Memory in piglets following gas exposure

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Abstract

Euthanasia in piglets is necessary on farms. Although the Blunt Force Trauma method is effective when it is well performed, it can be psychologically difficult for the person who is performing it. Methods of euthanasia are using carbon dioxide, which is effective but aversive for the piglets. Another method using a mixtures of nitrous oxide could be a good alternative, regarding its anaesthetic effects and less aversiveness. However, if there is a technical issue and the procedure fails, it is unknown whether the piglets would remember the gas chamber if the method is used again. If they do remember, they would experience stress during the second exposure, this would threaten piglets’ welfare. This project seeks to assess the memory in piglets under three different gas treatments, to determine the least aversive method for euthanasia in piglets. The data showed that in all treatments, piglets exhibited escape behaviour which could be interpreted as resulting from memory and aversion to the gas chamber. Furthermore, piglets receiving the nitrous oxide treatment exhibited behaviours that could be interpreted as resulting from its anaesthetic effects. The behaviour they exhibited could also be interpreted as demonstrating less aversiveness than the carbon dioxide treatment. However, more studies are needed.

Key words: memory, piglets, behaviour, nitrous oxide, carbon dioxide, aversion
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1. INTRODUCTION

The use of gas as an alternative method for euthanasia in piglets is still under study, mainly from an animal welfare point of view, because the methods which use gas have been found to be aversive to varying degrees for most gases. However, research to date has shown that nitrous oxide seems to be a good option. But every process that uses gas could fail to euthanize a piglet successfully. If that happens and the piglets have to endure the process and associated apparatus again, probably they will suffer additional stress before they are finally euthanized. In order to assess the extent of the stress they will suffer, it is necessary to assess if the piglets have memory of the failed process or not. The main alternative to the use of gas which is also the traditional and common process is the Blunt Force Trauma method, which shows an immediate death, provided the process is well performed.

1.1. Background - Euthanasia in pig industry

Euthanasia in piglets is a necessary practice on farm, from an animal welfare point of view. This is because in a livestock industry, there are always injured, ill or weak animals. The pig industry is no exception. Leaving suffering animals alive is not a humane or ethical practice (American Association of Swine et al., 2001). In response to animal welfare concerns, the aim of the euthanasia is to kill the animals with the least stress and aversive method, quick loss of consciousness and a quick death (American Association of Swine et al., 2001, Baumans et al., 1998).

The traditional and recommended methods for euthanasia are the Blunt force Trauma process (Whiting et al., 2011), electrocution (Vansickle, 2012), anesthesia overdose or injection of pentobarical (Whiting et al., 2011, Baumans et al., 1998), carbon monoxide [but it can be hazardous for human manipulation] (Lambooy and Spanjaard, 1980), and carbon dioxide (Sadler et al., 2013a, Sadler et al., 2014, Rault et al., 2013b, American Association of Swine et al., 2001, Baumans et al., 1998). These methods have been shown to be effective for euthanasia in piglets, but they are limited because of their initial cost for the farmers, or the additional or complex training that they require, or they have been shown to be aversive for the piglets.

The most commonly used method is the Blunt Force Trauma process, because it is economic, and there is no need for difficult training (Whiting et al., 2011). But the disadvantages of this method are the anesethia and the potential negative psychological effects experienced by the person who is performing the procedure (American Association of Swine et al., 2001).

In response of these disadvantages of the Blunt Force Trauma approach, other methods have been tested, such us non-penetrating captive bolt (Casey-Trott et al., 2013), and hypobaric hypoxia (Buzzard, 2012). These methods are very effective and less aversive, but they require complex training and extra equipment for doing the process of euthanasia. However, a number of approaches using various gases have also been researched. They seem to be more effective and act more quickly. Key examples include argon (Fiedler et al., 2016), carbon dioxide (Baumans et al., 1998) and nitrous oxide (Rault et al., 2015, Rault et al., 2013b). Although have been shown to be aversive for piglets in some circumstances (Sadler et al., 2013a), the use of nitrous oxide has been shown to be less aversive because of its anesthetic effects (Rault et al., 2013b).
1.2. Justification

Although with good manufacture practices and effective procedures, the manipulation of gas or equipment always involves a possibility of failing. It is a complex practice and needs good training and knowledge of the relevant manual and procedures including the manipulation of pressures inside a gas chamber, flow rate of wash in and wash out of gases, concentrations and other technical considerations (AVMA, 2013). In fact, manipulation of some gases can be dangerous for humans, mainly while controlling the flow rates, regulating the levels of concentration that the gas needs to achieve, and expulsion of the gas after the process. This is particularly the case with carbon monoxide, which is highly toxic, hazardous and difficult to detect when humans are exposed to it while it is being manipulated (AVMA, 2013), or if there is a gas leak. That is one of the reasons why the use of carbon monoxide is not recommended (Makowska and Weary, 2009). In contrast, the manipulation of other gases has been shown to be less risky, because of the detectable nature of the gas if there is a leak (Lafortune et al., 2013). This has led to recommendations to use other gases rather than carbon monoxide (American Association of Swine et al., 2001).

Besides human risk resulting from leaking gas, other technical problems may occur which cause processes that use gas to fail. For example, the concentration of the gas might not reach the required level inside the box (AVMA, 2013). Where the process fails for these or other reasons, piglets may survive, and the process may have to be repeated in order to euthanize them successfully.

The proper handling of animals and their welfare is compromised if the process of euthanasia fails. If the process fails, it would need to be repeated. This would mean that the animals have to be manipulated again, they could have to be placed inside the chamber for a second time and they could spend more time inside it. It could mean more stress that the normal stress that they are experiencing because of the separation from the sow (Hvozdik and Dezorzova, 1998).

In addition, it is currently unknown what the consequences that this repetition of the process can have on the behaviour of the piglets and how it could affect their welfare before euthanasia is finally successful.

Moreover, it is unknown if the animals would be able to remember their previous experience, and if this would cause them additional trauma if they were subjected to the process again. The main reason for doing this project is to investigate these issues that are critically important from an animal welfare point of view.

1.3. Hypothesis

The hypothesis of this project was, “piglets exposed to a chamber filled with one of three gas treatments (carbon dioxide, nitrous oxide or medical air) would be able to remember the experience if they have to be placed in the chamber again”.

1.4. Objective

The objective of this project was “to evaluate the behaviour and memory in piglets after being exposed to one of three different gases treatments when they have to be placed in the gas chamber again, and seek the least aversive method for euthanasia in piglets”.

