Effects of diazepam on the behaviour of weaned pigs in three putative models of anxiety

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Abstract

The present study examined the effects of diazepam (a widely used anxiolytic benzodiazepine) on the behavioural response of pigs to three novel experimental situations used to measure anxiety-related behaviour in rodents. Twelve weaned pigs (two pairs from each of the three litters) were tested in an elevated plus-maze at the age of 6 weeks, a light/dark test at the age of 7 weeks and an open-field test at the age of 8 weeks. Six of the pigs were pre-treated with diazepam (valium) and the other six with saline (control). In the elevated plus-maze, diazepam-treated pigs had a higher number of entries into open arms \(P = 0.04\), spent more time on open arms \(P = 0.07\), and had a higher number of total arm entries \(P = 0.05\) than pigs from the control group. However, diazepam had no significant effects on behaviour in the light/dark test (i.e., latency to enter lit compartment, number of entries into lit compartment and the time spent in lit compartment) or the open-field test (i.e., number of lines crossed, number of entries into centre). In summary, the anxiolytic effects of diazepam on the pigs’ behaviour were only demonstrated in the elevated plus-maze, where the time spent on open arms and the number of entries into open arms could be interpreted as measures of anxiety in pigs. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Fear and anxiety are important aspects of welfare in farm animals. The range of potentially fear or anxiety-eliciting events in pig husbandry include exposure to a new environment (Mormède et al., 1984; Beattie et al., 1995; Erhard and Mendl, 1999), exposure to and handling by humans (e.g., Hemsworth et al., 1986), isolation from litter mates (Forkman et al., 1995), and mixing with unfamiliar pigs (Jensen, 1994; Jensen and Yngvesson, 1998). A common element in these situations is novelty, which is often associated with a negative emotional response in animals (Boissy, 1995). Different methods have been developed to study fear responses of pigs during handling (e.g., Hemsworth and Barnett, 1991, 1992; Hemsworth et al., 1986; Tanida et al., 1995), but there is a considerable lack of validated methods for measuring fear-related behaviour in other novel situations. One approach to studying fear and anxiety in swine would be to apply the large body of knowledge provided by research on laboratory animals.

Two commonly used and well-validated models of anxiety in rodents are the elevated plus-maze (Pellow et al., 1985; Lister, 1987; Rodgers and Cole, 1993; Rodgers and Johnson, 1995; Cruz et al., 1994; File et al., 1994) and the light/dark test (Rodgers and Shepherd, 1993; Cheng et al., 1994; Costall and Naylor, 1994, 1995; Costall et al., 1987; Smythe et al., 1996; Hendrie et al., 1997). The elevated plus-maze is composed of four elevated arms of equal size radiating from a central platform. Two opposite arms have no walls while the other two have walls along their sides and ends. The model is based on the observation that although animals tend to explore a novel environment, the height and the open space of open arms are perceived as aversive and avoided (Montgomery, 1955; Pellow et al., 1985; File, 1992). In the elevated plus-maze, increases in the number of entries into open arms and time spent on open arms are associated with a lower level of anxiety (Pellow et al., 1985). The light/dark test, composed of a bright and dark room connected by a small opening, creates a conflict between the tendency to explore a novel environment and to avoid the open, brightly-lit compartment (Crawley and Davis, 1982). An anxiolytic effect in the light/dark test is indicated by an increase in the percent of activity and time spent in the aversive brightly-lit compartment (Costall et al., 1987; Onaivi and Martin, 1989) or the number of transitions between the two compartments (Smythe et al., 1996). Modifications of the open-field test developed by Hall (1936) have been used to a great extent to assess fear-related behaviour in many domestic species (pigs: Von Borell and Ladewig, 1992; Beattie et al., 1995, foxes: Bakken, 1994; poultry: Jones et al., 1995; Heiblum et al., 1998 and cattle: de Passillé et al., 1995; Jensen et al., 1997), but only a few have evaluated the method (Boissy and Bouissou, 1995; de Passillé et al., 1995; Marin et al., 1997). File (1992) states that proper validation of behavioural models of anxiety should combine behavioural, pharmaceutical, and physiological methods. These procedures are ubiquitous in validating models of aversion for laboratory animals but are blatantly lacking in farm animal studies. Anxiolytic drugs should thus be helpful tools in revealing which behavioural elements are related to anxiety in different experimental tests for domestic species.

