BEHAVIOURAL AND PITUITARY-ADRENAL CHARACTERISTICS OF PIGS DIFFERING BY THEIR SUSCEPTIBILITY TO THE MALIGNANT HYPERTERMIA SYNDROME INDUCED BY HALOTHANE ANESTHESIA. 1. Behavioural measures
R. Dantzer, P. Mormede

To cite this version:

HAL Id: hal-00901038
https://hal.science/hal-00901038
Submitted on 1 Jan 1978

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
BEHAVIOURAL AND PITUITARY-ADRENAL CHARACTERISTICS OF PIGS DIFFERING BY THEIR SUSCEPTIBILITY TO THE MALIGNANT HYPERThERMIA SYNDROME INDUCED BY HALOTHANE ANESTHESIA

1. Behavioural measures.

R. DANTZER and P. MORMEDE

Station de Pharmacologie-Toxicologie, I.N.R.A.,
180, chêmin de Tournefeuille, 31300 Toulouse, France.

The porcine stress syndrome is typically characterized by sudden death in pigs, preceded by increased heart rate, hyperventilation, hyperthermia, rigidity of muscles and blood acidosis. The extent of losses varies both from breeds and stocks. The symptoms usually develop in pigs subjected to stress associated with mixing of animals, transport, physical exertion or high temperatures exposure. Stress susceptibility has been tentatively identified by the inability of porcine animals to maintain homeostasis in a warm environment (Charpentier and Goutefongea, 1969; Forrest et al., 1968; Judge et al., 1968) or to withstand physical exercise (Topel et al., 1968). Similar symptoms have also been described in subjects submitted to halothane anesthesia: susceptible pigs develop muscular rigidity and a rise in body temperature (malignant hyperthermia syndrome or MHS) within a few minutes when exposed through a face mask to a mixture of halothane and oxygen; this will subsequently lead to death if the anesthetic is not withdrawn (Hall et al., 1966; Harrison et al., 1969; Sybesma and Eikelboom, 1969; Allen et al., 1970). Such a test has the advantage of allowing field application for routine testing and therefore has been the subject of intensive research from the point
of view of genetic control, endocrine correlates and basic mechanisms of apparition of the pathological condition (cf. for a recent review Cassens et al., 1975). Interestingly halothane-positive pigs (i.e. subjects which develop malignant hyperthermia when exposed to halothane) have been shown to be more susceptible to stress (Sybesma and Eikelenboom, 1969; Allen et al., 1970; Christian, 1974, 1977). Moreover the MHS appears to be inherited as an autosomal recessive (Ollivier et al., 1975; Christian, 1977).

Stress induces a complex set of interrelated responses, including peripheral and central modifications (Yuwiler, 1976). Behavioural responses may be very effective to cope with stress situations and have profound influences on the metabolic changes accompanying the stressfull experience (Henry, 1976). Little is known about the behavioural characteristics of stress-susceptible pigs when confronted with aversive stimuli. The present experiments were therefore initiated in an attempt to delineate behavioural traits of halothane-positive subjects compared with halothane-negative animals, using conditioning procedures. Pituitary-adrenal function was simultaneously assessed since there are definite relationships between pituitary-adrenal hormones and behaviour (de Wied et al., 1972).

In this first report, we shall present the results of behavioural experiments. Our efforts have concentrated on aversive conditioning procedures since they have been shown to be very sensitive to stress variables (Mormède and Dantzer, 1977). However exploratory behaviour and latent learning measures have also been assessed to allow to take into account eventual differences due to activity, reactivity or learning capacity.

Methods.

Subjects.

Two strains of pigs were compared, a Pietrain strain selected for meatness and a Large White control strain. Pigs from both strains were provided by the Animal Genetics Department of I.N.R.A. (Domaine expérimental d'Avord, Cher) and were tested for halothane sensitivity at 6-8 weeks of age: 2.5% halothane (Fluothane, ICI-Pharma) with oxygen as the only carrier was administered through a face mask for a maximum of 5 mn. after initiation of the anesthetic state (Monin et al., 1976). When muscular rigidity and hyperthermia were diagnosed, anesthesia was immediately stopped and the pigs left to recover. Sixteen Pietrain positive, sixteen Pietrain negative and sixteen Large White negative (LW) pigs were randomly selected within the stock. Each experimental group contained 8 females and 8 castrated males weighing 16-25 kg. Pigs were transported by lorry to Toulouse one week before the beginning of the behavioural observations. Each pig received an intramuscular injection of 40 mg of Azaperone (Stresnil, Janssen) before the transport.