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1.5. **Specific objectives**
Assess the memory in piglets through the observation of their behaviour response and vocalizations.
Determine which method is the least aversive for euthanasia in piglets following gas effects.

1.6. **Questions addressed by the project**
In order to test and assess the hypothesis, this project formulated these questions:
Do the piglets have spatial memory after being exposed to one of three different kind of gasses?
And if the piglets have spatial memory, which method shows to be the least aversive?

2. **LITERATURE REVIEW**

2.1. **Behaviour in piglets**
A key task of this project is to use observations of the behaviour of piglets in order to assess whether they remember that they have been previously subjected to a failed attempt to euthanize them using gas. As part of this task, behavioural observations will also be used to assess how aversive piglets find the second treatment. These are not simple tasks. As we shall see in this literature review, behaviour is very complex to assess, because of the many factors involved and the diverse stimuli that affect behavioural responses (Martin and Bateson, 2007). However, various researchers have used the expression of particular behaviours and vocalizations as indicators of whether the welfare of particular animals is being looked after appropriately.

Assessing behaviour can be particularly effective where the environment surrounding the animal changes, and where changes in behaviour are more likely to be able to be attributed to the changed environmental circumstances. In these kinds of situations, particular vocalizations may be associated with particular behaviours and with specific changes in the stimuli and environment. For example, if a piglet is suffering and showing behaviours associated with stress (da Silva Cordeiro et al., 2013), the vocalization could be expressed as a long and frequent grunting, squealing and even screaming. In fact, these three categories of vocalization have been tested and studied under a variety of circumstances (Von Borell et al., 2009). The outcome of this kind of research can be extremely useful for the researcher undertaking the kind of project that is the subject of this research. The researcher can use the research to explain the state which is being experienced by a piglet when it is making a particular kind of vocalization. Unfortunately, however, the literature review has disclosed that only a limited amount of research has been undertaken into these kinds of issues in the past.

We will begin our detailed examination of this literature by examining what it has to tell us about the kinds of behaviour that pigs exhibit under normal conditions:
2.1.1. Piglets under normal conditions

Piglets are very social animals under normal conditions and the existence of social behaviours are good indicators that piglets are experiencing positive welfare (Morgan et al., 2014). Examples of social behaviours include that most activities of piglets are done in a group, and that they regularly socialize and communicate by nose contact, physical contact and vocalizations. On the other hand, they are generally organized into hierarchies that start to be established when they are juveniles. In fact, this hierarchy starts when they begin the suckling of milk from the sow, and they chose one of the nipples for the whole lactancy (Fericean et al., 2011). The choosing of nipples can lead to fights between piglets. Normally, the more aggressive piglets can take the best nipple positions, which are the ones located in the middle of the udder, creating in that way the first dominant signals for the hierarchy of the piglets within the litter (Skok et al., 2014). In many cases, the leading piglet would establish the behaviour of the rest of the litter, because the rest of the piglets would tend to imitate and follow the behaviour of the leader (D'Eath, 2005).

Also piglets have very good hearing, sharper than their sight. They communicate with each other using different vocalizations. The normal sounds between them are grunts, squeals and barks. For example, piglets can squeal to the sow when they want to suckle milk (Fericean et al., 2011). These kinds of behaviour show that piglets are very expressive animals.

Piglets are also very curious animals, and they tend to exhibit exploratory behaviour whenever they experience new situations (Fericean et al., 2011). Every new situation can affect the piglets positively or negatively. When they encounter new situations, piglets tend to exhibit behaviours that indicate whether the new situation affects their welfare negatively or positively.

2.1.2. Piglets under positive experiences

Although new situations can be stressful for piglets, they can show positive or normal and natural behaviours in new circumstances, including exploring, playing or socializing. I will deal with each of these behaviours in turn:

- **Exploration**

One of the positive kinds of behaviour that piglets show in new situations is exploratory behaviour. These are very common and natural behaviours for piglets which are very explorative animals (Oliveira-Santos et al., 2016). They undertake exploratory behaviour in order to investigate what is around them. In fact they develop this skill during the first ten days of life (Fericean et al., 2011).

During exploration, piglets show high motoric activity, related to locomotion, when they are in an open field (Von Borell et al., 2009) in fact, when piglets are exploring, this additional locomotion is related to the minimization of aggressiveness and fighting between them (Martin et al., 2015). In these kinds of situations when piglets are exploring new things, they tend to make vocalizations that include long grunting and short squeals. These tend to be associated with a state of excitement associated with exploring new situations (Von Borell et al., 2009).

Although piglets have been shown to exhibit exploratory behaviour, mainly when they encounter new objects, they can also get stressed easily. They can be scared by new sounds or new places, or even by quick movements made by their own partners inside their pens. But after the scary event, they have the tendency to commence exploring again (Martin et al., 2015).
Also, piglets are often showing exploratory behaviour when they root the floors, walls, food, objects, using their nose, because of the association of the odours, textures and tastes. In fact, they are able to recognize four tastes when they are juveniles (Fericean et al., 2011). In fact, when piglets are encountering enriched environments, such as with toys or a different kind of floor, they can show exploratory behaviour which is of longer duration and more intensive. This tends to reduce the abdominal rooting or aggressive behaviour that piglets exhibit. This kind of enriched stimuli can also make the piglets express behaviour that is more independent (Martin et al., 2015).

- **Playing and socializing**

Socializing is generally considered to be a positive sign of the welfare of piglets. If piglets are interacting socially, it tends to suggest that they have good conditions of living. Socializing tends to be more common when the environment presents different forms of enrichment, for example, when toys are added to the place (Martin et al., 2015). In addition, these kinds of enriched environments also tend to make piglets behave normally and to play. This is also the case where piglets have more space. Play is another positive sign of the welfare of piglets (Rauw, 2013).

### 2.1.3. Piglets under negative experiences

Although piglets can face different kinds of stress, they can show stress as aversion when they face new situations in which there are stimuli that can affect them negatively. Examples are isolation, separation from the sow or the litter, weaning, or facing poor environments.

First signs of stress could be the touching or sucking of the abdomen area, when they have partners. Most of the time these can be easily confused with socializing, or exploring. However, studies indicate that these behaviours may be exhibited because they miss their mother (Hvozdik and Dezorzova, 1998). These kinds of behaviour can also be associated with other behaviour involving nose attacks and crushes. These can be confused with normal physical contact, when it is really associated with aggressiveness (Jarvis et al., 2008). Piglets are very sensitive to changes to the usual conditions of living. Another common cause of stress in piglets is isolation (Kanitz et al., 2014).

