentes-Jiménez A. Rescue behavior in white-faced capuchin monkeys during an intergroup attack: Support for the infanticide avoidance hypothesis. Am J Primatol. 2006 Oct;68(10):1012–6.

10. Decety J, Bartal IBA, Uzefovsky F, Knafo-Noam A. Empathy as a driver of prosocial behaviour: Highly conserved neurobehavioural mechanisms across species. Philos Trans R Soc B Biol Sci [Internet]. 2016 Jan 19 [cited 2020 Apr 28];371(1686). Available from: http://dx.doi.org/10.1098/rstb.2015.0077

11. Ben-Ami Bartal I, Rodgers DA, Bernardez Sarria MS, Decety J, Mason P. Pro-social behavior in rats is modulated by social experience. Elife [Internet]. 2014 Jan 14 [cited 2022 Dec 19];3. Available from: /pmc/articles/PMC3884117/

12. Horner V, Carter JD, Suchak M, De Waal FBM. Spontaneous prosocial choice by chimpanzees. Proc Natl Acad Sci U S A [Internet]. 2011 Aug 16 [cited 2022 Dec 19];108(33):13847–51. Available from: www.pnas.org/cgi/doi/10.1073/pnas.1111088108

13. Hein G, Silani G, Preuschoff K, Batson CD, Singer T. Neural responses to ingroup and outgroup members' suffering predict individual differences in costly helping. Neuron. 2010 Oct 6;68(1):149–60.

14. Sato N, Tan L, Tate K, Okada M. Rats demonstrate helping behavior toward a soaked conspecific. Anim Cogn [Internet]. 2015 Sep 22 [cited 2020 Nov 3];18(5):1039–47. Available from: https://link.springer.com/article/10.1007/s10071-015-0872-2

15. Cox SS, Reichel CM. Rats display empathic behavior independent of the

opportunity for social interaction. Neuropsychopharmacology [Internet]. 2020 Jun 1 [cited 2022 Dec 19];45(7):1097–104. Available from: https://www.nature.com/articles/s41386-019-0572-8

16. Eklund J, Andersson-Stråberg T, Hansen EM. “i’ve also experienced loss and fear”: Effects of prior similar experience on empathy : Pity and Social Sciences. Scand J Psychol [Internet]. 2009 Feb 1 [cited 2023 Feb 2];50(1):65–9. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1467-9450.2008.00673.x
17. Meyza KZ, Bartal IBA, Monfils MH, Panksepp JB, Knapska E. The roots of empathy: Through the lens of rodent models [Internet]. Vol. 76, Neuroscience and Biobehavioral Reviews. 2017 [cited 2020 Nov 3]. p. 216–34. Available from: https://www.sciencedirect.com/science/article/pii/S0149763415303432
18. Yamagishi A, Lee J, Sato N. Oxytocin in the anterior cingulate cortex is involved in helping behaviour. Behav Brain Res. 2020 Sep 1;393:112790.
19. Wu YE, Hong W. Neural basis of prosocial behavior. Vol. 45, Trends in Neurosciences. Elsevier Ltd; 2022. p. 749–62.
20. Schwartz LP, Silberberg A, Casey AH, Kearns DN, Slotnick B. Does a rat release a soaked conspecific due to empathy? Anim Cogn [Internet]. 2017 Mar 1 [cited 2020 Nov 3];20(2):299–308. Available from: https://link.springer.com/content/pdf/10.1007/s10071-016-1052-8.pdf
21. Silberberg A, Allouch C, Sandfort S, Kearns D, Karpel H, Slotnick B. Desire for social contact, not empathy, may explain “rescue” behavior in rats. Anim Cogn [Internet]. 2014 [cited 2020 Nov 3];17(3):609–18. Available from: https://link.springer.com/content/pdf/10.1007/s10071-013-0692-1.pdf
22. Vasconcelos M, Hollis K, Nowbahari E, Kacelnik A. Pro-sociality without empathy. Vol. 8, Biology Letters. Royal Society; 2012. p. 910–2.
23. De Wit S, Dickinson A. Associative theories of goal-directed behaviour: A case for animal-human translational models. Psychol Res [Internet]. 2009 Jul 7 [cited 2022 Dec 19];73(4):463–76. Available from: https://link.springer.com/article/10.1007/s00426-009-0230-6
24. Frank ET, Schmitt T, Hovestadt T, Mitesser O, Stiegler J, Linsenmair KE. Saving the injured: Rescue behavior in the termite-hunting ant Megaponera analis. Sci

