



Sveriges lantbruksuniversitet  
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and  
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Department of Clinical Sciences

# Porcine pain face

– identifying visible characteristics of pain in pigs

*Lina Göransson*

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## **Porcine pain face – identifying visible characteristics of pain in pigs**

Pain face hos gris – identifiering av möjliga markörer för smärta

*Lina Göransson*

**Supervisor:** Magdalena Jacobson, Swedish University of Agricultural Sciences, Department of Clinical Sciences

**Assistant Supervisor:** Pia Haubro Andersen, Swedish University of Agricultural Sciences, Department of Clinical Sciences

**Examiner:** Annette Backhans, Swedish University of Agricultural Sciences, Department of Clinical Sciences

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## SUMMARY

The sensation of pain is an important evolutionary adaptation, vital for the survival of an organism. Not only does it enable identification and subsequent avoidance of potentially harmful threats, it also allows for special tending of the affected and possibly injured area. However, the *suppression* of pain may also constitute an essential evolutionary adaptation, especially in prey animals, which tend not to show such vulnerability for as long as they can possibly disguise it. In pigs, pain may thus be difficult to identify due to the stoic nature of this species. However, alleviation of pain requires it to be acknowledged. Numerous methods have been evaluated as possible means of pain assessment in pigs, but the development of a precise and reliable, objective and efficient pain assessment tool is however yet to await.

In humans, facial behavioural changes associated with pain is considered to be a reliable and consistent method for pain evaluation, and a good alternative when patients cannot convey their pain through spoken words. The concept of pain face has recently been further studied in *e.g.* non-human primates, horses, rodents and rabbits, and the method seems to be valuable also in these individuals unable to verbally communicate. However, to our knowledge, a pain face has not yet been developed in pigs.

Through video recordings of six pigs, following the application of topical Capsaicin crème inducing a transient burning pain sensation, facial expressions of pain were evaluated in accordance with a previously developed protocol for pain face in horses. The pigs served as their own control, and were filmed without any noxious stimulus, prior to noxious challenge. Topical Capsaicin was thereafter applied, during separate trials, to an area on the left respectively the right shoulder. All trials were performed twice, with and without an observer in the room. The films were evaluated by two external assessors, and a drawing of a porcine pain face was produced based on these findings. Subsequently, a blinded evaluation was performed based on a number of still images from each film, to assess the presence or absence of these pain face features. Furthermore, an ethogram was constructed regarding certain gross pain behaviours observed.

The evaluation of various gross pain behaviours revealed no significant behavioural changes as a consequence of topical administration of the Capsaicin, and gross behaviours indicative of discomfort or pain were infrequently and only occasionally observed. The facial expressions of the porcine pain face seem to comprise an angled appearance of the eyes ( $P = 0.004$ ), lowered ears held back or in an asymmetrical manner, wrinkling of the snout and possibly also tension of certain muscles around the mouth and cheeks. Furthermore, ears held in an upright position, turned forward in an attentive manner, were less frequent during pain induction ( $P = 0.02$ ) than during the control trials.

The facial expressional changes observed in the pigs during noxious challenge may have been rather subtle. Moreover, various concentrations of Capsaicin need to be evaluated in relation to the age and weight of the pigs under investigation. The findings of this study does however indicate that also in pigs, a pain face can be identified – and that it may constitute a possible future method for the assessment of porcine pain.



## SAMMANFATTNING

Förmågan att kunna känna och uppleva smärta utgör en livsviktig evolutionär anpassning. Smärta gör oss uppmärksamma på tänkbara faror, samtidigt som förnimmelsen leder till omvårdnad av en eventuellt skadad kroppsdel. Att kunna *dölja* tecken på smärta utgör dock likaså en livsnödvändig evolutionär anpassning framför allt hos bytesdjur, som normalt maskerar alla tecken på sådan sårbarhet in i det längsta. Smärtbedömning hos gris är således oftast mycket svårt, med tanke på artens stoiska karaktär. Ett flertal metoder för att kunna identifiera och bedöma smärta hos gris har studerats, men utvecklingen av en tillförlitlig, objektiv och effektiv metod för smärtbedömning hos gris återstår dock fortfarande.

Specifika smärtrelaterade förändringar i ansiktsuttryck anses vara en tillförlitlig metod för smärtbedömning inom humanmedicin, till exempel då patientens förmåga att genom ord uttrycka smärta är begränsad. Smärtrelaterade ansiktsuttryck, även kallat pain-face, har likaså studerats inom veterinärmedicin, till exempel hos andra primater, häst, gnegare och kanin. Också hos dessa individer, oförmögna att kommunicera verbalt, förefaller smärtrelaterade förändringar i ansiktsuttryck att vara en tillförlitlig och användbar metod för bedömning av smärta. Ett pain face har dock ännu inte, såvitt vi vet, utvecklats hos gris.

Efter topikal applicering av Capsaicinsalva, vilken inducerar en övergående brännande smärtförnimmelse, filmades sex stycken grisar vars ansiktsuttryck analyserades med avseende på smärtrelaterade förändringar. Protokollat baserades på en motsvarande, nyligen genomförd studie av pain-face hos häst. Samtliga grisar fungerade som sin egen kontroll, och filmades initialt utan någon stimulus, och därefter i samband med smärtinduktion. Capsaicin applicerades på ett område på vänster respektive höger sidas bogblad, vid separata tillfällen. Grisarna filmades först med och sedan utan en observatör i rummet. Filmerna analyserades av två externa bedömare, varpå ett pain-face utformades baserat på deras fynd. Därefter gjordes en blindad bedömning av ett antal stillbilder, med avseende på förekomst eller frånvaro av dessa ansiktsuttryck. Dessutom bedömdes ett antal andra potentiellt smärtrelaterade beteenden med hjälp av ett etogram.

Fyndet i denna studie tyder på att grisens pain face inkluderar ett spetsigt eller vinklat utseende av området just ovanför ögonen ( $P = 0,004$ ), sänkta och asymmetriska eller bakåt lagda öron, ett ökat antal rynkor på trynet, samt möjligen även kontraktion av viss muskulatur kring mun och längs huvudets laterala sidor. Smärtinduktionen resulterade dessutom i en signifikant minskning av den tid som grisarna höll öronen uppåt och framåt ( $P = 0,02$ ). Förekomsten av övriga smärtrelaterade beteenden uppvisade inga signifikanta skillnader före och efter appliceringen av Capsaicin. Tecken på obehag i samband med applikationen av Capsaicin sågs endast hos enstaka grisar och blott ett fåtal gånger.