Other behaviours that piglets express when they are having a negative experience are particular vocalizations. For example, when piglets experience a threat after being separated from their mothers, they tend to make long vocalizations related to the frustration that they feel. Gruntings and squeals can also be related with this kind of event (Von Borell et al., 2009).

In addition, the sucking of objects or places can also be related to stress. But in some cases, these behaviours can be confused with exploration, mainly with rooting. This kind of behaviour could involve the piglet searching for rewards in order to re-comfort itself (Hvozdik and Dezorzova, 1998). These behaviours might be expressed when piglets have suffered a separation from the mother (Jarvis et al., 2008)

- **Panic and distress**

Piglets can experience different situations in different ways. They could be stressed, frustrated, panicked, or distressed. Or they could be contented if the new situation is more positive (Wemelsfelder, 2007). However, some piglets seem to be predisposed to get stressed or panic easily. It seems likely that genetic factors are involved in producing these predispositions.
Common behaviour that indicates a state of panic state could be the absence of socializing behaviour, or the freezing of movement in the piglets, or even defecating or urinating (Reimert et al., 2013). Sometimes these kind of behaviours can be confused with states of curiosity or with states of alertness or states of inactivity (Temple et al., 2011).

Panic behaviour can be a sign of frustration and anxiety disorder, even phobias, for example in dogs (Straus, 2006). However, it could also be related to defensive behaviour against a threat, but it is still showing an excessive fear following a stimulus (Perkins et al., 2011).

When piglets experience a distressing situation, they may make a screaming kind of vocalization. This can also be a response to a fearful or an unfamiliar environment, but then the squealing is likely to be longer and more energetic squealing and screaming (Von Borell et al., 2009). Castration is an example of the highly stressful situations where this kind of vocalization is made (Bates et al., 2014). Piglets can also show screams and high pitched vocalizations as an aversion response (Reimert et al., 2013). However, screaming doesn’t seem to be a good indicator of distress or panic, because in some cases piglets will copy the vocalizations, mainly if the place is unfamiliar (Von Borell et al., 2009).

In conclusion, depending on the stimuli, piglets can show different emotions when they face a new experience. This experience can be a positive one, in which case the piglets will tend to show positive signs, such as playing, barking or tail movements. On the other hand, if the piglets encounter a bad experience, then they would show aversion to an object or a place, exhibiting a tendency to defecate, freeze, urinate, attempt to escape, and/or make high pitched vocalizations (Reimert et al., 2013).

2.1.4. Behaviour under gas exposure

There are many differences between behaviours expressed under different type of gases. The gases that have been shown to be effective for euthanasia are nitrous oxide and carbon dioxide. Nitrous oxide has anaesthetic properties (Schubert et al., 2012) and seems to be less aversive. Carbon dioxide is more commonly used because of its effectiveness, although it seems more aversive. This project sets out to assess these two gases more closely than the literature does currently.

• Behaviours under carbon dioxide

Flailing is one of the characteristic behavioural responses to exposure to carbon dioxide. It involves erratic uncontrolled movements, jumps and the expression of panic (Rault et al., 2015). Piglets are very sensitive to the effects of carbon dioxide. In fact, they can flail within 5 seconds of being exposed to the gas (Rault et al., 2013b). This also demonstrates a high aversion to this gas, producing stress and even distress during the process of euthanasia under this gas (Sadler et al., 2013a) In addition, because piglets show flailing behaviour so quickly, they are not able to react or make attempts to escape, if they had those possible choices (Rault et al., 2013b).

Open mouth breathing, righting response and attempts to escape are other behaviours that are commonly seen when piglets are exposed to this gas. But when the rates of flow are faster, the expression of these behaviours tends to be reduced (Sadler et al., 2013b).

Another behaviour that may be expressed when piglets are exposed to this gas is gasping (Buzzard, 2012). This behaviour could be related to a sudden tightening of the neck and mouth.
muscles with strong inhalation (Rault et al., 2015). However, the behaviour doesn't seem to appear until some time after piglets are exposed to the gas.

Although ataxia tends to arise in a lower proportion in carbon dioxide treatments (Buzzard, 2012, Sadler et al., 2013a), it seems to be related to a loss of stability (Rault et al., 2015).

Meanwhile the heart rate seems to be maintained at similar levels during exposure to the gas. But in some cases, it seems to decrease when a piglet starts flailing (Rault et al., 2013b, Burkholder et al., 2010). Despite this, the mean of the heart rate tends to increase when piglets are stressed during gas exposure (Buzzard, 2012).

**Behaviours under effects of Nitrous oxide**

According to studies, exposure to this gas produces anaesthetic effects (Mawhinney et al., 2012). These effects can be expressed behaviourally as ataxia.

Exposure to nitrous oxide does not seem to produce distress or panic, which would appear to be less aversive for the piglets. Perhaps this is why exposure does not seem to change the heart rate much, although it could increase when the piglets are exposed to a higher concentration (Rault et al., 2015). However, the heart rate can increase when the piglets are presented with stressors prior to the treatment with the gas (Taverne and Randall, 1983). Whether or not vocalizations are made that indicate stress would depend on the extent to which the animal is stressed before and during the experiment (da Silva Cordeiro et al., 2013).

If piglets can remember being exposed to a gas chamber, the memory may cause them to become stressed if they are exposed to a gas chamber again. Whether or not they have such a memory and, if so, whether such a memory would cause them stress during a repeat exposure, is therefore critically important for this project. We will now turn to examining the literature that is relevant to these issues:

**2.2. Memory in animals and pigs**

There is no comprehensive and definitive evidence about memory in pigs. However, some studies have suggested that adult pigs can have similar intelligence to that of three years old human children. They are able to recognize a sound and a name if they are given one, and seem to be able to store images in a long term memory (Oliveira-Santos et al., 2016).

The pig is an animal that has been considered to have a notable long-term memory. Mainly, their memory has being tested in learning and developing tasks (Gieling et al., 2011). Even when they are piglets, it seems that they could be able to learn simple tasks (Rault et al., 2013b), with or without stimuli. In fact, when they are presented with relevant stimuli, adult pigs have been shown to have a memory of objects for at least one hour (Paz et al., 2006).