Adv [Internet]. 2017 Apr 1 [cited 2022 Dec 19];3(4). Available from: https://www.science.org

25. Carvalheiro J, Seara-Cardoso A, Mesquita AR, de Sousa L, Oliveira P, Summavielle T, et al. Helping Behavior in Rats (Rattus norvegicus) When an Escape Alternative Is Present. J Comp Psychol [Internet]. 2019 [cited 2022 Dec 19]; Available from: /record/2019-15237-001

26. Paulsson NI, Taborsky M. Reaching Out for Inaccessible Food Is a Potential Begging Signal in Cooperating Wild-Type Norway Rats, Rattus norvegicus. Front Psychol. 2021 Aug 30;12.

27. Rice GE, Gainer P. “Altruism” in the albino rat. J Comp Physiol Psychol [Internet]. 1962 Feb [cited 2020 Nov 3];55(1):123–5. Available from: /record/1963-02725-001

28. Ben-Ami Bartal I, Decety J, Mason P. Empathy and pro-social behavior in rats. Science (80-) [Internet]. 2011 Dec 9 [cited 2021 Feb 10];334(6061):1427–30. Available from: https://science.sciencemag.org/content/334/6061/1427

29. Burkart JM, Allon O, Amici F, Fichtel C, Finkenwirth C, Heschl A, et al. The evolutionary origin of human hyper-cooperation. Nat Commun [Internet]. 2014 Aug 27 [cited 2020 Nov 3];5(1):1–9. Available from: www.nature.com/naturecommunications

30. Lalot M, Delfour F, Mercera B, Bovet D. Prosociality and reciprocity in bottlenose dolphins (Tursiops truncatus). Anim Cogn [Internet]. 2021 Sep 1 [cited 2022 Jan 24];24(5):1075–86. Available from: https://link.springer.com/article/10.1007/s10071-021-01499-z

31. Márquez C, Rennie SM, Costa DF, Moita MA. Prosocial Choice in Rats Depends on Food-Seeking Behavior Displayed by Recipients. Curr Biol [Internet]. 2015 [cited 2023 Feb 6];25(13):1736–45. Available from: https://www.sciencedirect.com/science/article/pii/S0960982215005928

32. Goumon S, Špinka M. Emotional contagion of distress in young pigs is potentiated by previous exposure to the same stressor. Anim Cogn [Internet]. 2016 May 11 [cited 2019 Sep 9];19(3):501–11. Available from: http://link.springer.com/10.1007/s10071-015-0950-5

33. Reimert I, Bolhuis JE, Kemp B, Rodenburg TB. Indicators of positive and negative emotions and emotional contagion in pigs. Physiol Behav. 2013 Jan 7;109(1):42–50.

34. Pérez-Manrique A, Gomila A. The comparative study of empathy: sympathetic concern and empathic perspective-taking in non-human animals. Biol Rev [Internet]. 2018 Feb 1 [cited 2023 Feb 2];93(1):248–69. Available from: https://onlinelibrary.wiley.com/doi/10.1111/brv.12342

35. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions [Internet]. Vol. 21, Endocrine Reviews. 2000 [cited 2020 Jan 29]. p. 55–89. Available from: https://academic.oup.com/edrv/article-abstract/21/1/55/2423840

36. Ben-Ami Bartal I, Shan H, Molasky NMR, Murray TM, Williams JZ, Decety J, et al. Anxiolytic treatment impairs helping behavior in rats. Front Psychol [Internet]. 2016 Jun 8 [cited 2021 Jan 19];7(JUN):850. Available from: http://journal.frontiersin.org/Article/10.3389/fpsyg.2016.00850/abstract

37. Moscovice LR, Gimsa U, Otten W, Eggert A. Salivary Cortisol, but Not Oxytocin, Varies With Social Challenges in Domestic Pigs: Implications for Measuring Emotions. Front Behav Neurosci [Internet]. 2022 May 23 [cited 2022 Dec 19];16:899397. Available from: /pmc/articles/PMC9169876/

38. D'Eath RB, Turner SP. The Natural Behaviour of the Pig. In: Marchant-Forde JN, editor. The Welfare of Pigs [Internet]. Springer Netherlands; 2009. p. 13–45. Available from: https://link.springer.com/chapter/10.1007/978-1-4020-8909-1_2

39. Marx G, Horn T, Thielebein J, Knubel B, Von Borell E. Analysis of pain-related vocalization in young pigs. J Sound Vib. 2003 Sep 18;266(3):687–98.