De förändringar i ansiktsuttryck hos gris som observerats i samband med smärtinduktion må vara subtila. Vidare krävs utökade studier för att utvärdera en optimal koncentration av Capsaicin i relation till vikt och ålder hos grisarna. Resultaten av denna studie tyder dock på att ett pain face kan identifieras och kanske i framtiden utgöra en alternativ metod för smärtbedömning även hos gris.



## INTRODUCTION

Alike the evolutionary adaptation for survival that the sensation of pain constitutes, the expression of pain – as well as the ability to detect it in others – may be considered equally important (Prkachin, 2009; Williams, 2002). The communication of pain between individuals does not only serve to warn the receiver about potential threats in the surroundings, but may also enable the observer to aid with escape, healing and recovery from the pain (Williams, 2002). The recognition of pain may become problematic, especially when the signs are subtle or well concealed (Bateson, 1991; Weary et al., 2006). Due to survival instincts, prey animals in particular tend not to show such vulnerability for as long as they can possibly disguise it (Williams, 2002; Weary et al., 2006). To perceive pain in these animals, defined and appropriate measures for pain assessment are necessary.

Today, most people would agree upon that the sensation of pain is not exclusively experienced by humans, but by other animal species as well (Bateson, 1991; Weary et al., 2006). Yet in pigs, routine husbandry procedures such as castration and tail docking, known to induce pain, are commonly performed – but the use of apt analgesia is often not sufficient for adequate pain relief (Hay et al., 2003; Kluivers-Poodt et al., 2013; Lonardi et al., 2015; Prunier et al., 2005; Sutherland et al., 2011). Many pigs suffer from problems such as shoulder ulcers (Dahl-Pedersen et al., 2013; Larsen et al., 2015) and tail biting lesions (D'Eath et al., 2015). Pigs are furthermore commonly used as an experimental animal model for human biomedical research (Simon and Maibach, 2000), which all constitutes additional situations in which the need for proper recognition and alleviation of pain becomes essential.

Human facial expressions have been thoroughly studied (Ekman and Friesen, 1978) and have been applied in clinical medicine to evaluate pain in individuals with limited capacity to verbally communicate with their surroundings (Kunz et al., 2007; Lints-Martindale et al., 2007). In clinical veterinary medicine, the concept of pain face is however rather new (Gleerup et al., 2015; Holden et al., 2014; Keating et al., 2012; Miller et al., 2015). Previously, within the walls of research labs, facial expressions in for example rats (Sotocinal et al., 2011), mice (Leach et al., 2012) and chimpanzees (Vick et al., 2007) have been considered as a novel tool for pain assessment. In horses (Gleerup et al., 2015), cows (Gleerup et al., 2014) and cats (Holden et al., 2014), studies of facial behavioural changes as a method of clinical pain assessment are quite recent – and the concept of pain face is successively breaking new grounds as an important complementary approach to evaluate pain.

A number of different means to detect and evaluate pain in pigs currently exist, comprising various physiological, physical and behavioural parameters (Hay et al., 2003; Kluivers-Poodt et al., 2013; Lonardi et al., 2015; Marx et al., 2003; Prunier et al., 2005). Problems arise however as these signs might often be predominantly associated with acute or substantial pain (Hay et al., 2003), and may in addition vary depending on the cause and location of the pain (Leach et al., 2012). Many farmers as well as veterinarians agree upon that recognising pain in pigs can be difficult (Ison and Rutherford, 2014; Wilson et al., 2014). As a complement to current pain assessment tools, a method enabling and facilitating the identification of more

subtle signs indicating pain would thus be desirable – perhaps such as the development of a pain face in pigs.

## **Objective**

The identification of such facial behavioural changes would not only facilitate the assessment of pain in pigs. It would hopefully also emphasize the need for improved pain identification and management in this species.

The purpose of this study was to investigate whether pigs experiencing pain display certain characteristic facial expressions, known in several other mammals as pain face. The aim was to identify and describe these facial expressions as an additional method of pain evaluation in pigs. Furthermore, gross pain behaviour was also analysed.

## **LITERATURE REVIEW**

### **Pain sensation in animals**

It is universally acknowledged that the sensation of pain constitutes an evolutionary adaptation central to the survival of an organism, as it enables detection and subsequent behavioural changes and avoidance of noxious stimuli and potentially harmful insults (Williams, 2002). All mammals possess all the neuroanatomical and neuropharmacological requirements enabling them to feel pain (Mathews et al., 2014). The complex mechanisms that the physiology of pain comprises are however not included in the scope of this literature review, but rather the concept of the perception and assessment of it.

Regardless of species, pain is a subjective experience, unique to the beholder (Mathews et al., 2014). It is defined by the International Association for the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (IASP, 1994). Furthermore, the IASP emphasises that the inability to express pain verbally does nothing to diminish the significance of the sensation in an individual. The latter is of great importance, as animals rather use auditory, physiological and physical means for the purpose (Weary et al., 2006). Through measurement of different physiological, physical and behavioural parameters, animal pain may be estimated, but pain is nevertheless without a unit and thus impossible to quantify in an exact manner, which complicates the evaluation of it (Weary et al., 2006; Tranquilli et al., 2007, p. 41). In humans, the gold standard for pain assessment is self-report (Prkachin, 2009). In veterinary medicine, no such thing exists. The quality and magnitude of pain in animals is thus dependent on the human perception of it (Tranquilli et al., 2007, p. 41).

The presence of pain is important when considering animal welfare. It constitutes one of the Five Freedoms: “Freedom from pain, injury, and disease by prevention or rapid diagnosis and treatment” (Farm Animal Welfare Council, 2009). The quality of an animal’s life and well-being may become greatly reduced due to the experience of pain, and the misery associated with not being able to avoid it (Weary et al., 2006). Thus, animal welfare compromised by

pain can only be improved through the correct identification of that pain, enabling proper alleviation of it.