During all the experimental procedure, pigs were separated according to the strain and the reaction to halothane. In the experimental piggery, they were held by pen of four animals. Water was available ad libitum, while food was rationed to about 4-5% of the body weight. Temperature was maintained around 20°C and light was on from 7.00 a.m. to 8.00 p.m.

Behavioural tests.

All pigs were individually submitted to the following experimental routine:

1) EXPLORATION OF A NEW ENVIRONMENT (day 1).

Exploratory behaviour was studied in a two-compartment cage held in a sound-insulated box by counting during a 20 min. session the number of crossings from one compartment to the other (responses) per 2 min. intervals (Dantzer, 1977).

2) LATENT LEARNING (day 2).

Pigs were deprived of food during 24 hours after the exploration session. At the end of this period, they were reintroduced into the two-compartment cage and the latency of approach of a bowl containing food and located at the far end of the cage was recorded to the nearest sec. as well as the latency of feeding from the bowl and the amount of food eaten.
3) PASSIVE AVOIDANCE (day 2 to day 5).

After being trained to eat from the food bowl in the two-compartment cage during two daily 10 min. sessions on two successive days, pigs were punished with an electric shock on the fifth session as soon as they began to eat from the bowl. The electric shock (25 mA and 5 sec. duration) was given between an electrode fastened on the pig's back by means of a nylon strap and the metal floor of the cage. The pig was removed from the experimental cage 1 min. after the delivery of the electric shock. If the pig did not eat within 2 min. after the beginning of the experimental session, the shock was delivered independently of its behaviour and the session ended as usual. The next day, each subject was reintroduced into the experimental cage with food available and the latencies of approach and feeding responses were recorded as well as the amount of food eaten. A score of 600 sec. was given to subjects which did not display the approach response during the 10 min. test session.

4) CONTINUOUS AVOIDANCE (days 8-11).

Pigs were submitted to 4 training sessions in the two-compartment cage according to a continuous avoidance procedure with a shock-shock interval of 10 sec. and a response-shock interval of 30 sec. (Mormède and Dantzer, 1977). Number of responses emitted and number of shocks received were recorded during each daily 20 min. session.

Statistical analysis.

Number and/or latency of responses were compared among experimental groups by analysis of variance, with experimental group and sex as main factors. Differences between means were further analyzed by means of the Newman-Keuls test (Lellouch et Lazar, 1974).

Results.

1. EXPLORATORY BEHAVIOUR

The mean number of responses per 2 min. intervals was submitted to a 3-way analysis of variance (10 periods x 3 groups x 2 sexes). It was higher between the 2nd and the 8th min. and lower at the very beginning and at the end of the experimental session (F=4.57, d.f.=9,420, P<0.001) [Fig. 1]. Pietrain negative pigs were more active than either Pietrain positive or Large White pigs (1.95 vs. 1.56 and 1.66 responses respectively, F=3.33; d.f.=2,420; P<0.05); however the only significant difference by the Newmann-Keuls method was between the two extremes (P<0.05). Castrated males did not differ from females and first and second order interactions were not significant.

2. LATENT LEARNING

The results of the latent learning test are summarized in Table 1. The latency of the approach and the feeding responses differed according to the experimental group: Pietrain positive and Pietrain negative pigs had a better score than LW pigs, but did not differ from each other, except on the latency of the approach response.

3. PASSIVE AVOIDANCE

The results of the passive avoidance test are summarized in Table 2. Only the data from pigs which had learnt to feed within 120 sec. after their introduction into the experimental cage were taken into account. The experimental groups did not differ in their performance on the last session before the presentation of the electric shock (F=0.91; d.f.=5,36). The punishment of the feeding response resulted in an increased latency to both approaching the food bowl and feeding in the next session. However the experimental groups did not differ in...
Table 1.—Latent learning; latency of approach response and feeding response, according to the experimental group.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Number of subjects</th>
<th>Approach response</th>
<th>Feeding response</th>
</tr>
</thead>
<tbody>
<tr>
<td>P+</td>
<td>16</td>
<td>20 (6-45)</td>
<td>76 (11-304)</td>
</tr>
<tr>
<td>P—</td>
<td>16</td>
<td>65 (6-199)</td>
<td>125 (11-370)</td>
</tr>
<tr>
<td>LW</td>
<td>16</td>
<td>126 (4-600)</td>
<td>188 (9-600)</td>
</tr>
</tbody>
</table>

a) Median latency, in seconds. In brackets is the range of variation.
b) P+/P—: P<0.02; P+/LW: P=0.002 by the Mann-Whitney U test.
c) P+/LW: P=0.10; P—/LW: P<0.10 by the Mann-Whitney U test.