40. Briefer EF, Sypherd CCR, Linhart P, Leliveld LMC, Padilla de la Torre M, Read ER, et al. Classification of pig calls produced from birth to slaughter according to their emotional valence and context of production. Sci Rep [Internet]. 2022 Dec 1 [cited 2023 Feb 2];12(1):3409. Available from: https://doi.org/10.1038/s41598-022-07174-8

41. Schön PC, Puppe B, Manteuffel G. Automated recording of stress vocalisations

as a tool to document impaired welfare in pigs. Anim Welf [Internet]. 2004 May 11 [cited 2023 Feb 2];13(2):105–10. Available from: https://www.cambridge.org/core/product/identifier/S096272860002683X/type/journal_article

42. Ruis MAW, Te Brake JHA, Engel B, Buist WG, Blokhuis HJ, Koolhaas JM. Adaptation to social isolation acute and long-term stress responses of growing gilts with different coping characteristics. Physiol Behav. 2001 Jul 1;73(4):541–51.
43. R Core Team. R: A language and environment for statistical computing [Internet]. R Foundation for Statistical Computing. Vienna, Austria; 2018. Available from: https://www.r-project.org/
44. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models using lme4. J Stat Softw [Internet]. 2015 [cited 2018 Sep 21];67(1). Available from: http://www.jstatsoft.org/v67/i01/
45. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, et al. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 2017;9(2):378–400.
46. Lüdecke D, Ben-Shachar M, Patil I, Waggoner P, Makowski D. performance: An R Package for Assessment, Comparison and Testing of Statistical Models. J Open Source Softw [Internet]. 2021 Apr 21 [cited 2023 Feb 28];6(60):3139. Available from: https://doi.org/10.21105/joss.03139
47. Fox J, Weisberg S. An R Companion to Applied Regression: Appendices [Internet]. Second. Robust Regression in R. Thousand Oaks, CA: Sage; 2014. 1–17 p. Available from: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion/appendix.html
48. Lenth R. emmeans: Estimated Marginal Means, aka Least-Squares Means. https://CRANR-project.org/package=emmeans. 2020;
49. Rutte C, Taborsky M. Generalized reciprocity in rats. PLoS Biol. 2007;5(7):1421–5.
50. Kettler N, Schweinfurth MK, Taborsky M. Rats show direct reciprocity when interacting with multiple partners. Sci Rep [Internet]. 2021 Dec 1 [cited 2022 Dec 19];11(1):3228. Available from: https://doi.org/10.1038/s41598-021-82526-4

51. Rutte C, Taborsky M. The influence of social experience on cooperative behaviour of rats (Rattus norvegicus): Direct vs generalised reciprocity. Behav Ecol Sociobiol [Internet]. 2008 Feb 21 [cited 2023 Feb 2];62(4):499–505. Available from: https://link.springer.com/article/10.1007/s00265-007-0474-3
52. Therneau T. A Package for Survival Analysis in R. https://CRANR-project.org/package=survival. 2023;
53. Therneau T, Crowson C, Atkinson E. Using Time Dependent Covariates and Time Dependent Coefficients in the Cox Model. R Proj https//cran.r-project.org › vignettes › timedep [Internet]. 2022 [cited 2023 Feb 6];1–8. Available from: https://www.researchgate.net/publication/265041278_Using_Time_Dependent_Covariates_and_Time_Dependent_Coefficients_in_the_Cox_Model
54. Hollis KL, Nowbahari E. Toward a behavioral ecology of rescue behavior. Evol Psychol [Internet]. 2013 [cited 2023 Feb 9];11(3):647–64. Available from: www.epjournal.net
55. Yamamoto S, Humle T, Tanaka M. Chimpanzees' flexible targeted helping based on an understanding of conspecifics' goals. Proc Natl Acad Sci U S A. 2012 Feb 28;109(9):3588–92.
56. Adriaense JEC, Koski SE, Huber L, Lamm C. Challenges in the comparative study of empathy and related phenomena in animals. Vol. 112, Neuroscience and Biobehavioral Reviews. Elsevier Ltd; 2020. p. 62–82.
57. Silva PRR, Silva RH, Lima RH, Meurer YS, Ceppi B, Yamamoto ME. Are There Multiple Motivators for Helping Behavior in Rats? Front Psychol. 2020 Jul 29;11.
58. Podgórski T, Lusseau D, Scandura M, Sönnichsen L, Jędrzejewska B. Long-lasting, kin-directed female interactions in a spatially structured wild boar social network. PLoS One. 2014 Jun 11;9(6):e99875.
59. Düpjan S, Tuchscherer A, Langbein J, Schön PC, Manteuffel G, Puppe B. Behavioural and cardiac responses towards conspecific distress calls in domestic pigs (Sus scrofa). Physiol Behav. 2011 Jul 6;103(5):445–52.
60. Mafessoni F, Lachmann M. The complexity of understanding others as the evolutionary origin of empathy and emotional contagion. Sci Rep. 2019 Dec 1;9(1).