### **Pain evaluation and assessment in pigs**

There is currently no ideal method enabling an objective and reliable assessment of pain in pigs, but a number of studies have been performed, aiming to find a sensitive and consistent method. As castration is a well-known source of pain in piglets, frequently performed as a routine husbandry procedure, several studies have used this as a model for pain assessment (Hay et al., 2003; Kluivers-Poodt et al., 2013; Lonardi et al., 2015; Prunier et al., 2005; Sutherland et al., 2011). Preferably, these findings could be extrapolated to function as general indicators of pain in pigs also in other situations.

Physiological parameters analysed in blood and urine from castrated piglets have been studied as possible indicators of pain. In a recent study by Lonardi et al. (2015), blood sampling was performed within the first 24 hours after castration. Although blood sampling as such might induce a bias, the study nevertheless did show that plasma cortisol increased significantly immediately following the procedure, compared to in piglets not castrated. These findings supported previous results by Prunier et al. (2005), which in addition displayed a corresponding increase in plasma adrenocorticotrophic hormone (ACTH). No differences in plasma glucose were found between the treatment and control groups in either study. On the contrary, Prunier et al. (2005) however detected a significant increase in plasma lactate following castration. Hay et al. (2003) analysed spontaneously voided urine in piglets during the first five days following castration. Urinary cortisone significantly increased the first day after the procedure, as did noradrenaline during the second day only, but no significant differences were found regarding cortisol, norepinephrine and epinephrine. What must be considered however, is that an activation of the hypothalamus-pituitary-adrenal axis and a subsequent rise in stress hormones may be elicited by a number of factors associated with castration, such as the anxiety due to handling, fear and pain, as well as the inflammation induced by the tissue damage (Lonardi et al., 2015; Prunier et al., 2005).

This inflammatory reaction, as well as the stress associated with handling, may similarly result in an increased eye as well as rectal temperature. However, Lonardi et al. (2015) concluded that eye temperature might be relevant when estimating porcine pain, as it significantly increased and peaked four hours following castration, compared to in piglets not subjected to the procedure. The method is however troublesome and time consuming, as it requires the handling of individual pigs.

Several behavioural changes have also been considered as indicators of pain in piglets following castration. As such, Lonardi et al. (2015) evaluated and found three possibly useful behavioural aspects, comprising a latency to move, alterations in walking pattern, and postural changes. The latter included kyphosis, hind tip-toe walking and non-weight bearing hind limbs. Significant differences were observed between the piglets castrated and not castrated, however only within the first four hours after the procedure. Kluivers-Poodt et al. (2013) studied the effects of lidokain and meloxicam, by attending merely to behavioural

changes during the first five days after castration. Piglets treated with meloxicam displayed significantly less pain-related behaviours, such as prostration, stiffness, and “trembling”, compared to the piglets castrated with lidokain only or without any analgesia whatsoever. Also, the former displayed more unspecific behaviour considered not to be pain-related, such as “suckling”, “playing”, “walking” and “sleeping”. Likewise, Hay et al. (2003) reported similar results when evaluating behavioural signs of pain during the first five days following castration.

As an alternative approach, Marx et al. (2003) recorded and analysed pain-related vocalisations in piglets subjected to castration. Calls were classified as grunts, squeals or screams, and these were subsequently analysed considering a number of defined sound parameters. Grunts and squeals appeared to be significantly different from screams in particular, and the latter was clearly associated with the pain induced by castration. Through the development of autonomic classification of different call types, the authors suggest that these findings may thus become a useful pain assessment tool in pigs.

As a general indicator of welfare, tear staining has recently been studied by Telkänranta et al. (2015). Tear staining, also known as chromodacryorrhea, results from a porphyrin-pigmented secretion from the Harderian gland, located beneath the inner corner of the eye where a dark stain can sometimes be seen in pigs. The authors investigated the association between the magnitude of the dark stained area, and the amount of tail and ear damage, revealing significant positive correlations between these factors. However, the correlation coefficient was low, and the authors suggest that the stress induced by tail biting might in itself cause increased tear staining. Additionally, other common stress factors might make one particular individual more prone to ample tear staining as well as becoming tail or ear bitten. Nevertheless, the authors regard tear staining as a possible future welfare indicator in pigs, but emphasise the need for additional research to validate the method.

Finally, it has been suggested that changes in facial expressions displayed by piglets may be detected in association with pain following surgical castration, as scoring of cheek tension before and after the procedure was significantly higher in the latter (unpublished data, Lonardi et al., 2013). However, no comprehensive study on this has yet been published.

### **Facial expressions of pain**

The advantages of analysing facial expressions as an alternative or complementary method of pain assessment have been emphasised (Leach et al., 2011; Prkachin, 2009; Rahu et al., 2013). With increased knowledge and further development, it may become an effective way of identifying pain, considering that facial indicators are fewer and remains the same, despite the origin of the pain – as opposed to a full body behavioural assessment. The latter requires a considerable amount of behaviours to be assessed, which furthermore may vary depending on the location and type of pain (Leach et al., 2011; Leach et al., 2012). Moreover, the human tendency to primarily attend to the face of an individual in pain has been demonstrated (Leach et al., 2011). Thus, it becomes increasingly important to further enhance our knowledge on

*what* to look for in the face of another individual when attempting to identify pain, as valuable signs might otherwise be overlooked.

Charles Darwin was probably the very first to observe and describe the presence of facial expressions in, and their resemblance between, humans and other species (Darwin, 1872). Furthermore, he recognised distinct facial expressions in humans experiencing pain: “the mouth may be closely compressed, or more commonly the lips are retracted, with the teeth clenched or ground together... The eyes stare wildly as in horrified astonishment, or the brows are heavily contracted.” Several decades later, further attention was given to this notion, which led to numerous studies of mammal facial expressions exhibited during pain

The communicative function of facial pain expressions appears to be the primary one (Prkachin, 2009; Williams, 2002). In addition, the actual movement or contraction of facial muscles may draw attention away from the hurting area and thus decrease the sensation of pain, although it cannot protect from or eliminate the cause of pain. It has been suggested that facial pain expressions may be suppressed as a survival advantage, especially in prey animals, but that they however cannot be completely concealed through voluntary control (Gleerup et al., 2014; Williams, 2002).