Memory in pigs seems also be demonstrated by their ability for social recognition which seems to be related to their olfactory capacity (Kristensen et al., 2001). High olfactory capacities seem also to be related to a spatial memory.

Studies into learning and memory in piglets have generally been developed using mazes and conditioning methods. However, most have used piglets older than 8 weeks. In contrast, some studies using piglets have shown that they can have a positive response (Gieling et al., 2011), when there are stimuli involved, such as moving through mazes to obtain water or food as reward or conditioning. Studies have shown that these capacities are related to having a good spatial memory to the extent that they enable an animal to return once it knows where the
reward is located (Hai-feng et al., 2007). However, these approaches and findings have been taken from other experiments with rats or mice, because there are not many studies in piglets (Barnhart et al., 2015). In addition, pre-weaning piglets seem to have shown spatial memory when they are exposed to fear and stressed conditions (Siegford et al., 2008).

### 2.2.1. Spatial memory

In general, the spatial memory of pigs has not being tested. The methods that are most commonly used to test spatial memory in animals are those used in relation to rats and other rodents. For these reasons, studies that develop a proper protocol that is appropriate for use with pigs is required. Although the methods used in other species might be applicable to some extent, animals differ in complex ways, so methods need to be adjusted to reflect the special characteristics of piglets.

Although there are few relevant studies with piglets, they have been shown to have memory, and they seems to be able to recognize their own dam by odour, location and vocal cues (D'Eath, 2005).

One study using adult pigs appeared on the surface to show that they might have a memory that enabled them to recognize a particular place in an open area. However, other interpretations are possible: they might merely have been responding to a particular preference for a particular kind of place (Oliveira-Santos et al., 2016).

In rats, studies seem to demonstrate the existence of a spatial memory by showing that rats spend more time in a novel place, compared with the time they spend in a place that is already know to them (and is presumably remembered by them) (Howland and Cazakoff, 2010).

### 2.2.2. Aversion to the place

It is difficult to understand the aversion behaviour of piglets, particularly given that there are no protocols to follow, and little research about relevant aspects of their behaviour and memory. However, studies in other species have shown that animals tend to explore their environment, and when they have a spatial memory and recognize a particular place that is associated with negative stimuli, they could show aversion to it. Furthermore, one of the most common behaviours that could indicate this aversion is attempts to escape (Barnhart et al., 2015, Singh et al., 2016, Reiss et al., 2014, Xiong et al., 2013).

### 2.2.3. Memory and the gas effects

The learning performance seems to be involved with the effects of the gas and locomotion. They attempt to escape, when they show aversion to a gas (Brajon et al., 2016).

The normal effect of the gas nitrous oxide is anaesthesia (Mawhinney et al., 2012). From a welfare point of view this is positive, because the animals do not suffer and they do not show aversiveness (Rault et al., 2013a). Furthermore, exposure to nitrous oxide can have an effect on memory, at least in some animals. In fact, a study in rats showed a significant deficit of spatial memory and memory retention, when the rats were exposed for 4 hours to nitrous oxide 48 hours prior to the assessment (Xiong et al., 2013).
In the case of carbon dioxide, the negative effects seem to be worse, because it could cause more anxiety and impaired learning and memory. This possibility was tested in rats, and carbon dioxide seemed to be more aversive in juveniles (Kiray et al., 2014).

2.2.4. Other factors affecting memory

Studies show a possible difference between memory in juveniles and adults. In fact, in experiments using recognition of objects to assess memory, adults tended to do better than young animals, because young animals have a greater tendency to engage in exploratory behaviour (Oliveira-Santos et al., 2016).

Piglets may be sensitive to emotional contagion. In fact, piglets have a tendency to follow or copy the behaviour of leaders, in relation to both positive or negative events (Reimert et al., 2013). This can produce a bias when behaviour is being assessed.

Arousal effects and emotional learning can affect the process through which an animal learns to recognize a particular place. Studies into memory in rats have shown that a previous stress can be motivating for recognition of the place (Brajon et al., 2016).

As it has been discussed in this chapter, it is important for the purposes of this study to assess if piglets have spatial memory or not. If they do, it would seem that it is likely that they will find it aversive if they are placed in a gas chamber again, after the first attempt to euthanize them in the chamber has failed for technical reasons. If they would find it aversive, it is important to test different gases to find the least aversive method. Although this issue needs more studies in piglets, studies in some other species have been done. These studies tend to suggest that animals that seem somewhat comparable to pigs do have a spatial memory, and would find re-introduction to a gas chamber aversive. Although these studies do not allow definitive conclusions to be reached about piglets, they do increase the desirability from an animal welfare perspective of investigating these issues in piglets.

This project is a first step in this direction. It seeks to assess the memory and behaviour of piglets under gas effects, in order to determine the least aversive method for euthanasia in piglets using gas, from a welfare point of view.
3. MATERIALS AND METHODS

The project was performed at a large commercial piggery in NSW, Australia, and it had the ethic approval for the manipulation of the piglets. In order to determine if piglets have memory after a gas exposure, piglets were exposed to one of the three gas treatments in a gas chamber on day 1, before being placed back into the gas chamber and exposed to medical air on day 2. The behaviour of piglets in response to exposure to medical air in the gas chamber on day 2 was recorded.

3.1. Animals

There were 72 two week old piglets from a Large White x Landrace crossbreed, from 12 different litters. These piglets were assessed inside a gas chamber and located in pairs (female-male) during the exposure to the gases, in order to minimize the stress caused by the isolation (Kanitz et al., 2014) (Siegford et al., 2008). After exposure to the one of the three different gases, then the piglets were returned back to their pens.

3.2. Gas chamber and equipment

The gas chamber was a retrofitted commercial gas chamber, based in a black plastic box. This box was fitted according to the necessities of the experiment, adding a glass side for the observation, a hole with a globe for rescuing the piglets, if it was needed, as showing in Figure 1. Also it was added an air blowout hole was added for the expulsion of the gas. The dimensions of this box were 82 cm. x 40 cm. x 40 cm.


Figure 1. Commercial gas chamber retrofitted for the experiment.
3.3. Treatments

The piglets were exposed to one of the three different gases. These gases were selected according to previous results (Rault et al., 2015). Each gas represented one of the three treatment.