61. Camerlink I, Turner SP. The pig's nose and its role in dominance relationships and harmful behaviour. Appl Anim Behav Sci. 2013 May 1;145(3–4):84–91.

Tables and Figures

Table 1. Results of a zero-inflated negative binomial model predicting the likelihood of helping. Significant predictors are indicated in bold.

Predictor	Estimate	Std Error	χ^2	df	*P*
(Intercept)	-2.48	0.27			(†)
Proportion time looking in window	**0.57**	**0.09**	**43.43**	**1**	**< 0.001**
Proficiency during familiarization‡	**0.38**	**0.16**	**5.66**	**1**	**0.017**
Curiosity during familiarization‡	-0.14	0.16	0.75	1	0.385
Opened door during separation§	0.11	0.42	0.07	1	0.793
Potential helper sex_Female¥	0.37	0.32	1.30	1	0.251
Related to trapped pig#	-0.53	0.28	3.57	1	0.059
Test order in group	-0.01	0.13	<0.01	1	0.952

† Not indicated due to limited interpretability
‡ Data were square-root transformed
§-# Estimates refer to comparison with reference categories:
§ Did not open door during separation
¥ Potential helper sex_Male
Not related to trapped pig

Table 2. Results of a survival analysis predicting the likelihood and latency for trapped pigs to be helped. Hazard ratios are indicated by the exponentiated coefficients. Significant predictors are indicated in bold.

Predictor	coef	exp(coef)	se(coef)	robust se	χ^2	df	*P*
Signals distress_Yes[†]	0.86	2.37	0.41	0.40	4.65	1	**0.031**
Rate window investigations[‡]	-0.18	0.84	0.22	0.20	0.81	1	0.368
Trapped pig sex_Female[§]	0.05	0.95	0.34	0.33	0.03	1	0.868
Time in compartment	0.03	1.03	0.16	0.14	0.04	1	0.836
Location compartment _Side[¥]	0.39	1.48	0.34	0.34	1.37	1	0.242
Test order in group	0.15	1.16	0.16	0.16	0.91	1	0.341
Group identity_1B[#]	1.46	4.31	0.92	0.80	16.26	7	**0.023**
Group identity_2A[#]	-0.14	0.87	0.67	0.72			(Þ)
Group identity_2B[#]	1.00	2.72	0.62	0.65			(Þ)
Group identity_3A[#]	0.51	1.66	0.64	0.69			(Þ)
Group identity_3B[#]	0.73	2.07	0.61	0.62			(Þ)
Group identity_4A[#]	-0.04	0.97	0.62	0.67			(Þ)
Group identity_4B[#]	1.33	3.78	0.65	0.60			(Þ)

[†,§,¥,#] Estimates refer to comparison with reference categories:
[†] Trapped pig did not signal distress
[§] Trapped pig sex_Male
[¥] Location compartment_Front
[#] Group Identity_1A
[‡] Data were square-root transformed
[Þ] Refer to chi-square result under “Group Identity_1B” for over-all effect of Group identity on the likelihood of being helped

Figure 1. Diagram of the experimental set-up, showing two identical compartments that were attached to opposite sides of the home pen during familiarization and testing. The door of each compartment opens into the home pen when the handle is lifted high enough to release an inner latch.