Table 2.—Passive avoidance: Mean of the approach and feeding responses during the last session before conditioning and during the test session.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Number of subjects</th>
<th>Before conditioning</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Approach *</td>
<td>Feeding *</td>
</tr>
<tr>
<td>P+ males</td>
<td>8</td>
<td>12±5.9</td>
<td>20±7.2</td>
</tr>
<tr>
<td>P— females</td>
<td>7</td>
<td>9±2.2</td>
<td>10±3.0</td>
</tr>
<tr>
<td>P— males</td>
<td>7</td>
<td>10±6.6</td>
<td>19±6.8</td>
</tr>
<tr>
<td>P— females</td>
<td>7</td>
<td>4±1.8</td>
<td>10±2.7</td>
</tr>
<tr>
<td>LW males</td>
<td>8</td>
<td>19±7.2</td>
<td>24±6.9</td>
</tr>
<tr>
<td>LW females</td>
<td>5</td>
<td>23±14.3</td>
<td>31±15.3</td>
</tr>
</tbody>
</table>

a) Latency in seconds. Means are ± S.E.M.

Table 3.—Continuous avoidance: Results of 3-way analysis of variance on the number of responses emitted, the number of shocks received and the number of inter-response intervals.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Responses *</th>
<th>Shocks *</th>
<th>Inter-response * intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>strain</td>
<td>2,168</td>
<td>12.54 c</td>
<td>14.23 c</td>
<td>12.27 c</td>
</tr>
<tr>
<td>sex</td>
<td>1,168</td>
<td>6.57 b</td>
<td>5.36 b</td>
<td>5.58 b</td>
</tr>
<tr>
<td>session</td>
<td>3,168</td>
<td>7.83 c</td>
<td>7.16 c</td>
<td>21.30 c</td>
</tr>
<tr>
<td>strain×sex</td>
<td>2,168</td>
<td>0.33</td>
<td>0.17</td>
<td>1.18</td>
</tr>
<tr>
<td>strain×session</td>
<td>6,168</td>
<td>0.48</td>
<td>0.56</td>
<td>1.18</td>
</tr>
<tr>
<td>sex×session</td>
<td>3,168</td>
<td>0.36</td>
<td>0.39</td>
<td>0.38</td>
</tr>
<tr>
<td>strain×sex×session</td>
<td>6,168</td>
<td>0.20</td>
<td>0.43</td>
<td>0.22</td>
</tr>
</tbody>
</table>

a) F value. b) P<0.05. c) P<0.01.
their performance on the day of test (approach latency: $F=0.13$, d.f.$=5,36$; feeding latency: $F=0.37$, d.f.$=5,36$).

4. CONTINUOUS AVOIDANCE

Three-way analysis of variance (3 groups $\times$ 2 sexes$\times$4 sessions) on the number of shocks received, the number of responses emitted and the number of inter-response intervals$^1$ revealed that the three main factors were all significant, unlike the interactions (Table 3): Pietrain positive pigs and Pietrain negative pigs had a more efficient performance than LW pigs; they emitted significantly more responses and received significantly less shocks (Fig. 2). Pietrain positive pigs were slightly superior to Pietrain negative pigs, but the difference did not reach significance. On average females performed better than castrated males. In every experimental group, the performance improved from session 1 to session 4, indicating that learning was taking place.

1. Responses can be emitted following either a shock or another response: only in this last case is an inter-response interval recorded.

Discussion.

The discussion will first focus on the significance of the different behavioural measures used before attempting to put together in an unitary interpretation the results obtained.