Diazepam is considered to be one of the most powerful anxiolytic drugs. When used at low or moderate doses, the sedative and muscle relaxant effects are few and of low intensity (Flaten and Gräwe, 1986). In rodents, diazepam reduces open arm avoidance.
without altering activity in the elevated plus-maze (Cole and Rodgers, 1995), and reduces avoidance of the lit compartment in the light/dark test (Costall et al., 1987; Hendrie et al., 1997). On the contrary, diazepam is reported to have sedative effects on motor activity in the open-field (Hughes, 1993; Fisher and Hughes, 1996; Marin et al., 1997), which suggests that the effects of this drug can differ between tests. In pigs, diazepam reduces the aversive effect of non-reward and releases operant behaviour which has been suppressed by punishment (Dantzer, 1976, 1978). As anxiolytic effects of diazepam are documented in pigs (Dantzer, 1976, 1977; Arnone and Dantzer, 1980), this drug was chosen to assist in detecting anxiety-related behavioural elements in the present experiment.

The aim of the present experiment was thus to examine the effect of diazepam on the response of weaned pigs to three novel, aversive experimental tests to facilitate identification of anxiety-related behavioural elements.

2. Materials and methods

Twelve pigs were subjected to three experimental tests: an elevated plus-maze, a light/dark test and an open-field test at the age of 6, 7 and 8 weeks of age, respectively. Half of the animals were pre-treated with diazepam and the other half with saline (control).

2.1. Animals

Two pairs of weaned pigs (Landrace × Yorkshire, castrated males and females) from each of the three litters were chosen at the age of 6 weeks, so that pigs in each pair were of equal weight (maximum weight difference was 0.5 kg). One pig from each pair was treated with diazepam, and the other with saline. The mean weight of pigs used in the experiment was 15.0 ± 1.8 kg. After weaning at the age of 5 weeks, the three litters were moved to the research building. All pigs were kept in litter groups throughout the whole experimental period, and housed in pens (2.2 × 2.6 m) containing a lying area with solid floor in 2/3 of the pen and slatted floor in the rest. An infrared lamp used as a heater was suspended in one corner of the pen, and a small amount of litter (sawdust) was supplied every morning. Solid pen partitions prevented any visual or physical contact between litters. The pigs were fed ad libitum with standard concentrate from a food dispenser, and had free access to water from a nipple drinker. Air temperature was between 18°C and 20°C, and artificial light was kept on between 0800 and 1500 h. During the rest of the day and night, the windows and the infrared heaters were the only sources of light.

2.2. Drug administration

The pigs were weighed 30 min before each test, and injected intramuscularly with diazepam (Valium®, Roche; 0.8 mg/kg) from commercially available vials (10 mg/2 ml) or an equivalent volume of saline (control treatment). For pigs weighing between 12
and 50 kg. 1 mg/kg diazepam is a common dosage level (e.g., Dantzer, 1978; Arnone and Dantzer, 1980). The reason for reducing the dosage to 0.8 mg/kg in the present study, was that a preliminary study using 1 mg/kg resulted in severe side effects. These side effects included inactivity, problems with walking and shivering. At a dosage of 0.8 mg/kg, however, no side effects were observed.

2.3. Elevated plus-maze

The elevated plus-maze was a modification of the apparatus described by Lister (1987) for mice, and adjusted to the size of 6-week-old pigs. The apparatus consisted of two open, elevated (1.0 m above the floor) arms with a 2-cm high threshold, 0.60 × 3.0 m, and two enclosed arms, 0.60 × 0.30 × 0.60 m, arranged in such a way that the two open arms were opposite to each other (Fig. 1). The walls of the enclosed arms were made of transparent plexiglas.