- Treatment 1 (T1), or Carbon dioxide treatment: in this treatment the gas chamber was filled with 90% Carbon dioxide CO₂ and 10% Medical Air.
- Treatment 2 (T2), or Nitrous oxide treatment: in this treatment the gas chamber was filled with 100% Nitrous oxide N₂O.
- Treatment 3 (T3), or Medical air treatment: in this treatment the gas chamber was filled with 100% Medical Air. (Rault et al., 2015)

The Treatment 3 (medical air) was used as a control to assess the behaviour of the piglets inside the box, without the effects of any gas in normal conditions of handling.

3.4. Experimental design

Before the experiment, (day0), three female and three male piglets were chosen randomly, from each of the 12 litters. The males were marked on the back along their shoulders, while the females were marked in the hindquarter with a stock marker, in order to identify them once they were inside the gas chamber.

From each group of six piglets chosen from each litter, one male and one female were chosen and were designated in pairs for each treatment. Each treatment was identified with a mark using different colours stock markers. In case of Treatment 1, carbon dioxide, they were marked with yellow colour; the Treatment 2, nitrous oxide, they were marked with blue colour; and Treatment 3, medical air, they were marked with green colour.

The experiment was run over in 2 days.

On day 1, the piglet pairs were placed into animal carriers and transported to the gas chamber, this took approximately 5 minutes. The piglet pair where then placed into the gas chamber and exposed to one of the three gases. Piglets in T1 and T2 were exposed to the gas until they showed the signs of the gas exposure and were then rescued from the gas chamber. Piglets in treatment 3 were exposed to the gas chamber for 10 minutes.

- T1, Carbon dioxide treatment, the 12 pairs of piglets were exposed until they showed behaviours indicating effects of the gas such as flailing and squealing. According to previous studies, the usual response to this treatment is around three minutes (Rault et al., 2013b), but they started showing the effects of the gas in less than two minutes. Once a piglet showed distress behaviour they were rescued.
- T2, Nitrous oxide treatment, the 12 pairs of piglets were exposed until they showed the behaviours related with the gas effects of anaesthesia. According to previous studies, piglets can show the signs after 15 minutes, when they had been exposed to 60% N₂O and 30% O₂ (Rault et al., 2015). But they were rescued in around 270 seconds.
- T3, Medical air treatment, the 12 pairs of piglets were exposed to medical air for 10 minutes.
After each rescued, the piglet which showed first the effects of the gas was identified as the focal piglet, and then it was marked with a red spot in the back. Then, a blood sample was taken from each piglet of all of the litters.

On day 2, the piglet pairs from each treatment were placed back in the gas chamber, and all piglets from each of the treatments were exposed to medical air for 5 minutes.

3.5. Data collection

In order to analyse the behaviour of the piglets inside the box, two video cameras were located inside the gas chamber, and another one in front of the glass side of the gas chamber. These cameras recorded the behaviour of the piglets during gas exposure in gas chamber.

Also, to analyse the vocalizations of the piglet pair, a researcher counted the number of squeals with a hand click counter.

3.6. Data analysis

There were two types of data to analyse, the behaviour and vocalizations.

3.6.1. Behaviour analysis

Behaviour of the piglets were recorded using small digital video cameras. This behaviour were defined and standardized in an ethogram, as in Table 1. Where postures, behaviours, events and gas effects were identified and defined. In addition, behavioural states are considered mutually exclusive within categories, interruptions shorter than 3 seconds were considered in the same bout of behaviour, and finally, the recording of the videos were analysed once the two sides of the box were totally closed.

<table>
<thead>
<tr>
<th>Category</th>
<th>Behaviour</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stand</td>
<td></td>
<td>Up on 4 legs</td>
</tr>
<tr>
<td>Lie</td>
<td></td>
<td>Lie down on belly or side in contact with floor</td>
</tr>
<tr>
<td>Sit</td>
<td></td>
<td>One or two hind legs folded underneath the body and supporting weight on the two front legs. “Sit like a dog”</td>
</tr>
<tr>
<td>Lay over</td>
<td></td>
<td>Lay over companion pig</td>
</tr>
<tr>
<td>Kneeling</td>
<td></td>
<td>One or two front legs folded underneath the body, hind legs straight.</td>
</tr>
<tr>
<td>Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locomotion</td>
<td></td>
<td>Any movement moving two or more feet, walk or run.</td>
</tr>
<tr>
<td>Inactive</td>
<td></td>
<td>Immobile, not moving head, body or legs; head may be up or down, but always stationary.</td>
</tr>
<tr>
<td>Root floor</td>
<td></td>
<td>Snout in contact with floor. Touch, sniff, rub, chew.</td>
</tr>
<tr>
<td>Root walls</td>
<td></td>
<td>Snout in contact with walls. Touch, sniff, rub, chew.</td>
</tr>
<tr>
<td>Social contact</td>
<td></td>
<td>Interact by snout contact to any part of the body of companion pig in a calm manner.</td>
</tr>
</tbody>
</table>

Table 1. Ethogram for assessing Behaviour in piglets following gas exposure.
Alert: Immobile, freezing response, with head and ears up and oriented in the same direction.
Eliminate*: Defecation or urination.
Escape attempts: Rear on its hind legs, jump (all limbs lose contact with the floor) or scratch with front legs against the walls or the floor, or scrambling over companion pig.
Other: Any other behaviour not listed above. Write a comment.
Non-visible: The behaviour of the focal pig cannot be determined confidently.
Footage issue: The focal pig is not sufficiently or clearly visible to determine behaviour confidently.
Squeal*: High-pitch vocalization; Extended sound (0.5 to 2 s) of both high amplitude and high frequency with an open mouth.
Grunt*: Low-pitch vocalization; Sound of low to medium amplitude with a closed mouth.

<table>
<thead>
<tr>
<th>Gas effect</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck stretch</td>
<td>Extend its neck as much as possible, head up.</td>
</tr>
<tr>
<td>Ataxic*</td>
<td>Lack of muscles coordination in basic movements, lost of balance on one of more feet.</td>
</tr>
<tr>
<td>Heavy breathing</td>
<td>Forceful and quick repetition of flank movements, mouth closed.</td>
</tr>
<tr>
<td>Open mouth breathing</td>
<td>Jaw held open with the top lip pulled back, exposing gums or teeth</td>
</tr>
<tr>
<td>Righting response*</td>
<td>Unsuccessful effort to right up on 4 legs.</td>
</tr>
</tbody>
</table>

*Behaviours recorded as events due to their brief nature, rather than as states.
Adapted from (Sadler et al., 2014, Blumetto Velazco et al., 2013, Rault et al., 2013b).