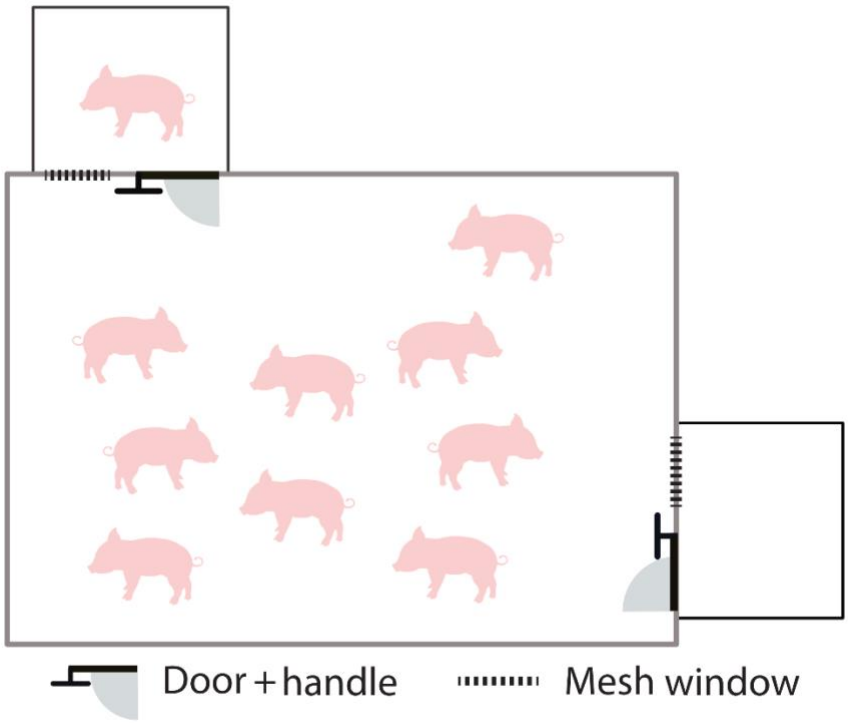

Figure 2. Latency (median and interquartile range) for helper pigs (indicated in circles) to release trapped pigs across test trials within each group. Colours indicate different social groups. Triangles indicate the trials where pigs were released by humans (not included in latency calculations).