The pigs were placed individually into the centre of the maze facing one of the closed arms. From the time that the animal was placed onto the maze the following parameters were observed for a period of 5 min: number of entries into open arms, number of entries into closed arms, and time spent on open arms.

2.4. Light / dark test

The light/dark box used in this experiment was a modification of the apparatus used for mice by Cheng et al. (1994), adjusted to the size of 7-week-old pigs. The box consisted of a brightly illuminated (60 W Halogen light) compartment (1.2 × 1.2 m) where the walls were painted white, a dark compartment (1.2 × 1.2 m) with dark brown walls, and an opening (0.4 × 0.4 m) between the two compartments (Fig. 2). An infrared light was positioned above the test apparatus. The light intensity in the lit and dark area was 90 and 2.5 lux, respectively.

The subjects were individually tested for 5 min at the age of 7 weeks. The pigs were placed into the dark compartment of the box, and the latency to enter the lit compart-
ment, number of entries into the lit compartment and the time spent in the lit compartment were recorded.

2.5. Open-field test

The open-field test was a modification of the test for rats developed by Hall (1936), adjusted to the size of pigs at the age of 8 weeks. A circular open-field arena with a
The diameter of 3.0 m and an 0.6 m high wall of plywood was used (Fig. 3). The pigs were individually placed in area number 1 and the number of lines crossed and the number of entries into the centre of the arena were recorded during a 5-min period. After the pig was removed from the apparatus, the number of fecal boli dropped was counted (defecation frequency).

The following points were the same for all three behavioural tests. The test apparatus was situated in a separate test room without windows and having a solid door, which the handler immediately exited after placing the animal onto the test apparatus. All behaviour was observed from a monitor in an observation room, connected to a video camera vertically mounted above the centre of the test apparatus, and the apparatus was cleaned before each animal was tested.

2.6. Statistics

To analyse the differences in behaviour between the diazepam and control group, a pair-wise $t$-test was conducted.

3. Results and discussion

Diazepam treatment increased the number of entries onto open arms, time spent on open arms and the total number of arm entries (Table 1). This corresponds to a large extent with the results from work on rodents (Pellow et al., 1985; Lister, 1987; Rex et al., 1993; Cole and Rodgers, 1995; Hendrie et al., 1997), and suggests that open arm