In addition, all of the postures, behaviours and events were measured by duration and frequency. The duration was measured in units of seconds for the behaviours. And the frequency were measures in times that the behaviours appeared during the whole experiment (Martin and Bateson, 2007).

Also, the video recordings were analysed, based in the criteria of the ethogram using Observer xt software (version 5.0 Noldus).

### 3.6.2. Vocalizations analysis

Squeals and grunting were recorded from each of the 36 pairs from the experiment, squeals and the grunting.

### 3.6.3. Statistical Analysis

All of the data obtained during the experiment, behaviour and vocalizations, both were analysed in Minitab for the statistical analysed using ANOVA with a p value of <0.05 was considered significant for the statistical comparisons, then through comparisons with Tukey test 95% of confidence.
4. RESULTS

Among the two days it was found the following results:

4.1. Day 1

During day 1 the piglets were exposed to one of the three different gases inside the gas chamber as discussed in Material and Methods, the results obtained from day 1 are related to the behaviours of the gas effects, indicated in the ethogram (Table 1) from Materials and methods.

The Tukey test 95% confidence showed that the duration of each treatment is significantly different, then the duration of each treatment was 102.6004 ± 14.103 seconds for the treatment carbon dioxide, 245.0025 ± 31.34 seconds for the treatment of nitrous oxide and ten minutes for the treatment of medical air.

The duration of heavy breathing, neck stretch, frequency of neck stretch and ataxic behaviours were significantly different, according to ANOVA p value of <0.05. It is shown in the Figure 3 from Material and methods. As expected, the medical air treatment didn’t show any of these gas effects behaviours.

Treatments that don’t share a letter means they are significantly different, according to Tukey 95%.

Source: Rault, J.-L. 2016 Data analysed in Minitab through ANOVA p value of <0.05.
Figure 2. Comparisons between behaviours under gas effects and treatments. a) Duration in seconds of heavy breathing behaviour, b) Duration in seconds of Neck stretch behaviour, c) Frequency in times of Neck stretch behaviour, d) Frequency in times of Ataxic behaviour.

4.2. Day 2

The data analysed with Minitab by model ANOVA p value of <0.05 showed there is no significant different in the duration and frequency of sit, layover, rooting floors, attempts to escape, slip, nose contact and physical contact, according to Tukey 95%. Also there were no significant differences, between treatments and sex, focal and time of the experimentation.

Source: Rault, J.-L. 2016 Data analysed in Minitab through ANOVA p value of <0.05.

Figure 3. Comparisons between behaviours following gas effects and treatments. a) Duration in seconds of attempts to escape behaviour, b) Frequency in times of Attempts to escape behaviour.

Significant differences between treatment medical air and carbon dioxide in frequency of nonvisible behaviour, inactive (Figure 4 and 5), locomotion (Figure 5) and root walls (Figure 6). Also these significant differences were found in the duration of inactive and nonvisible (figure5). In the case of nitrous oxide treatment showed to have non significant differences between T1 and T3 in the duration of inactivity (Figure 4), and in frequency of rooting walls (Figure 6).
Treatments that don’t share a letter means they are significantly different, according to Tukey 95%.
Source: Rault, J.-L. 2016 Data analysed in Minitab through ANOVA p value of <0.05.

Figure 4. Comparisons between behaviours following gas effects and treatments. a) Duration in seconds and Frequency in times of non-visible of behaviour, b) Duration in seconds and frequency in times of inactive behaviour.
Treatments that don’t share a letter means they are significantly different, according to Tukey 95%.
Source: Rault, J.-L. 2016 Data analysed in Minitab through ANOVA p value of <0.05.

Figure 5. Comparisons between behaviours following gas effects and treatments. a) Frequency in Times of Locomotion activity, b) Duration in seconds of Stand posture,

Although there wasn’t a significant difference between treatments in duration of rooting walls, a significant difference was found in the frequency of rooting walls. This frequency shows to be higher in the treatment carbon dioxide and it doesn’t have significant difference with the nitrous oxide, as well, but a significant difference with medical air(Figure 6). Probably it can be related with other factors, apart from rooting walls itself.

Treatments that don’t share a letter means they are significantly different, according to Tukey 95%.
Source: Rault, J.-L. 2016 Data analysed in Minitab through ANOVA p value of <0.05.

Figure 6. Comparisons between behaviours following gas effects and treatments. Frequency in times of Rooting walls behaviour.
Finally, the analysis from ANOVA p value of <0.05 and comparisons between treatments and sex, litters, focal, didn't have any significant difference in vocalizations of squeals.
5. DISCUSSION

The key issue is whether the piglets behaved in ways that suggest they have spatial memory of being previously exposed to a gas. The data that are most relevant to this issue are those from Day 2. The data analysed from the second day are relevant to whether the piglets’ memory could have been affected by the gas, when the piglets were exposed to it during the previous day (Day 1). The experimental data collected and analysed in this project can be interpreted as supporting the hypothesis that the piglets could have spatial memory after being exposed to a chamber filled with one of three gas treatment (carbon dioxide, nitrous oxide or medical air). It could also be interpreted as supporting the view that carbon dioxide is the more aversive method. However, as I will discuss in more detail below, other interpretations are also possible, and further experimentation would be necessary to arrive at a definitive position on these issues. We will begin this broader discussion by analysing the data from each of the two days of the experiment.

5.1. Gas effect on Day 1

During day one, the lengths of the treatments showed different durations, as was expected. This is because each piglet was rescued as soon as it showed the signals of the effect of the gas. These signals were flailing in the case of carbon dioxide treatment and anaesthetic effects in the case of the nitrous oxide. Furthermore, although both piglets were rescued in the same treatment, each piglet in a treatment did not have the same duration of exposure to the gas. This was because in each treatment, the first of the piglets to show the effects of the gas on its behaviour (the focal piglet), was rescued first.

On Day 1, piglets showed significant different in their behaviours (ANOVA p value of <0.050). However, this can be explained by the different durations of exposure to the gas and by the different effects of the different gases, as illustrated in Figure 1 from Materials and methods. For example, ataxic behaviour is higher in the nitrous oxide treatment, because it is a common behavioural consequence of the anaesthesia effect of this gas.

