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Evaluation of animal welfare by the
self-expression of an anxiety state

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Summary
Although mental well-being has long been accepted as an important aspect of animal welfare, the subjective feelings of farm or laboratory animals are regarded as lying beyond the scope of scientific enquiry. We now report that pharmacological conditioning of pigs with a drug, pentylenetetrazole, known to induce anxiety in man, permits investigation of the presence or absence of this psychological state during exposure to a variety of environmental stimuli encountered during normal husbandry. Such pharmacological conditioning therefore provides a valuable means to assess and improve elements of animal welfare and should be applicable to other species that show operant behaviour.

Keywords AnxietY conditioning; drug discrimination; pentylenetetrazole; pig; animal welfare

Previous behavioural studies to alleviate suffering in farm or laboratory animals have either manipulated environments to minimize the abnormal behaviour thought to be indicative of poor welfare (Broom 1986, Maestripieri et al. 1992) or have relied on the subjects learning to modify or select their environment (Baldwin & Ingram 1967, Dawkins 1976, Dawkins 1977, Stephens et al. 1985). Although such studies can be used to optimize individual parameters of housing conditions, the results obtained will depend on the prior experience of the animals and on their motivation to perform other behaviours, such as feeding, egg-laying or seeking conspecifics (Wood-Gush et al. 1975, Dawkins 1977, Duncan 1978, Dawkins 1983, 1990). Indirect measures of presumed suffering can also be made by monitoring activity of the sympathetic nervous system together with the cardiovascular and metabolic responses characteristic of alarm or by monitoring activity of the hypothalamo-pituitary-adrenal axis, which mediates the general adaptation syndrome of the stress response (Broom 1988). However, these physiological measures also need to be interpreted with caution. The intensity of the alarm reaction as opposed to the adrenocortical response to potential stressors appears to vary between different species (Wood-Gush et al. 1975) and in the unweaned calf, kid or lamb, for example, increased sympathetic activity accompanies the act of drinking or sucking milk (Bloom et al. 1975), which can hardly be described as aversive. Also and perhaps most importantly, it is clear from studies in the pig that suffering may arise before activation of the alarm (Marcuse & Moore 1944) or stress (Baldwin & Stephens 1973) responses. We now show that the above ambiguities in the assessment of animal welfare can be overcome by employing the anxiogenic drug pentylenetetrazole in a pharmacological conditioning procedure which enables self-expression of the psychological state of anxiety.

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At subconvulsant doses, pentylenetetrazole (PTZ) causes anxiety in man (Rodin 1958) and induces an interoceptive stimulus in animals that can be revealed by pharmacological conditioning (Emmett-Oglesby et al. 1990). We have shown that pigs can be trained to discriminate the effects of PTZ from saline by a conditioning procedure which permits detection of the onset and offset of this drug-induced psychological state (Carey et al. 1992). Moreover, the conditioned emotional state elicited by anticipation of a mild, non-injurious electric shock to the skin, a form of conditioned anxiety (Estes & Skinner 1941), generalizes to the PTZ discriminative stimulus (Carey & Fry, 1993), confirming the interoceptive cue as a state of anxiety. With this knowledge, we have applied our test of anxiety to evaluate aspects of pig husbandry, by examining the discriminatory response of PTZ-conditioned pigs to a variety of putative aversive stimuli that might normally be encountered at the farm or abattoir.

Materials and methods

Animals

The subjects were 4 male pigs (15–20 kg, initial weight) of the Large White breed, 8 weeks old at the start of the study. They were housed individually in straw-floored pens in a barn which was maintained at 18–20°C under normal daylight conditions. Animals were fed a restricted diet of pellets ('Spotlight' stage-1 pellets; Charnwood Milling Co. Ltd) at 1.5–2.0% of body weight. At the end of each experimental day, the animals received the amount of this food which was not consumed during pharmacological conditioning. Water was freely available in the home pens.