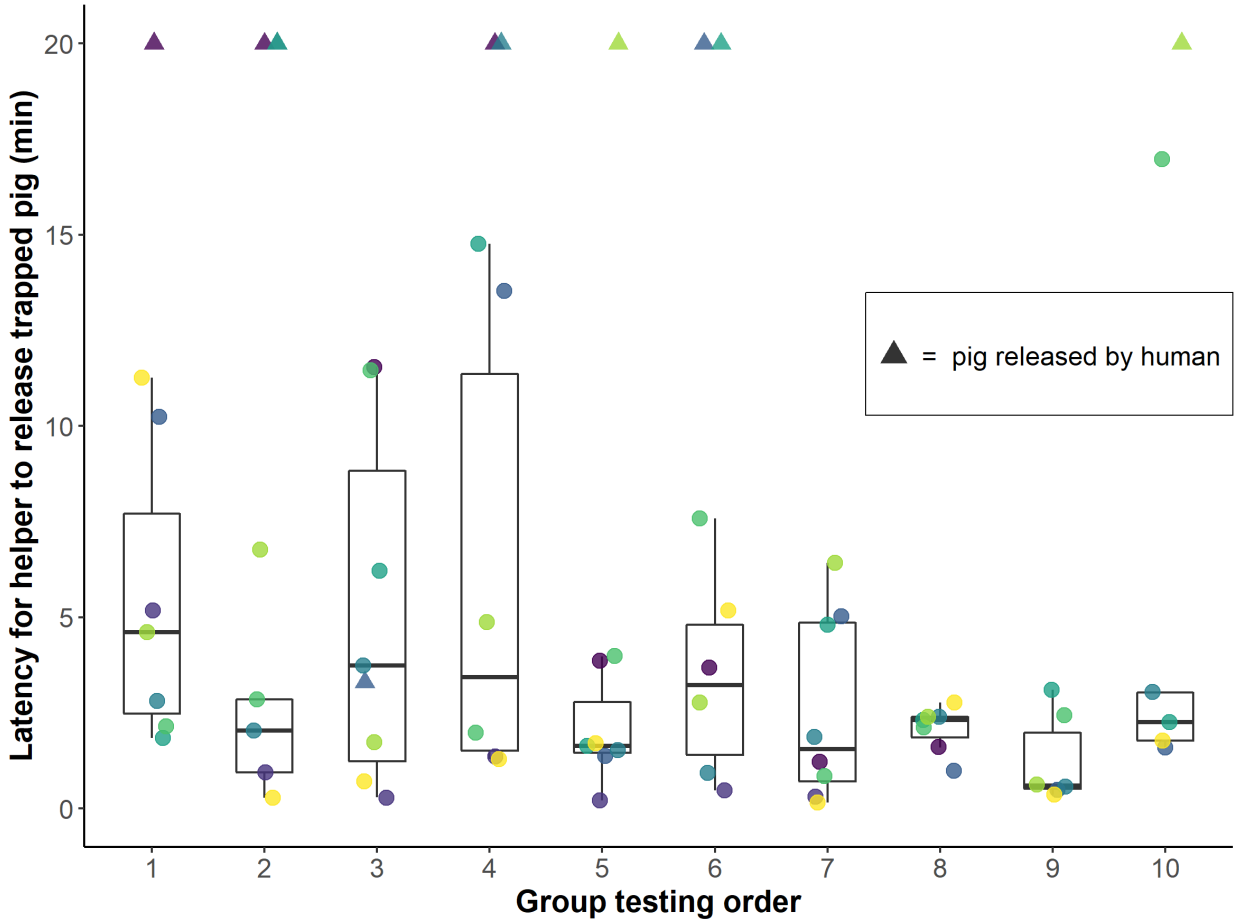

Figure 3. Relationship between each potential helper's social attention to the trapped pig and their likelihood of helping, averaged across their 6–9 exposures to trapped pigs. Colours indicate different social groups. The dashed line depicts the fitted model with control predictors centred around 0. For full model results see Table 1.