Exploratory behaviour is a complex set of behavioural attitudes including ambulatory and non ambulatory (e.g. sniffing, rooting...) patterns, occurring when an animal is introduced into a new environment. Experimental psychologists usually limit themselves to measurement of the locomotor activity displayed in the new environment (e.g. for rats the number of squares crossed in an open-field) and/or vegetative signs such as the number of fecal boluses (Archer, 1973). Most of the results are interpreted within a two-process model in which the subject’s behaviour is assumed to be a reflection of the fear evoked by the novel environment on one hand and the tendency to explore it on the other hand. However there is no unique relationship between locomotor activity and fear since for instance the subjects can attempt to escape the experimental situation by fast running, which will manifest itself by an increase of activity. In pigs, the locomotor activity displayed in the two-compartment cage sometimes is accompanied by some evident distress signs such as escape attempts and vocalizations (Fraser, 1974; Dantzer, 1977). The higher activity displayed by Pietrain negative pigs can therefore be taken as the consequence of either a lower or an higher emotionality. More important is the observation that the gradual temporal decline of activity within the exploratory session was the same for all experimental groups (no interaction between strain or sex on one hand and time on the other hand). This would suggest that the environment lost its novelty at the same rate whatever the strain. However this measure may be still contaminated by activity factors and exploration itself cannot be directly assessed by this only test.

The latent learning procedure allows to differentiate between locomotor factors and true exploratory effects: an animal introduced into a new environment collects available information when moving. On the test day this animal is exposed to a complex
of stimuli to which it has already been exposed and to a single, novel stimulus, the food bowl. It is expected that the more the animal has explored the environment on the first day, the faster he will get around to approach the food bowl and to eat. It would therefore appear that the Pietrain positive pigs have taken the same profit of their stay in the two-compartment cage on the first day than Pietrain negative pigs, in spite of the higher activity displayed by this last group. Large White pigs behaved as if they had little prior experience of the experimental situation. However this reasoning assumes that a strange object, the food bowl, in a familiar place should evoke approach and investigation in all experimental groups (Barnett and Cowan, 1976). This is not necessarily the case for both strains, and an alternative interpretation would be that LW pigs tend to avoid new objects even in a familiar place ("neophobia"). As a matter of fact, casual observation of LW pigs revealed that, although they oriented towards the food bowl, they looked very reluctant to approaching it.

In the passive avoidance situation, pigs are first trained to feed from the bowl in the two-compartment cage. They are then given an electric shock as soon as they begin to eat. Animals trained in this way show on the test session obvious signs of conflict between approach and avoidance tendencies due respectively to hunger and fear of the shock and evidenced by an increase of the approach and feeding latencies compared with control sessions. The experimental groups do not differ in their performance, which means either that they display the same level of fear or that fear induces the same level of response suppression in the experimental situation.

In the continuous avoidance procedure, animals are shocked at 10 sec. intervals unless they emit the appropriate response, i.e. shuttling from one compartment to the other. This response postpones the occurrence of the next shock by 30 sec. The subjects must therefore keep up a high enough rate of responding to avoid the scheduled shocks. Two-factor theory (Mowrer, 1947; Miller, 1948) has suggested that avoidance responses are learned because they are rewarded by a reduction of fear conditioned to some internal stimuli related to the passage of time. There have been however several objections to this theory, mainly based on the demonstration that animals are able to detect a reduction in the frequency of occurrence of electric shocks, in the absence of any other signalling stimulus (e.g. Herrnstein, 1969). Other theories have emphasized the fact that the electric shock by itself elicits a set of innately determined defensive reaction patterns or "species-specific defense reactions" (Bolles, 1970): if one of these coincides with the requirements of the experimental situation, the probability of emission of this response will increase, largely because the remaining set of defense reactions will be punished. According to the Mowrer-Miller theory, experimental groups should differ by a factor of fear while, in the Bolles theory, avoidance responses of LW pigs are disrupted because of the intrusion of defense reactions to the electric shock which are incompatible with the performance of the shuttling response (e.g. freezing responses or erratic escape attempts). Sex differences in avoidance behaviour have already been noted in rodents, females tending to be superior to males. These differences do not appear to be due to the presence of the sex hormones, at least outside an early post-natal critical period (Gray, 1971).