Pharmacological conditioning

The pigs were trained to discriminate the effects of pentylenetetrazole (PTZ) from saline as previously described (Carey et al. 1992). In brief, the operant chamber consisted of a modified Skinner box with 2 manipulanda for the pig to press to obtain food reward. This chamber was housed in an insulated room, maintained (unless otherwise stated) at 20–22°C and illuminated by a 58W fluorescent tube, with constant background noise provided by 3 ventilation fans. During the initial, pre-drug conditioning, both levers were set to reward in an alternate fashion [alternation training] such that at the end of this stage of training the animals had learned that when reinforcement had been obtained by pressing one lever at a fixed ratio of 20 presses per food reward (FR 20), the next food reward could be obtained only by pressing the alternate lever at a FR 20.

Once pigs were responding in this fashion, training was suspended to allow the surgical implantation of jugular catheters [under azaperone (2 mg/kg, i.m.)/metomidate (10 mg/kg, i.p.)/N₂O anaesthesia]. After 2 days to recover from this surgery, the animals received a further period of alternation training until they had satisfied a criterion of not making more than one incorrect lever selection in 10 consecutive sessions. These training sessions were of 10 min duration and incorrect selections defined as 5 or more presses on the non-rewarded lever. Finally, the animals were conditioned to respond on one lever only (FR 20) following an injection of PTZ (2.8–10 mg/kg, administered intravenously through the indwelling jugular catheter) and to respond on both levers alternately (FR 20) following an intravenous injection of the same volume of the saline vehicle alone. The criterion of successful discrimination training was 4 out of 5 consecutive saline sessions with no more than one incorrect selection in each consecutive 10 selections and 4 out of 5 consecutive PTZ sessions with no incorrect selections. An incorrect selection was again defined as 5 or more presses on the non-rewarded lever. For each pig, the position of the designated drug lever was kept constant throughout this study: the right-hand lever for pig numbers E1, E3 and E5; the left-hand lever for pig number E4.

Detection of anxiety in response to environmental stimuli

When the pigs satisfied the criterion of PTZ/saline discrimination, they were
Fig 1 Detection of anxiety on exposure to novel stimuli. (A) Cumulative record of the effect of a novel wooden floor in the Skinner box on the discriminatory response of a pig (number ES; PTZ lever = A). A significant ($P<0.001$) preference for the PTZ lever was observed in the first 300 sec followed by a stable alternation of lever selection response. On the records, closed symbols (•) represent lever presses, the lines joining the symbols represent the rate of lever pressing and the vertical lines represent food reinforcement. During some reinforcements, movement of the food hopper solenoid triggered artefactual lever presses. These have been edited from the data and account for ratios of 18 or 19, rather than 20 lever presses per food reward. (B) Cumulative record of the effect of a novel object (a small rubber ball) in the food bowl at the start of a test in the Skinner box on the discriminatory response of a pig (number ES; PTZ lever = A). The test was terminated after 450 sec because the animal turned around in the box to face the exit. The record shows a significant ($P<0.001$) bias toward the PTZ lever. For details of cumulative record see legend to Fig. 1A
Fig 2 Effects of aural stimuli on anxiety state in the pig. (A) Cumulative record of the response of a pig (number E1; PTZ lever=A) to a tape recording of the squeals of another, non-experimental pig which had been restrained by the hind legs. A stable alternation of lever selection response was evident before the tape playback. The tape recording of duration 100 sec induced a suppression of response which persisted for 380 sec. (B) The effect of a tape recording of a barking dog on the discriminatory response of a pig (number E1; PTZ lever=A). The test animal was allowed to respond undisturbed for 300 sec, when it displayed an alternation of lever selection. The recording was then played for a duration of 89 sec. During the tape playback, a suppression of response was observed and the animal did not resume responding until 144 sec after the tape recorder was turned off. This response after the tape playback showed a significant (P<0.05) bias toward the PTZ lever. For details of cumulative record, see legend to Fig. 1A.
presented with putative anxiogenic stimuli and their discriminative responses examined. Some stimuli were presented to the animals during a test session in the Skinner box while others were presented outside. In the latter case, tests of discriminative behaviour were carried out in the Skinner box immediately after exposure to the stimulus. All tests were conducted with both levers set to reward. The precise details of each stimulus presentation and corresponding discriminative behaviour test are given in Results. Training sessions with saline and PTZ were conducted between test sessions to confirm persistence of the discriminative behaviour.