<table>
<thead>
<tr>
<th>Test variable</th>
<th>Saline</th>
<th>Diazepam</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated plus-maze</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of entries into open arms</td>
<td>5 ± 1 (2–8)</td>
<td>8 ± 2 (3–16)</td>
<td>0.04</td>
</tr>
<tr>
<td>Entries into the open arms (%)</td>
<td>56.0 ± 5.2 (40–80)</td>
<td>55.8 ± 7.2 (36–71)</td>
<td>0.49</td>
</tr>
<tr>
<td>Time spent on open arms (%)</td>
<td>31.6 ± 5.2 (15.7–50.3)</td>
<td>47.5 ± 7.7 (31.0–80.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>No. of entries into closed arms</td>
<td>4.2 ± 1.0 (1–7)</td>
<td>6.0 ± 1.1 (2–9)</td>
<td>0.16</td>
</tr>
<tr>
<td>Total no. of arm entries</td>
<td>9.2 ± 1.9 (5–14)</td>
<td>14.0 ± 2.8 (7–25)</td>
<td>0.05</td>
</tr>
<tr>
<td>Light / dark test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to enter light area (s)</td>
<td>148.2 ± 49.3 (10–293)</td>
<td>238.5 ± 40.8 (68–300)</td>
<td>0.11</td>
</tr>
<tr>
<td>No. of entries into light area</td>
<td>1.8 ± 0.6 (0–4)</td>
<td>1.5 ± 1.1 (0–7)</td>
<td>0.40</td>
</tr>
<tr>
<td>Time spent in light area (%)</td>
<td>20.8 ± 8.6 (0–55.3)</td>
<td>3.4 ± 8.5 (0–41.0)</td>
<td>0.32</td>
</tr>
<tr>
<td>Open-field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of lines crossed</td>
<td>90.7 ± 10.2 (540–117)</td>
<td>98.8 ± 14.1 (54–117)</td>
<td>0.31</td>
</tr>
<tr>
<td>No. of entries into centre</td>
<td>4.5 ± 1.2 (0–7)</td>
<td>3.2 ± 0.7 (1–6)</td>
<td>0.17</td>
</tr>
<tr>
<td>Defecation frequency</td>
<td>0.7 ± 0.2 (0–1)</td>
<td>1.2 ± 0.2 (1–2)</td>
<td>0.04</td>
</tr>
</tbody>
</table>
entries and time spent on open arms are indicators of anxiety in pigs. The number of and percent entries into open arms and time and percent of time spent on open arms are considered the most important indicators of anxiety for rodents in the plus-maze, as they to a lower extent are sensitive to changes in locomotor activity (Hogg, 1996). In factor analysis of murine elevated plus-maze behaviour these four variables all load strongly on the factor which is anxiety-related (see Rodgers and Johnson, 1995). In the present experiment, however, diazepam did not increase the percent of entries into open arms as one would expect if this element was anxiety-related. The reason for this is unclear. Since the interpretation of behavioural elements for rodents in the elevated plus-maze is to a large degree based on the results from factor analysis, this discrepancy also illustrates a need for factor analysis of porcine elevated plus-maze behaviour (see Andersen et al., 1999). Similar to the findings of Cole and Rodgers (1995) for mice, the number of entries into closed arms was not significantly affected by diazepam treatment. As closed entries are considered a purer measure of the locomotor activity than total number of arm entries (Lister, 1987; File, 1992; Rodgers and Johnson, 1995; Hogg, 1996), it can be inferred that diazepam did not affect the activity level per se. This could also explain why there were no significant effects of diazepam on open-field activity (i.e., number of lines crossed, the number of entries into the centre; Table 1). It is also possible that rodents and pigs show different behavioural responses in the open-field test. This can only be addressed by including more behavioural parameters when studying open-field behaviour in pigs. Furthermore, we need to analyse the behavioural alteration in pigs after administration of anxiolytic drugs and how these changes are related to the pigs fear responses in other experimental tests. Some authors have reported sedative effects of diazepam on motor activity in the open-field (Hughes, 1993; Fisher and Hughes, 1996; Marin et al., 1997), but others suggest that this effect is dose dependent (Schmitt and Hiemke, 1998). As found by Pohorecky and Roberts (1991), diazepam treatment resulted in a higher frequency of defecation in the open-field, which may be interpreted as a side effect of the drug. There were no differences between the diazepam and the control group in any of the variables in the light/dark test (Table 1). In fact, the diazepam-treated pigs tended to show a greater latency to enter the lit compartment. The wild boar and feral pig show a peak of activity at dusk and dawn (Caley, 1997; Russo et al., 1997), but unlike rodents, there is very little documentation of preference for a dark area in the domestic pig (Braude et al., 1958). The lack of documentation of aversion to light in swine, combined with the fact that diazepam did not influence the response to light in the present experiment, indicates that pigs may not perceive light as aversive. The light/dark test may thus have less biological relevance and practical value for pigs than for rodents.

In conclusion, anxiolytic-like effects of diazepam on pig behaviour were found in the elevated plus-maze, but not in the other experimental tests. The results from the elevated plus-maze suggest that time spent on open arms and number of entries into open arms are related to anxiety in pigs. Generally, before applying anxiety models used in rodents to evaluate fear responses in pigs, these models should be validated. This can be provided firstly by using anxiolytic drugs to detect the fear-related behavioural elements, and secondly by examining the relationship between behaviours from a number of different tests of anxiety.
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References