### ***Pain face in humans***

A century after Darwin’s publications, the “Facial Action Coding System” (FACS) was provided by Ekman and Friesen (1978). This system has since publication been widely used as a method for measuring facial behavioural changes in humans, and subsequently also in several other animal species. Based on studies on functional anatomy of the facial musculature, Ekman and Friesen (1978) identified a number of so called “Action Units” (AUs), which represents the movement of certain muscles. These AUs provide an objective way of describing facial movements regarding frequency of occurrence and intensity. Through further studies, distinct AUs have successfully been correlated to certain pain-related facial behavioural changes, enabling the development of a pain assessment tool in humans.

One of the first studies on this topic was conducted by LeResche (1982). Although the study was small and based only on 16 photographs coded using FACS, the author identified a number of AUs consistently associated with pain: “[b]row lowering with skin drawn in tightly around closed eyes, accompanied by a horizontally-stretched, open mouth, often with deepening of the nasolabial furrow.” Other studies further confirmed these results, with only a few distinct facial expressions consistently present during pain, such as contraction of muscles around the eyes, tightened eyelids and eye closure, furrowed or lowered brows, raising of the upper lip and cheeks, and nose wrinkling (Hadjistavropoulos et al., 2002; Kunz et al., 2007; Rahu et al., 2013). However, not all AUs repeatedly associated with pain may be, or is actually rather unlikely to be, expressed in every individual, or simultaneously at any one time (Kunz and Lautenbacher, 2014; Williams, 2002). Hence, Kunz and Lautenbacher (2014) proposed to group single AUs observed on an individual basis during pain into distinct “facial activity patterns”. The authors described four patterns of facial movements repeatedly associated with pain: “narrowed eyes with furrowed brows and wrinkled nose”, “opened

mouth with narrowed eyes”, “raised eyebrows” and “furrowed brows with narrowed eyes.” These findings indicate that there is not one universal human pain face, but at least four different combinations of the AUs most commonly associated with painful sensations.

Although human pain assessment relies largely on self-report (Prkachin, 2009), there are individuals unable or with limited capacity to communicate their pain through spoken words (Prkachin, 2009). Due to this, Kunz et al. (2007) and Lints-Martindale et al. (2007) evaluated facial expressions as an alternative method of pain assessment in adults with dementia respectively Alzheimer’s disease. In both studies, FACS was used for coding of facial expressions in patients during induced pain, revealing that AUs commonly associated with pain appeared consistently during noxious stimulus, with no significant difference between the patient group and the control group. This was further supported by a study on facial expressions in intubated patients during painful endotracheal suctioning (Rahu et al., 2013).

### ***Pain face in animals***

In a similar manner to humans unable to communicate verbally, pain assessment in animals requires alternative methods to self-report. As Darwin observed, changes in facial expressions during pain are not only seen in humans, but similar changes can be detected in other animal species as well (Darwin, 1872). During the last decade, an increasing number of studies have proposed to analyse facial expressions in several animal species, often in order to develop objective methods for the evaluation of pain.

The human FACS has been adjusted to study facial expressions in a number of non-human primates. Vick et al. (2007) were among the first to show that the human FACS can be extrapolated to other species, as they constructed the “ChimpFACS.” These findings were supported by Parr et al. (2007), who further validated the application of ChimpFACS. More recent studies have likewise modified the human FACS to enable an objective analysis of facial expressions in other primates, such as gibbons (Waller et al., 2012), orangutans (Caeiro et al., 2013) and barbary macaques (Julle-Danière et al., 2015). These studies demonstrate that, despite certain differences between species regarding superficial facial anatomy such as bone structure, facial hairiness and coloration, all non-human primates exhibit similar facial expressions and movements compared to each other and to humans.

Distinct facial behavioural changes in association with pain have been investigated in laboratory rodents. Coding of facial expressions in mice during spontaneous pain following surgery was first documented by Langford et al. (2010), who developed the “Mouse Grimace Scale” (MGS), further evaluated by Leach et al. (2012) and Miller et al. (2015) as a method for pain assessment. The characteristic pain face in mice includes orbital tightening, folding of the ears with an outward and forward rotation, bulging of nose and cheek, and forward movement of the whiskers (Langford et al., 2010). Sotocinal et al. (2011) subsequently modified the MGS to construct a corresponding “Rat Grimace Scale” (RGS). Similar features as in mice could be seen in rats, however with a distinct flattening of the nose and cheek instead of bulging. Both the MGS and the RGS have consequently been concluded to be reliable and applicable methods for pain assessment in laboratory rodents. An equivalent

“Rabbit Grimace Scale” (RbtGS) was developed based on facial behavioural changes following ear tattooing (Keating et al., 2012), and the facial expressions identified in rabbits were alike the ones previously observed in especially rats.