It is apparent from the preceding discussion that the results of a single behavioural test do not give an unique answer, but that different tests have to be combined in order to find the clue. Emotionality neophobia, ability to suppress responding, fear and defense reactions to aversive events have successively been advocated to account for the results observed within each procedure. If the experimental groups differ by a factor of fear, they should display differential responding not only in active avoidance but also in passive avoidance conditioning. The same would be true with learning ability: if Pietrain pigs associate better the consequences of their responses with a reduction in the frequency of shocks, they should also learn better the association between punishment and eating response in the passive avoidance situation, i.e. they should exhibit a greater response suppression. The comparison of the results of the passive avoidance test with those of the continuous
avoidance procedure therefore allows to set aside fear or learning ability as an intervening variable. It would appear that the different strains of pigs have the same ability to suppress their responding when confronted with aversive events but are not able to react positively to aversive events to the same extent. As a matter of fact, some authors have put forward the idea that purposive or goal-directed behaviour is controlled by antagonistic mechanisms including a behavioural facilitation system (reward system) and a behavioural inhibition system (punishment system) (Gray, 1971; Wise et al., 1973; Laborit, 1974). According to this line of reasoning, the performance deficit of LW pigs in both the latent learning procedure and the continuous avoidance situation may be ascribed to a lower ability to initiate active responding when confronted with aversive events (novel stimuli or electric shock). In Pietrain pigs like in LW pigs, the behavioural inhibition system dominates when subjects are exposed to punishing stimuli. However in the former strain the influence of the punishments system is counterbalanced by the operation of the behavioural facilitation system in situations requiring active approach or defense reactions (latent learning procedure and continuous avoidance sessions). The anatomical localization and the neurochemical basis of these systems are still debated (Gray, 1971; Wise et al., 1973; Laborit, 1974) but it is generally agreed that the reward system involves noradrenergic neurotransmission and the punishment system serotonergic and/or cholinergic synapses. It would therefore appear that LW pigs are suffering from a deficit in central noradrenergic activity and it could be predicted that their performance should be improved by such drugs as scopalamine or amphetamine. Since Pietrain positive pigs tend to exhibit better performance than Pietrain negative pigs, they should also be characterized by a somewhat higher central noradrenergic activity. Interestingly such pigs have been described as having excess sympathetic activity, based mainly on clinical evidence and protection afforded against MHS by \(\alpha\)-adrenergic blocking agents (Lister et al., 1976; Williams, 1976).

Taken as a whole, the results of the present experiments indicate that differences between strains are more pronounced than differences eventually related to susceptibility to MHS. Pietrain pigs would appear to behave in a more positive way in response to aversive events than LW pigs, which would suggest that the former should adapt more easily to new surroundings. The same would be true although to a lesser extent when Pietrain positive pigs are compared to Pietrain negative pigs. The question of the physiological mechanisms accounting for these differences can only be answered by further research.

Accepted for publication, June 5th 1978.

Acknowledgements.

This study has been made possible thanks to the help of the Animal Genetics Department of I.N.R.A. which provided the experimental subjects and screened them for MHS susceptibility. Many thanks are due to the skilful technical assistance of Mrs. R.M. Bluthé and Mr. M. Caussette. This research was supported by funds from I.N.R.A.-Recherches Vétérinaires and a grant from the Ministry of Agriculture (DGRST n° 787-0680) (as part of a joint research project with Laboratoire de Physiologie, Ecole Nationale Vétérinaire de Toulouse, Prof. Y. Ruckebusch). Mr. A. Parise participated to the experiments as part of his research training program.

2. These experiments do not, and cannot, provide any explanation of the mechanisms involved in the malignant hyperthermia syndrome. They are only ways of testing overt behavioural reactions to arbitrarily selected aversive situations. However, given the observed trend for Pietrain positive pigs to display better performance than Pietrain negative pigs, it would appear that malignant hyperthermia sensitivity, at least in Pietrain pigs, is an undesirable trait only because of its lethal potential: halothane positive subjects do not suffer any unfavourable consequence from their pathological condition as long as the syndrome itself is not triggered by the environmental events. In our experimental conditions, the stress conditions were sometimes very severe but habituation to daily handling and gradual progression in the severity of the aversive procedures were sufficient to ensure protection against MHS.
Summary

Behavioural responses were compared in Pietrain pigs and in Large White pigs differing by their susceptibility to the malignant hyperthermia syndrome induced by halothane.

Non reactive Pietrain pigs (P―) displayed a higher level of locomotor activity in a new environment than either reactive Pietrain pigs (P+) or Large White pigs. Pietrain pigs performed better in a latent learning procedure (latency of approach or feeding response, after exploration of the new environment) and in a continuous avoidance task run in a two-compartment cage; differences between P+ and P― did not reach significance, but P+ pigs tended to be superior to P― animals. The three groups did not differ on a measure of passive avoidance.

These results suggest that Large White pigs are characterized by a lower ability to initiate active responding when confronted with aversive events. The same would be true when Pietrain positive pigs are compared with Pietrain negative pigs, although to a lesser extent.

References