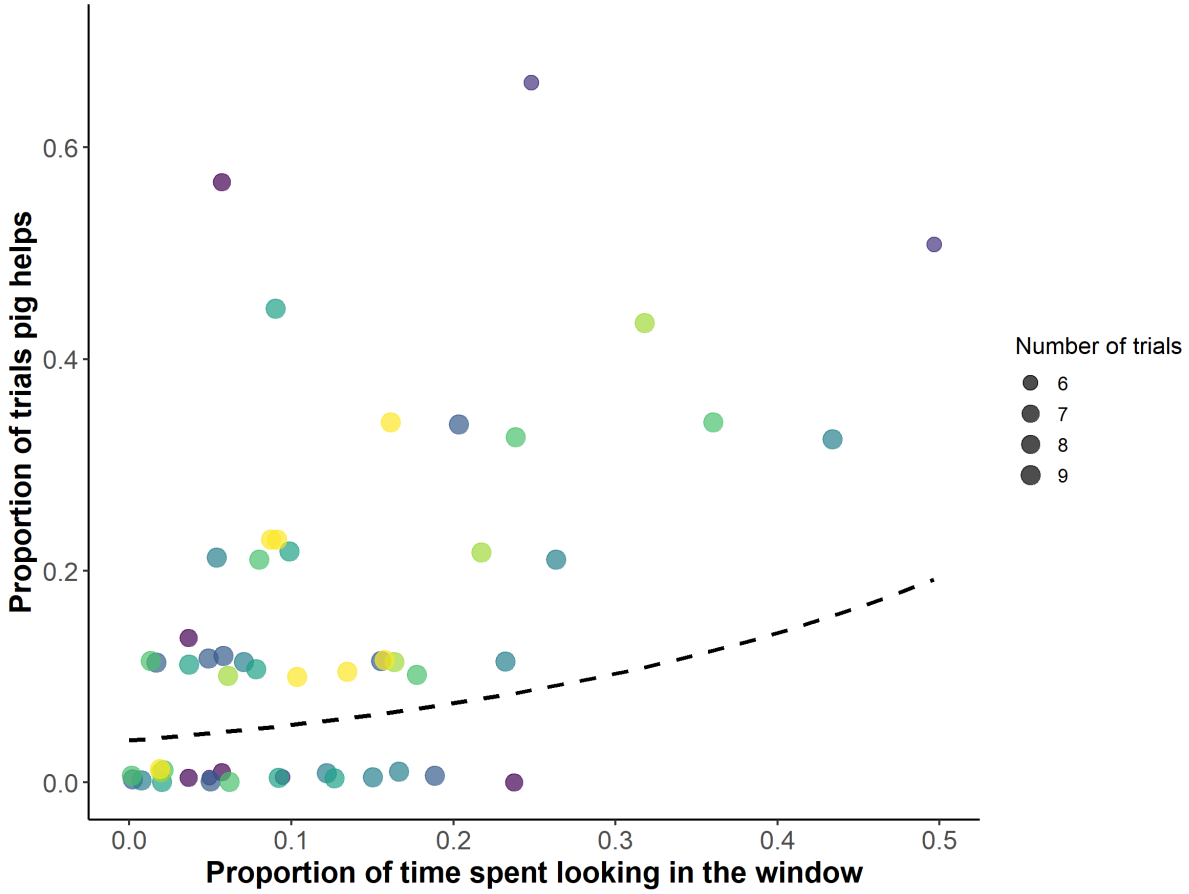

Figure 4. Comparison of post-release salivary cortisol concentrations in trapped pigs and their helpers.