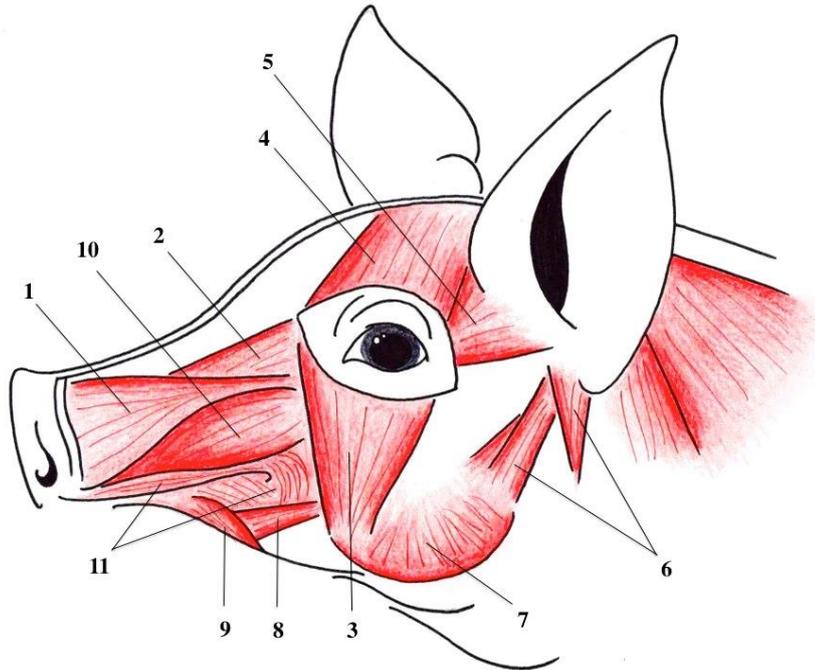
In horses, through dissections of underlying facial musculature (Gleerup et al., 2014; Wathan et al., 2015) and observations of facial expressions, Wathan et al. (2015) identified a range of facial movements and AUs homologous to the ones previously seen in primates, and subsequently developed “EquiFACS.” Dalla Costa et al. (2014) and Gleerup et al. (2015) more specifically identified facial behavioural changes seen during pain, constructing the “Horse Grimace Scale” respectively an equine pain face. Gleerup et al. (2015) used topical Capsaicin crème 10% in six adult horses. All horses served as their own control, and were filmed during control trials and during induced pain, with and without an observer present in the room. These video recordings were evaluated and alterations in facial expressions identified to describe an equine pain face. Subsequently a number of still images were scored by a blinded observer for the presence or absence of these features, which were significantly higher in the images from the noxious challenges compared to the control trials. The pain face described by Gleerup et al. (2015) includes orbital tightening and tension above the eyes, producing an angled appearance, outward rotation and lowering of the ears, tense stare, mediolaterally dilated nostrils, and tension of the lips, chin and chewing muscles. Similar to earlier findings (Kunz and Lautenbacher, 2014), not all features could be seen at any one time or in all individuals. In addition to the equine pain face, Gleerup et al. (2014) have also evaluated facial expressions in dairy cattle as part of a “Cow Pain Scale”. Facial behavioural changes observed were similar to those described in horses, comprising the aforementioned changes in ear position, orbital tightening, tense stare, tension of certain facial muscles and dilated nostrils.

In dogs, Waller et al. (2013) analysed underlying facial musculature through dissections and observed a wide range of facial movements to develop the “DogFACS”, providing a comprehensive list of AUs in accordance with the human FACS. In cats, Holden et al. (2014) more specifically analysed and described the presence of facial expressions associated with acute pain. The findings of the latter indicate that changes around the eyes and ears, mouth and nose were most prominent. The observers however, found it difficult to discriminate cats in pain from painless cats.

### **Facial anatomy of the pig**

The most distinctive anatomical feature of the porcine face is the rostrum, or snout, which is the mobile tip of the muzzle. Movements of the snout are primarily due to the contractions of *m. levator labii superioris*. The lips are short and rather immobile, and pigs cannot open their long and rather narrow mouth widely. The eyes are small and deeply located (Dyce et al., 2010, p. 755).

Figure 1. *Superficial musculature of the porcine head (Ill. Lina Göransson).*



1, *m. caninus*; 2, *m. levator labii superioris*; 3, *m. malaris*; 4, *m. levator anguli oculi*; 5, *m. frontoscutularis*; 6, *m. parotidoauricularis*; 7, *m. masseter*; 8, *m. depressor labii inferioris*; 9, *m. mentalis*; 10, *m. depressor labii superioris*; 11, *m. orbicularis oris*.

### **Capsaicin as a noxious stimulus**

Capsaicin is the pharmacologically active substance found in chilli pepper. It binds to a polymodal receptor known as the *transient receptor potential vanilloid subfamily member 1 (TRPV1) receptor*, sensitive to mechanical, chemical and thermal stimuli (Magerl et al., 2001; Ohta et al., 2005). This receptor is found in sensory neurons of *e.g.* the skin, cardiovascular structures, the gastrointestinal and urinary tract, as well as the central nervous system (Sharma et al., 2013), and has been identified in a number of mammals including humans, pigs, rabbits and dogs (Ohta et al., 2005). Stimulation of TRPV1 leads to the activation of nociceptive fibres and a burning pain sensation, as well as subsequent hyperalgesia and allodynia (Caterina et al., 1997; Giordano et al., 2012).

Capsaicin does also have an analgesic effect and may be used for symptomatic treatment of neuropathic pain. Through prolonged and repeated stimulation of TRPV1 it induces a refractory state, during which the neurons become insensitive to stimuli (Caterina et al., 1997; Sharma et al., 2013). This phenomenon is known as *defunctionalisation*, caused by several mechanisms such as loss of membrane potential, depletion of neuropeptides and a retraction of cutaneous nerve fibre terminals (Anand and Blay, 2011). Although studies suggest that low-concentration Capsaicin has little or no analgesic effect (Derry and Moore, 2012; Sharma et al., 2013), a Capsaicin crème 0.075% (*Capsina*, Bioglan AB, Malmö, Sweden) is available for this indication. A high-concentration Capsaicin dermal patch 8% (*Qutenza*, Astellas Pharma AB, Malmö, Sweden) seem to be more effective in soothing neuropathic pain (Anand and Blay, 2011; Henrich et al., 2015; Magerl et al., 2001), although some studies suggest that

a higher concentration such as 8% does not provide a better analgesic effect (Bischoff et al., 2014).

Topical application of Capsaicin has previously been used to induce neurogenic inflammation and experimental hyperalgesia in porcine skin, in order to sensitize the tissue before applying a thermal stimulus (Di Giminiani et al., 2014). In a pilot study, the authors concluded that lower concentrations did not induce any (0.1-1%) or inconsistent (5%) cutaneous reactions. However, a concentration of 20% caused substantial local erythema, when applied to the flank for 30 minutes. Furthermore, the authors concluded that topical Capsaicin induced significant hyperalgesia following thermal stimulus, however only in a group of younger pigs (27±5 kg). No such change in nociceptive thresholds was observed in the older pigs (57±3 kg). Behavioural studies revealed no significant rubbing behaviour following the application of Capsaicin. The authors mention a high level of individual variability in the nociceptive thresholds changes induced by Capsaicin, and suggest that the physical barrier constituted by the cutaneous layer of porcine skin might affect the penetration of the substance.