However, as mentioned above, it is the behaviours exhibited by the piglets on Day 2 that is most relevant to addressing the key issues dealt with by this project, including the effects of the different gases. We now turn to an analysis of the data from day 2:

5.2. Day 2

5.2.1. Memory in piglets

In all the treatments undertaken on Day 2, piglets exhibited one of the most important behaviours from this study, which are attempts to escape. This behaviour is important because, according to the literature review, it could be an indicator of the spatial memory in piglets and their possible aversion for the gas chamber (Singh, et al 2015, Reiss, et al. 2014, Oliveira-Santos, et al. 2016). Although attempts to escape did not show a significant difference across treatments, they were higher in the Carbon Dioxide treatment, as outlined in Figure 3 from Materials and Methods.

In addition, all of the treatments showed non-significant differences between nose and physical contact, which is consistent with the piglets possessing social memories that enable them to recognize their partners (this kind behaviour could be more common in juveniles). Although socializing could be considered as part of exploration and normal behaviour, it could also be considered as part of a demonstration of memory (Kristensen et al., 2001).
Stress is a factor which could enhance memory capacities, particularly in the kinds of conditions that the piglets experienced in these experiments. Studies have revealed that piglets might have spatial memory when there are exposed to fear and stressed conditions (Siegford et al., 2008). We examine the relevance of the data to this factor in the next section:

5.2.2. Stress, distress and Panic in piglets

Although most of the behaviours show non-significant differences between treatments, there are some differences in certain behaviours.

In the treatment with carbon dioxide gas, the duration of ‘inactivity’ was lower, although the difference was not significant (ANOVA p value of <0.05). But the carbon dioxide treatment did show significant differences in other comparisons of behaviours between treatments. These included differences in the frequency of inactivity, locomotion, nonvisible and rooting walls. These could be related to higher movements and change of activity that could be related to distress and a possible aversion to the gas chamber. In fact, other research has suggested that high locomotion can indicate exploration and stress (Von Borell et al., 2009).

In addition, the higher ‘rooting walls’ in this treatment could be related to a possible confusion between suckling walls or the corner of the glass (Fericean et al., 2011). This could be related to stress, and could also be related to pressing the walls, which could be associated with attempts to escape. But this kind of behaviour could also result from other states.

Lower durations of inactivity could also be confused with expressions of other states, and could be interpreted differently. For example, in a stressful situation, inactivity could result from a freezing movement, which could be related to a panic state.

However, all of these results and possible behaviours are also showing the possibility that the piglets that received the carbon dioxide treatment did not like the gas chamber and experienced negative feelings about it, as a result of their exposure to the gas chamber the previous day.

On the other hand, the treatment that used medical air showed a higher duration of inactivity (ANOVA p value of <0.05), as well as a lower frequency of locomotion, inactivity, nonvisible and rooting walls. This could be related to less activity and movements, which in turn could result from less stress inside the gas chamber under normal conditions.

Nevertheless, the behaviours of piglets that received the medical air treatment still seems to express aversion to the gas chamber, even though at a lower frequency. This apparent stress could possibly be related to all of the manipulation of the animals. This seems plausible given that piglets are very sensitive animals which can get stressed easily. Every small change can affect them, and the handling and the transportation, the separation from the mother and the enclosure inside a box, is likely to be a very stressful event for them. So it may be that the aversion they show to the gas chamber through attempting to escape, could be due to the aversion they experience for the whole handling and manipulation process. The data from this project and the findings in the literature are insufficient to definitively evaluate these possibilities.

Finally, the nitrous oxide treatment showed some non-significant differences to the carbon dioxide treatment in the duration of inactivity and in rooting walls. The duration of inactivity was higher for nitrous oxide and the frequency of rooting walls was lower. Both these differences could be due to the anaesthetic effects of nitrous oxide. Nevertheless, piglets receiving the nitrous oxide treatment had a non-significant higher frequency of inactivity, nonvisible and locomotion than for piglets receiving the medical air treatment, which could all
result from an expression of stress as an aversion to the gas chamber. However, this stress and possible aversion seems to be lower than for the carbon dioxide treatment. It is possible to interpret the frequency of escape attempts made by piglets in the nitrous oxide treatment as due to memory of the experiences of Day 1. However, these attempts are lower in frequency than for the medical air treatment. This could be due to the possibility that their memory of the experience has been impaired by exposure to nitrous oxide (there is evidence that nitrous oxide can impair memory in rats (Xiong et al., 2013)) Or it might be that they remember the experience of the previous day, but don’t feel high aversion to the gas chamber.

5.3. General factors
5.3.1. The influence of hierarchy within the litter

Although the focal and the companion piglets were assessed, it is unknown the extent to which the hierarchy from the litter influenced the behaviour of the piglets inside the box. It is established that piglets have a tendency to copy or repeat the behaviour of other piglets, mainly when they are not dominant in the litter. As a result of this tendency, the presence of one dominant and one subordinate piglet in the box could have biased the assessment of the behaviour of both piglets. In fact, the behaviour of the focal piglet could determine the behaviour related to the treatment and the box. However, analysis of the data indicated that there weren’t significant differences in the comparisons between treatments and focal according to Tukey 95%.

5.3.2. Exploration and aversion to the chamber as indicated by vocalizations.

Grunting and squeals were also assessed during the experiment. However, they were assessed for the whole chamber rather than for each individual piglet, due to the difficulty of assessing them individually. The vocalizations didn’t show any significant difference between treatments. This suggests that piglets in all of the treatments could experience the need to explore a new place, frustration due to separation from the mother or the litter, or stress or distress associated with the gas chamber. However, in order to assess the significance of these and other factors in eliciting grunting and squeals, more experimental research and data would be necessary.

According to a pilot study, pigs learn at an early age, and in a group of two week old piglets, learning involved a memory process (Rault et al., 2013b). Furthermore, it has been show that pigs in general have a good long term memory (Gieling et al., 2011).

Although nose and physical contact is often interpreted as relating to socialization and exploration, it can be confused with abdominal touch, which could be a behaviour related to stress caused by the absentia of the mother. Like suckling, or root abdominal space, it could be hard to identify them, due to the confusion with crushing while they walk. (Hvozdik and Dezorzova, 1998, Jarvis et al., 2008)

The study did not find any significant difference between the sex and treatments (ANOVA p value of <0.05). Possibly this is due to the fact that the piglets were not mature enough for differences between males and females to make a significant difference.
5.3.3. Least aversive method, from the point of view of piglets’ memory.