Statistical procedures
For each test session, the statistical significance of any lever bias (i.e. deviation from an alternation of lever selection response) induced by the application of an environmental stimulus was determined by Chi-square analysis. An expected ratio of presses on both levers was determined by assuming as a null hypothesis that the environmental stimulus did not effect the alternation of lever selection.

Results
Effects of novel stimuli
Replacing the usual wire mesh floor of the Skinner box with a smooth wooden board induced a significant \((P<0.05)\) preference for the PTZ lever (Lever A), corresponding to anxiety, during a 10 min session in 3 out of 4 pigs tested (Fig. 1A). To examine the effect of novel stimuli encountered during ingestive behaviour, a novel object (a small rubber ball) was introduced into the food bowl of the box at the start of a 10 min session in 3 out of 4 pigs tested (Fig. 1B). This induced an anxiety response in 2 out of 3 pigs tested (Fig. 1B). During the time that the animals displayed the latter response, the food reinforcements delivered into the food bowl were not eaten.

Effects of changes of ambient temperature and lighting conditions
Reducing the temperature of the Skinner box from its normal temperature of 20–22°C to 5°C elicited an anxiety response in 2 out of 4 pigs tested, whereas changes in the colour or reductions in the intensity of the ambient lighting of the conditioning chamber had no significant effect (not shown).

Effects of aural stimuli
A recording of the vocalizations of a restrained pig was played for 100 sec to animals which had previously shown an alternation of lever selection response for 300 sec. This recording induced a suppression of further operant response in 2 out of 3 pigs tested (Fig. 2A). The anxiogenic effect of the sound of a dog barking was also examined. When a recording of a dog bark was presented 300 sec after the start of a test session, a complete suppression of operant response was observed in all 3 pigs tested. Following the tape playback, which lasted for 89 sec, one of these animals then displayed a significant \((P<0.05)\) preference for the PTZ lever (Fig. 2B). In order to provide a control, non-specific auditory stimulus, a recording was also made of the sounds present in the barn of the test pigs when these animals were undisturbed. Presentation of this recording at the same sound level as the stimuli above did not induce an anxiety response or a suppression of operant responding (not shown), providing evidence that the behaviour observed in response to the auditory stimuli of squealing pig and barking dog reflects a psychological disturbance specific to these stimuli.

Discriminative behaviour in response to transportation, interactions with strange pigs and olfactory stimuli
Pigs were transported individually in a trailer at a speed of 10–30 mph for a period of 20 min and then subjected to a 10 min test session in the Skinner box. An anxiety response was observed in only one of 3 animals tested (Fig. 3B).