*Rubor* is one of the cardinal signs of inflammation, associated with the vasodilatation and subsequent hyperaemia mediated by the release of different inflammatory mediators, caused by various endogenous and exogenous stimuli. The topical application of Capsaicin constitutes such an exogenous stimuli, as it triggers the release of cytokines and other inflammatory mediators from *e.g.* endothelial cells, epithelial cells and various inflammatory cells (Zachary and McGavin, 2012). Besides vasodilatation and hyperaemia, the effects include increased vascular permeability (swelling), chemotaxis, fever, pain and further tissue damage. To exert its effect on cutaneous sensory neurons and induce its inflammatory effects, Capsaicin like all other topical substances must penetrate the physical epithelial barrier of the skin – resulting in the commonly seen cutaneous erythema and flare following its topical administration (Di Giminiani et al., 2014).

The similarities between human and porcine skin are significant, and pigs are commonly used as an animal model in human medicine research (Simon and Maibach, 2000; Summerfield et al., 2015). Summerfield et al. (2015) describe how both species have limited hair covering, a thick epidermis (50-120 µm in humans and 30-140 µm in pigs) and dermis, with similar dimensions and dermal-epidermal thickness ratio (10:1-10:3). Moreover, both human and porcine skin is firmly attached to the subcutaneous connective tissue. Also the amount and distribution of dermal vascularization seem to be very much alike in the two species. The subdermal fatty tissue is generally thicker in pigs, but described as abundant also in human skin (Summerfield et al., 2015). Considering topical penetration of substances through the porcine skin, such as Capsaicin, Simon and Maibach (2000) however emphasize the fact that breed, age, size and weight of the pigs may induce varying results.

Application of topical Capsaicin has also been used as an effective noxious stimulus in horses (Gleerup et al., 2015). The equine skin has more pigmentation and hair covering than human and porcine skin, but the amount of subcutaneous fat tissue is rather meagre (Wong et al., 2005). The epidermis is on average 53 µm thick. The thickness of the dermal layer varies greatly between 1 to 6 mm (average 3,8 mm), as horses have a unique, especially compact

and thick fibrous component of the dermis in some parts of the body such as the mane, dorsal thorax and back, and croup (Wong et al., 2005).

## **MATERIAL AND METHODS**

The study was approved by the Ethics Committee for Animal Experimentation, Uppsala, Sweden.

### **Animals and facilities**

Six specific pathogen-free pigs (three gilts and three boars) from the Swedish Livestock Research Centre at the Swedish University of Agricultural Sciences (SLU), Lövsta, Uppsala, participated in the study. On arrival, the mean age of the pigs was approximately 10 weeks ( $68\pm 6$  days), and their mean weight was  $24,3\pm 2,1$  kg. Three weeks later, at the beginning of the experimental trials, mean weight was  $44,8\pm 3,3$  kg. The pigs were clinically healthy, and had not been subjected to any previous treatments. Two boars were purebred Yorkshire, whereas one boar and the three gilts were crossbred Yorkshire, Hampshire and Swedish Landrace. Age and equal gender allocation was requested for standardization of the study, whereas breed was selected by the employees at the Research Centre. Furthermore, to facilitate interpretation of facial expressions, all pigs selected had erect ears. The pigs were held in stables at SLU, pairwise in 4 m<sup>2</sup> pens with plenty of straw. They were fed twice a day (grounded complete feed, Piggfor, Sluka, Lantmännen Lantbruk, Malmö, Sweden) and had free access to water.

### **The study design**

The protocol was essentially designed in accordance with a previous study on the equine pain face by Glerup et al. (2015). Two boars participated in an initial pilot study to establish a suitable protocol. During an acclimatisation period of three weeks they were habituated to the facilities, handling and presence of staff. Initially, attempts were made to accustom the pigs to wearing a neck collar or harness, and thereafter to be standing tied in the aisle of the stable, similar to the horses in the study by Glerup et al. (2015). However, during the third week of acclimatisation the approach was altered as the pigs were still not comfortable with being tied up, despite plenty of training, and thus they were instead familiarised with a commercial pig weighing crate (120x40x80 cm), for the purpose of subsequent video recordings. The pigs were accustomed to standing in the weighing crate for up to 20 consecutive minutes, and acquainted with the cameras set in final position. Training was always conducted in the aisle, as were the video recordings later on. During all training sessions and video recordings, the other pigs remained in their pens. Positive reinforcement solely – dry cat food and verbal encouragement – was used in the study.

Each pig was filmed six times in total and all pigs were filmed according to the same schedule (Table 1). All pigs served as their own control, and were thus firstly filmed without any noxious stimulus. Thereafter each pig was filmed after exposure to a topical Capsaicin crème, used as noxious stimulus, to an area on the left respectively the right shoulder. All of these sessions were recorded *with* and thereafter *without* an observer in the room. The observer was

always the same person and someone the pigs were familiar with, sitting approximately one meter away from the weighing crate during filming. Recording started within three minutes after application of the Capsaicin crème, and continued for 20 minutes. Video recordings during the pilot study were conducted during three consecutive days, with each pig being subjected to filming once in the morning and once in the afternoon. Noxious stimulus was never applied to the shoulder of the same side during any one day, hence allowing the area exposed to Capsaicin to rest for a minimum of 24 hours.

Table 1. *Treatment schedule for all pigs*

<b>Trial</b>	<b>Noxious stimulus</b>	<b>Observer</b>
<b>1. Control I</b>	No noxious stimulus	With observer
<b>2. Control II</b>	No noxious stimulus	No observer
<b>3. Capsaicin I</b>	Left shoulder	With observer
<b>4. Capsaicin II</b>	Right shoulder	With observer
<b>5. Capsaicin III</b>	Left shoulder	No observer
<b>6. Capsaicin IV</b>	Right shoulder	No observer

Following completion of the video recordings in the pilot study, another four pigs arrived. Again, three weeks of acclimatisation and training were followed by one week of video recordings. Based on the previous experiences, some adjustments were made in the protocol. These four pigs were immediately accustomed to standing in the weighing crate. The aim was to make the pigs comfortable and calm enough in the weighing crate to allow video recordings of them lying down. These pigs were recorded during three days, however with a two-days break before the third and last day of filming. As during the pilot study, each pig was subjected to filming once in the morning and once in the afternoon, and similarly Capsaicin was never applied to the shoulder of the same side during any one day.