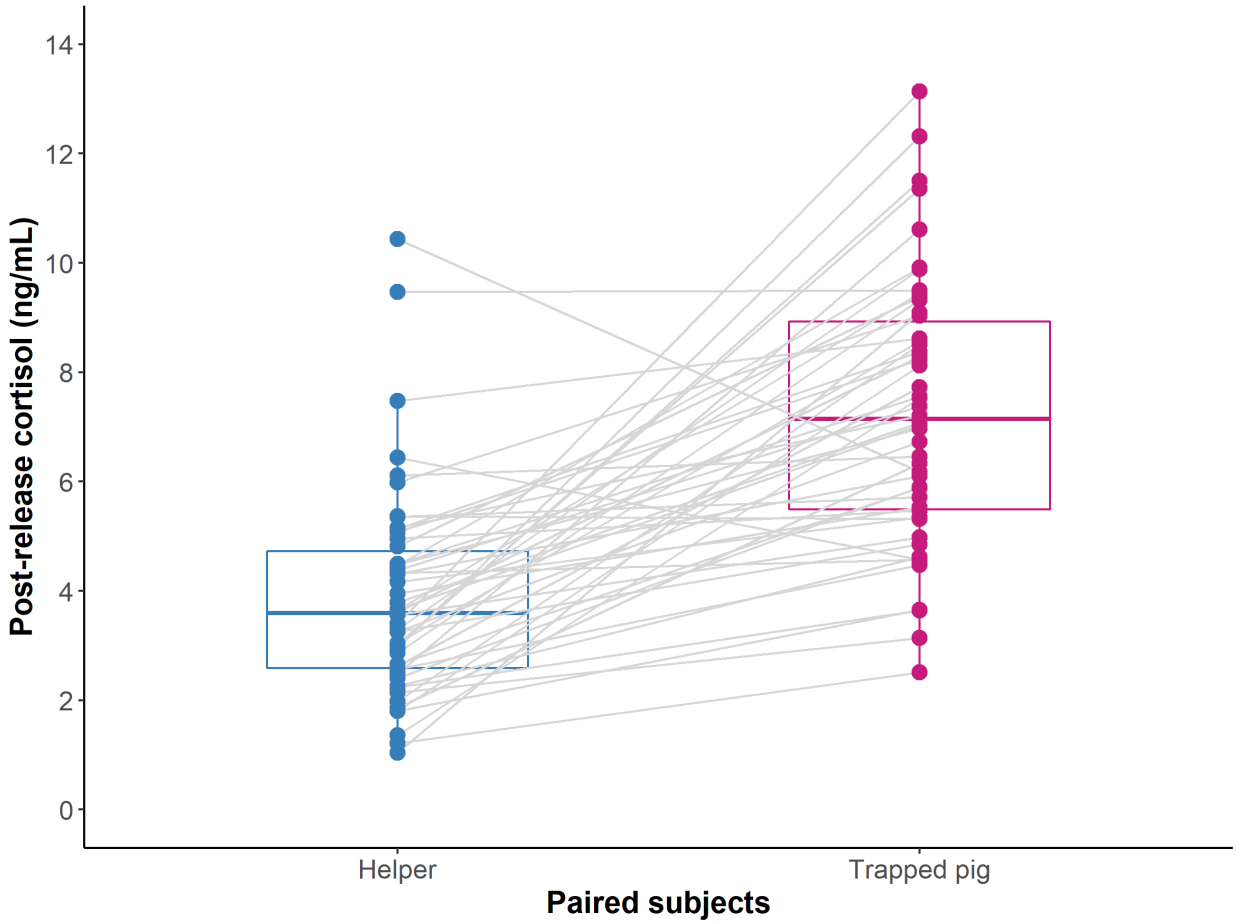
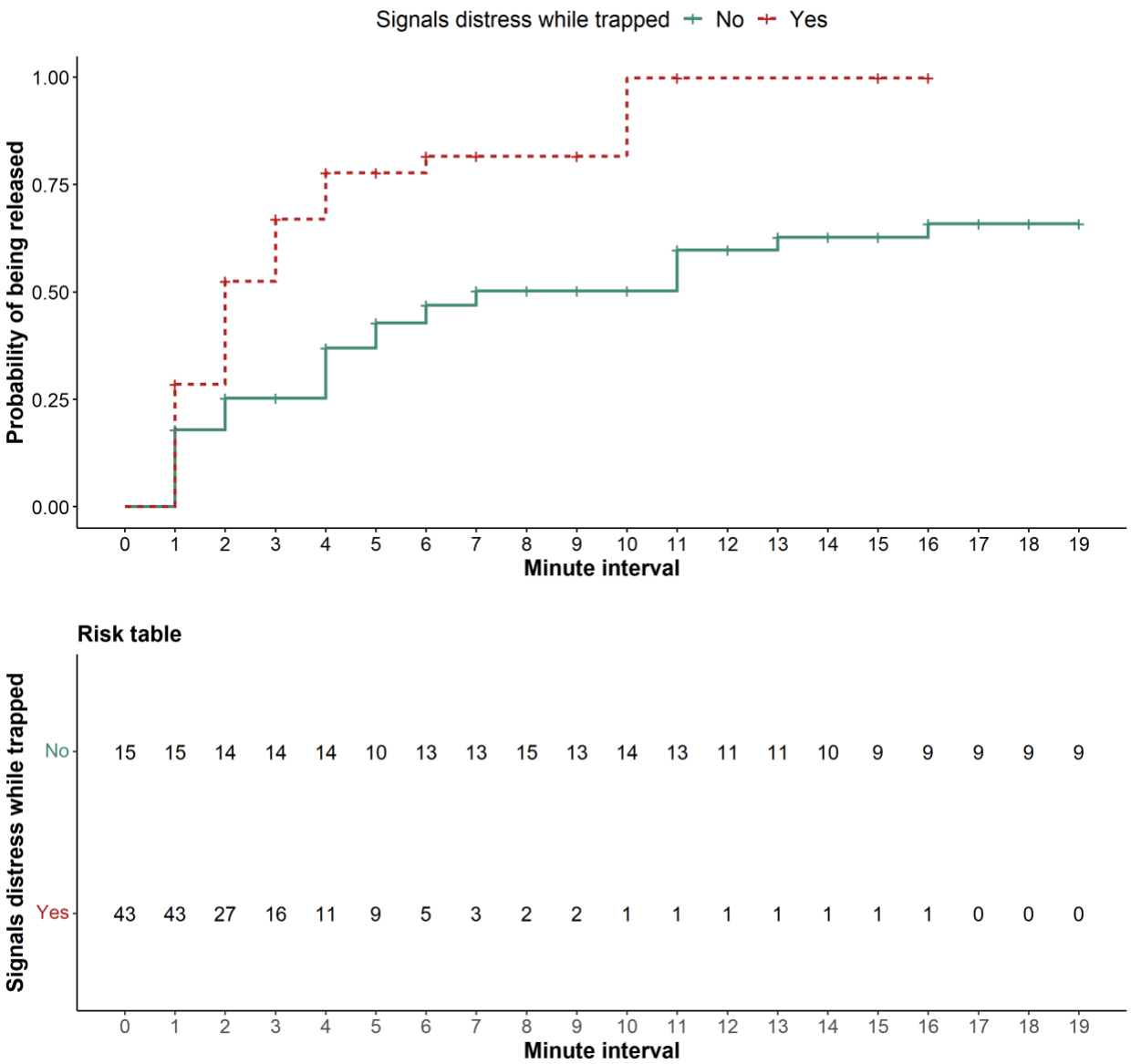
Figure 5. Survival curve showing the probability for n = 58 pigs who were trapped for at least one minute to be helped by a group member. At each minute interval, pigs who gave distress signals were more likely to be released than those who did not (Hazard ratio = 2.4, p = 0.03). For full results, see Table 2.