The effects of an encounter with strange pigs, were investigated by giving each test pig a control 5 min session in the Skinner box and then returning the animal to its
Fig 3  Effects of transportation and of mixing with strange pigs on anxiety state. (A) Cumulative record of the effect of individual transportation in a trailer on the subsequent discriminatory response of a pig (number E4; PTZ lever = B). A significant (P < 0.001) bias for the PTZ lever was evident in the initial 300 sec of the test immediately after removal from the trailer. (B) Cumulative record of the discriminatory response of a pig (number E4; PTZ lever = B) before and after an encounter in its home pen with 2 strange male prepubertal pigs. An alternation of lever selection response occurred in the test prior to the encounter and a significant (P < 0.02) bias for the PTZ lever in the test following the encounter. For details of cumulative record, see legend to Fig. 1A.
Fig 4  Detection of anxiety in response to olfactory stimuli. (A) Cumulative record of the discriminatory response of a pig (number E3; PTZ lever = A) to the presence of urine obtained from a sow in oestrus and rubbed on the front panel of the Skinner box. A significant ($P < 0.001$) bias toward the PTZ lever was observed in the first 5 min of the test period. (B) Cumulative record of the response of a pig (number E3; PTZ lever = A) to the presence of a freshly killed and split pig carcass placed out of view under the floor of the Skinner box. A generalization to the PTZ stimulus occurred in the initial 157 sec of the test period and thereafter the animal selected both levers alternately. The bias toward the PTZ lever in the first 5 min of the test was significant ($P < 0.001$). Records (A) and (B) show responding at a fixed ratio of 40 presses per food reward (FR 40) as opposed to a FR 20 because at this stage of the experiment, it was found that a FR 40 allowed a more stable discriminatory response in this particular animal. For other details of cumulative record see legend to Fig. 1A.
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home pen to encounter 2 strange male prepubertal pigs (from an adjacent barn) for a period of less than 10 min. In all cases the encounter of the test pig with the 2 strange pigs resulted in characteristic antagonistic behaviour [Signoret et al. 1975], with the animals circling one another and applying pressure shoulder to shoulder. Immediately following the encounter, the test animals were given a further 5 min session in the Skinner box. In the test prior to the encounter, an alternation of lever selection response was detected for all 4 animals (Fig. 3B). In contrast, an anxiety response was observed in 2 pigs in the test following the encounter (Fig. 3B).

Olfactory stimuli examined were from the following sources: (1) the urine of a sow in oestrous; (2) the secretion of the preputial gland of a strange boar; (3) freshly killed (<30 min) and split pig carcasses; and (4) melted thymol. To present the first 2 stimuli, the fluids concerned were rubbed evenly on the front panel of the Skinner box, but not on either of the 2 levers or the food bowl. Animals were then subjected to a 10 min test session in the box. Exposure to the smell of sow urine resulted in an anxiety response in 2 out of 4 pigs tested (Fig. 4A) whereas the preputial gland secretion induced a period of anxiety in one out of 3 pigs tested (not shown). The smell of a pig carcass was presented by placing it out of view under the floor of the Skinner box. Test pigs were given a 10 min test session with a carcass in place and an anxiety response was detected in 2 out of 4 pigs tested (Fig. 4B). The smell of melted thymol, contained in a beaker of hot water concealed under the floor of the Skinner box, did not induce anxiety during a 10 min session in any of the 4 animals tested (not shown).

Discussion

We have shown that prior pharmacological conditioning with an anxiogenic drug can be used to test for the presence or absence of a subsequent anxiety state in response to a variety of environmental stimuli that might be encountered during normal animal husbandry. In the present study, animals were conditioned with the anxiogenic drug pentylenetetrazole (PTZ), which is known from previous behavioural and pharmacological investigations to induce an interoceptive cue in the pig that corresponds to an anxiety state, rather than a non-specific arousal or pro-convulsant aura [Carey et al. 1992, Carey & Fry 1993]. Interestingly, although all the pigs retained their PTZ discrimination throughout the study, they did not all show the same discriminative responses to environmental stimuli. This indicates the individual differences that exist in anxiety responses and illustrates the value of a test that can be applied with statistically defined confidence to single animals.

Intensive animal husbandry involves housing large numbers of con-specifics in a controlled environment in which they have limited opportunities to explore. One consequence of such environmental restrictions is that animals may periodically be exposed during normal management, transport or abattoir conditions to unfamiliar stimuli. In the present study, anxiety was observed in response to a change in floor covering in the Skinner box or to the presence of a novel object in the food bowl. These anxiety responses presumably reflect the importance of investigative rooting behaviour in the pig [Signoret et al. 1975]. An additional factor when the novel object was in the food bowl would have been the frustration of non-reward, because the food reinforcements were not eaten in this test situation. Although not completely identical to the present experiments, a frustrating non-reward in which the expected reinforcement fails to appear is known to induce stress in the pig, as judged by elevated plasma corticosteroid concentrations [Blatchford et al. 1978, Dantzer et al. 1980].