### **Noxious stimulus**

Topical administration of Capsaicin crème 10% (Essex crème, Glostrup Apotek, Denmark) functioned as a noxious stimulus. It was chosen in accordance with the previous study on the equine pain face, also using Capsaicin 10% as a noxious stimulus, thus offering a standardized method to induce pain (Gleerup et al., 2015).

An area on each pig's shoulder was shaved with an electrical shaver. A square of approximately 5x5 cm was outlined with a marker pen, and a layer of the Capsaicin crème was applied to completely cover this square. After administration of the crème, the area was covered with a 10x10 cm gauze compress fastened with surgical tape. A gauze compress was also fastened with surgical tape during the control trials. After each completed recording the Capsaicin crème was removed with shower oil (Eucerin pH5 Shower Oil, Beiersdorf AG, Hamburg, Tyskland).

## **Video recordings**

Video recordings were performed with two cameras (CANON Legria HF R606, Canon Inc., Tokyo, Japan) placed on tripods (CAMLINK TP1700 Tripod, NEVIS B.V., 's-Hertogenbosch, The Netherlands). During each recording throughout the pilot study one camera was placed in the anterior end of the weighing crate straight in front of the pig, and another on the side of the scale diagonally in front of the pig. The pigs in the pilot study were mainly standing up, and could to some extent move back and forth in the scale. During the subsequent video recordings of the additional four pigs the cameras were both placed in front of the pigs, since the construction of the weighing crate prevented filming from the side when these pigs were lying down.

## **Pathoanatomical investigation**

When the study was completed, the last four pigs were euthanized immediately after the final video recording. Biopsies from the skin area exposed to Capsaicin on the right respectively left shoulder, as well as a control biopsy from an area not exposed to Capsaicin, were sent for histopathological examination at the Department of Biomedical Sciences and Veterinary Public Health, Section of Pathology, SLU, Uppsala, Sweden.

## **Data processing**

### ***Facial expressions of pain***

The films were sent to two external assessors, well experienced in analysing facial expressions, for an initial evaluation of a pain face and behavioural changes. The assessors evaluated all films independently of each other. To assess the normally occurring facial expressions, the control films were evaluated first, with particular focus on eyes, ears and snout. Thereafter the films of the pigs during pain induction were evaluated, aiming to detect any differences before and after the administration of Capsaicin.

Due to practical reasons, only the films *with* observer were assessed from the pilot study, and these were edited and cut to a length of approximately two minutes per film. Films from the recordings of the additional four pigs were not edited, and only the ones *without* observer were evaluated as the quality of these were superior. For all subsequent analyses, the same video recordings were used.

Based on the observations made by the two external assessors regarding facial behavioural changes displayed, a sketch of a porcine pain face was drawn. This exhibited all the possibly pain-related features observed in the pigs (Fig. 3).

### ***Blinded evaluation***

A blinded evaluation was performed based on a number of still images from each film, to assess the presence or absence of the previously identified pain face features (Table 2). Seven still images were taken from each film. The images from the video recordings of the last four pigs were taken with intervals of one minute, or as soon as a clear image could be obtained, starting two minutes into the film (approximately five minutes after Capsaicin had been applied). Since the films from the pilot study had been heavily cut and edited, seven still images were taken whenever a sharp image could be obtained. These images were coded and

sent to a blinded assessor, who evaluated the presence or absence of each feature in every image.

The blinded evaluation of the still images included “angled eyes” and “snout wrinkling.” Other specific pain face features were difficult to assess based on the still images. All images impossible to evaluate due to inferior quality were excluded. For the assessment of snout wrinkling, the number of wrinkles in each image was counted when clearly visible. The area above the eyes was defined as either “round” or “angled.” Prior to the numerical measurement of the eye angle, the images were grouped depending on the view from which the image was taken.

### **Other behaviours**

An ethogram was constructed based on certain gross pain behaviours observed. Behaviours were defined (Table 5) and quantified during a sequence of 10 consecutive minutes, starting two minutes into each film (approximately five minutes after Capsaicin had been applied) in accordance with the study by Gleerup et al. (2015). The observer was not blinded during these recordings. Due to editing of the films from the pilot study, these were excluded.

### **Statistical analysis**

All statistical analyses were conducted using Minitab® 17 (Minitab Inc., State College, Pennsylvania, USA). Differences were considered to be statistically significant if  $P < 0.05$ .

The association between an individual pig, treatment (control or Capsaicin) and eye appearance (round or angled) was investigated using a logistic regression analysis. The correlation between an individual pig, treatment, image (profile, front view, and 75° respectively 85° deviation from frontal view) and eye angle (numerical) was performed through a multiple regression analysis.

A multiple regression analysis was performed to assess the association between an individual pig, treatment (control or Capsaicin), and snout wrinkling.

The different gross pain behaviours were evaluated using a *two-sample paired t-test*, comparing the mean occurrence of each behaviour on an individual basis, during control trials respectively during noxious challenge.

## **RESULTS**

### **Animals**

During both the control trials and the trials with noxious challenge, the four last pigs would in general lay down in the weighing crate within approximately three minutes. Although there were some individual differences between the pigs, they would usually remain in this position throughout the trial, however occasionally standing or sitting up for a short while before lying down again. All four pigs eventually became drowsy, typically lying with their snout between

their extended front legs, often with their eyes closed and ears low, and occasionally sighing or grunting. The two pigs of the pilot study were not as familiarised with the weighing crate, and did move around a vast proportion of the time during filming.

The topical administration of Capsaicin induced an evident local erythema in all pigs within approximately five minutes after application, which subsided within an hour. Three of the pigs did occasionally display some rubbing or scratching behaviour after the application of Capsaicin, but there was no significant increase of such behaviour in the pigs when exposed to, compared to when not exposed to, noxious stimulus.

During the trials *with observer*, the person present in the room was sitting approximately one meter from the pig, looking in another direction and not interacting with the pig. The presence of the observer did not seem to elicit any behavioural differences in the pigs, compared to during the trials *without observer*, in terms of interaction with observer, drowsiness or vocalisation, although no statistical analyses on this were performed.