As I have indicated throughout the discussion, more experimental studies would be needed to resolve some of the critical issues raised by this project. However, supported by some of the research identified in the literature review, the results of this study are consistent with the possibility that the use of nitrous oxide could be the least aversive method. This is principally because of its anaesthetic effects on the piglets that might prevent them from remembering the process in the chamber if it fails and has to be repeated.

5.4. Limitations

- The literature and studies in this area are very limited. As a consequence, there are no established protocols or previous studies assessing the effects of nitrous oxide on the memory or piglets, particularly on spatial memory. As indicated, this study was not able to answer definitively some of the key issues. More experimental research is therefore necessary to complement this study and to supplement the data obtained in this experiment.

- The box used in the experiment was a commercial box, but its major limitation was that the piglets from the piggery farm were too big for their age. At 10 days old they weighed around 6 kilos. As a result of this, the box, which had good dimensions for the experiment, was too small for the piglets. To an extent this may have biased the data. Against that, however, the piglets were still able to move and show their behaviour.

- The method used to assess the grunting and squeals of piglets was limited, because the recording and counting of the vocalizations was made for the whole box, rather than on an individual basis. In further research, it may be possible to develop methods that allow the collection of this kind of data for individual piglets.

- An error in the cameras during the recording of the videos necessitated many shortcuts in making the observations. It is possible that this issue could have caused variation in the results while the behaviours were analysed through The Observer xt.

- Although there were cameras placed inside the box, the video images proved to be too blurred. In some cases, it wasn’t possible to identify them. However, these videos were not analysed in the project.

5.5. Further studies

Future studies could attempt to create a more comprehensive and accurate protocol or procedure for assessing the behaviour of the piglets. This is because the identification of the states and behaviours of the piglets can be very subjective, if there is not a more objective and repeatable method.

A very significant behaviour in the experiment is ‘attempt to escape’. It seems highly likely to be closely related to memory and place avoidance, which are central to the experiment. Future studies could attempt to measure with greater accuracy aspects of this behaviour that are more relevant. It could perhaps be measured in latency instead of frequency or duration, because many factors could be involved in the piglets’ intention to escape, not just aversion to the gas chamber.

Further studies are also desirable into the possible influence on behaviour of the litter size and its structure. This is because of the tendency of the dominant piglets to lead behaviours, and the tendency of subordinate piglets to copy behaviours. One approach would be to limit the
assessment to piglets who would not be affected by this factor. For example, it could be limited to piglets who feed from a nipple that is located in the middle of the sow. Then they would be assessed in closely similar conditions to reduce possible bias.
6. SUMMARY AND CONCLUSIONS

The data analysed from day 1, the treatments were significantly different between them, even in the comparisons of the duration of the treatment, but this results was expected, because each pair of piglets were rescued when they showed the behaviours of the gas effects. These signals were flailing in the case of carbon dioxide treatment and anaesthetic effects in the case of the nitrous oxide.

Although limitations, the results suggest attempts to escape could be related to the spatial memory of being exposed to one of the three different gases (carbon dioxide, nitrous oxide or medical air), explaining in that way, the hypothesis could be truth, because we believe that attempts to escape could be related with the memory and the aversion of the place, according to other studies in other species. In fact, all of the treatments presented this behaviour, and with a non significant difference, but it shows to be higher in Carbon dioxide treatment. And other condition that could be related to the memory is the stress, because piglets could improve their spatial memory under conditions of stress.

In fact, another behaviour that could be related the memory in piglets is the nose and physical contact, these behaviours express non significant differences between treatments. And could suggest that they could have social memory recognizing their partners. Although, the socializing could be related to normal behaviour or exploration.

In the treatment with carbon dioxide gas, the duration of ‘inactivity’ was lower, but it presented significant differences in frequency of inactivity, locomotion, nonvisible and rooting walls. These could be related to higher movements and change of activity that could be related to distress and a possible aversion to the gas chamber.

While medical air showed a higher duration of inactivity and lower frequency of locomotion, inactivity, nonvisible and rooting walls. This could be related to less activity and movements, which could be linked to less stress inside the gas chamber under normal conditions. And could be aversion, too. But it is uncertain which causes are exactly causing that stress, many factors could be involved in the whole manipulation of the piglets, handling, transportation, separation from the mother, enclosure inside a gas chamber, thus the attempts to escape, showing a possible aversion to the place.

Finally, the nitrous oxide treatment showed non-significant differences to the carbon dioxide treatment in the duration of inactivity and in rooting walls. Both these differences could be due to the anaesthetic effects of nitrous oxide. Also, a non-significant higher frequency of inactivity, nonvisible and locomotion than for piglets receiving the medical air treatment, which could all result from an expression of stress as an aversion to the gas chamber, then this stress and possible aversion seems to be lower than for the carbon dioxide treatment.

Other factors that can influence in the results are the hierarchy within the litter, studies revealed that piglets have the trend to repeat behaviour of the dominant piglets, then it is unknown if it could have been affected the results of the experiment.

Assessing behaviour in piglets could not have accuracy, because animals could show various behaviours to express different states. This could confuse the researcher and become a bias. For example, the exploration and stress could be misunderstood, if the behaviour rooting walls is assessed as rooting walls instead of suckling the corner of the wall, or it could get confusions with a signal of spatial memory when they slightly pressed the walls, expressing attempts to scape. Then more studies are needed to have more definitions of behaviours.
Also, other behaviours such as nose and physical contact could be related to socialization, and exploration, but if the touch is in the abdomen area, could be related to stress. However, all of the behaviours can show ambiguous meaning, mainly because of missing studies to assess the behaviour with more precision.

Grunting and squeals assessed in pairs during the experiment. Could have an influence in the results, although they didn’t show significant differences. in fact, other techniques would be needed, because vocalization could be a good complimentary variable to assess behaviour.

In fact, future studies could support the understanding of the behaviour of piglets, with new protocols, procedures and identify more accurate the states of the behaviour. attempt to create a more comprehensive and accurate protocol or procedure for assessing the behaviour of the piglets.

A very significant behaviour in the experiment is ‘attempt to escape’. It seems highly likely to be closely related to memory and place avoidance, which are central to the experiment. Future studies could attempt to measure with greater accuracy aspects of this behaviour that are more relevant. It could perhaps be measured in latency instead of frequency or duration, because many factors could be involved in the piglets’ intention to escape, not just aversion to the gas chamber.

Further studies are needed in other factors that could influence the behaviour of the piglets, such as the litter size and their structured, because a dominant hierarchy could avoid the expression of the behaviour in the subordinates.
7. REFERENCES