Anxiety responses were also seen on reducing the temperature of the Skinner box from 20-22°C to 5°C, but not on reducing the lighting of the conditioning chamber. These results are consistent with
the observation of pigs in a natural environment (Signoret et al. 1975), where they will start to seek shelter and huddle together when ambient temperatures fall below about 5°C and where they rarely use visual cues in the search for food.

When pigs are handled or restrained, they commonly respond by squealing and struggling. Although the animals appear excited, their heart rate seldom varies during such episodes, which have been termed tantrum or grandstand behaviours (Marcuse & Moore 1944). Nevertheless, a recording of such squealing was found to have a profound effect on the pigs tested, inducing a complete suppression of responding in 2 animals. We also examined the effects of a recording of a dog bark as this stimulus is thought to induce fear in other farm animals (Stephens 1980). Again, a complete suppression of responding was observed. However, responses did recover in one pig during the test session after exposure to the sound of the barking dog and revealed a significant anxiety state (Fig. 2B). In contrast to the above effects of specific auditory stimuli, presentation of a recording of background noise from their home barn had no significant effect on the test pigs. This relative insensitivity to non-specific auditory stimuli has been described by earlier workers (Stephens et al. 1985), who found that pigs would learn to switch off the vibration, rather than the noise, of a transport simulator.

It is known that transportation of pigs and abattoir conditions prior to slaughter can produce physiological changes indicative of stress which adversely affect meat quality. Indeed, some stress-susceptible strains of pig do not even survive the journey to the slaughter house (Wood-Gush et al. 1975, Lean 1988). In the present investigation, testing of pigs immediately after a 20 min journey in a trailer revealed anxiety in only one of 3 animals. This result is surprising as the movement and vibration of a transporter, as mentioned above, are known to be aversive (Stephens et al. 1985), but it may have been the case that anxiety faded quickly on removal from the trailer. Once at the abattoir, pigs are held in groups prior to slaughter. These animals have a well-developed capacity for individual recognition and the establishment of dominance hierarchies (Meese & Ewbank 1973). Thus, grouping of pigs from different units together usually results in violent fighting, especially between males. The observed anxiety after an encounter with strange pigs could have resulted from the physical interaction itself and/or from the threat which the presence of the strange animals imposed. Since olfaction plays a significant role in social interaction among pigs (Ewbank et al. 1974, Signoret et al. 1975), we examined the discriminatory response of the test pigs either to the presence of urine obtained from a sow in oestrous or to the secretion from the preputial gland of a strange boar. The latter secretion gives the boar its characteristic odour (Signoret et al. 1975). Anxiety responses were observed in response to both stimuli but not to the non-porcine olfactory stimulus of thymol. Thus, our results indicate that mixing of strange pigs could have an adverse effect on the psychological state of the animals in the absence of any physical interaction. Anxiety responses were also observed in response to another olfactory stimulus that may be present at the abattoir, the smell of a freshly killed and split pig carcass.

Inadequacies in the scientific assessment of mental well-being in animals are now well recognized (Dawkins 1990, Maestripieri et al. 1992). Indeed, Bekoff et al. (1992) have asserted that since ‘the animals' point of view is taken from the humans' point of view', a human element is inevitable and ‘cannot be transcended by scientific approaches'. Although anxiety can only ever be appreciated with reference to personal experience, our approach reduces the imposition of human values by providing the animal with a direct means of expressing its own psychological state. The PTZ discrimination paradigm therefore provides a valuable scientific tool for future studies of animal welfare and should be applicable to other animals capable of undergoing operant conditioning. As shown
in the present study with pigs, the presence or absence of anxiety can be evaluated in response to a variety of environmental stimuli. Thus, we can now take the desirable step of refining our attitudes to animal welfare (Dawkins 1989), by investigating the attitudes of the animals themselves.

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