### ***Pathoanatomical investigation***

A histological analysis of skin biopsies from two of the pigs was performed, which did not reveal any signs of acute inflammation or hyperaemia in any of the biopsies. During a blinded evaluation, there were no clear differences to be observed between the histological specimens. One of these dermal specimens had recently been exposed to Capsaicin at the time of biopsy collection, whereas one had been exposed to Capsaicin approximately five hours prior to biopsy collection. The control skin biopsy had never been exposed to Capsaicin. There was no damage to the *stratum corneum* of the epidermis to be seen. Furthermore, no significant increase in the number of inflammatory cells, or signs of increased vasodilatation was detected.

### **Facial expressions of pain**

The evaluation of the video recordings suggested that, although the signs were subtle, certain distinctive changes in facial expressions can be associated with pain in pigs. These facial behavioural changes include movement and position of the ears, appearance of the eyes and snout, as well as tension around the mouth and along the cheeks. The features identified are described in Table 2, and illustrated in the drawings in Figure 3.

Table 2. Descriptions of the porcine pain face features

Pain face feature	Description
<b>Angled eyes</b>	Tension of <i>m. levator anguli oculi</i> and the dorsal area of the eyes, producing an angled appearance immediately above the eyes.
<b>Asymmetrical/low ears</b>	Ears not in an upright position, placed facing different directions but clearly not attentive to the surroundings, and/or lowering of the ears with increased distance between the apex of the ears.
<b>Ears backwards</b>	Both ears turned backwards, almost horizontally (not in upright position).
<b>Wrinkled snout</b>	Contractions of <i>m. levator labii superioris</i> , resulting in a number of wrinkles and lines dorsal and dorsolateral of the snout.
<b>Tension of mouth and cheek muscles</b>	Strained appearance around the mouth and cheeks, possibly due to tension of <i>m. orbicularis oris</i> , <i>m. malaris</i> , <i>m. masseter</i> and <i>m. parotidoauricularis</i> .

Each pain face feature could at some time during the observations be detected in all pigs. However, during pain induction, these pain-related facial expressions were not continuously seen. Moreover, not all features could be identified simultaneously at any one time.

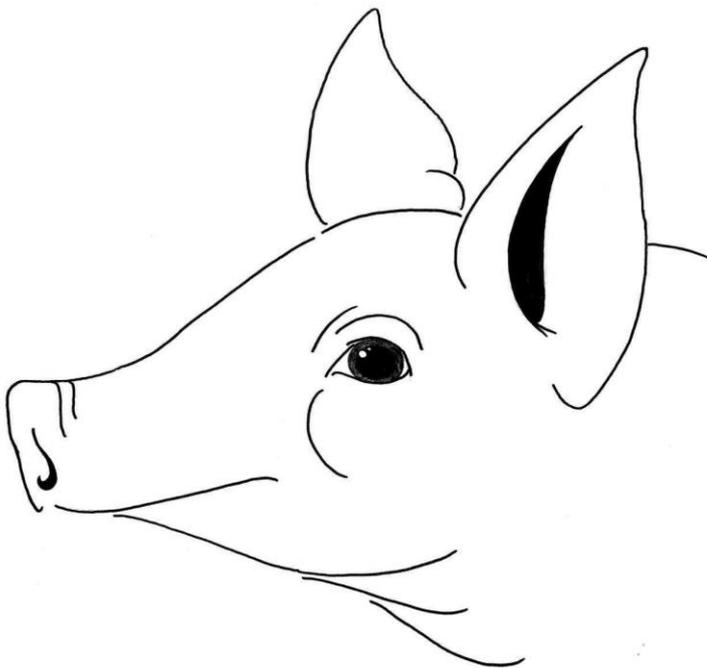


Figure 2. Facial expression of pig based on observations from the control trials (Ill. Lina Göransson).

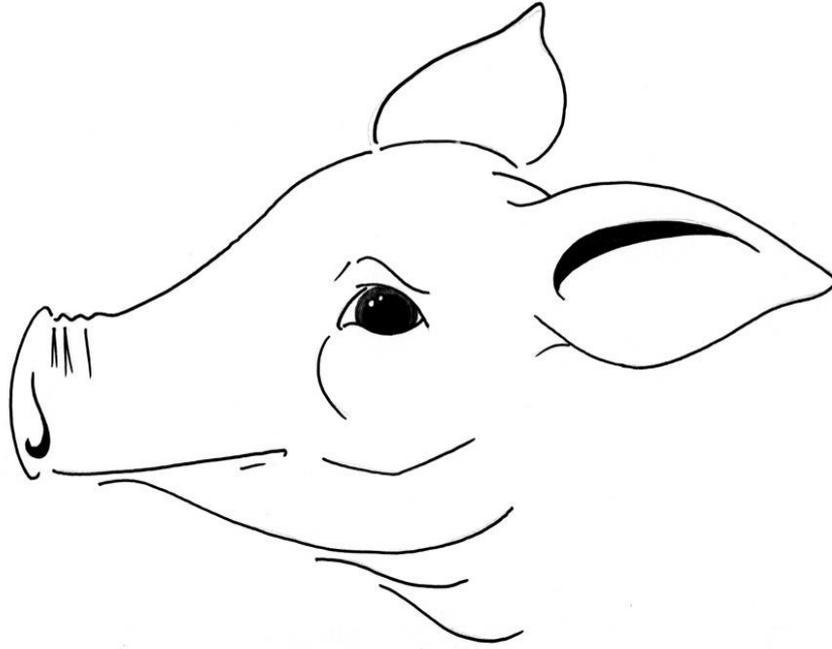


Figure 3. *Facial expressions of pig based on observations during pain induction, with a wrinkled snout, strained muscles around the mouth and along the cheeks, an angled appearance above the eyes, and ears held low and backwards (Ill. Lina Göransson).*

An initial evaluation of the porcine pain face suggested that, during pain induction, the area immediately above the eyes of the pigs would display an angled appearance (Fig. 3b), due to tension of muscles such as *m. levator anguli oculi*. This angled appearance differed from the more rounded appearance seen otherwise (Fig. 3a).



Figure 3. (a) *Pig during control trial with rounded appearance of the eye.* (b) *Pig during Capsaicin trial with angled appearance of the eye.*

Moreover, the initial assessment of facial behavioural changes during noxious stimuli, revealed an altered positioning and movement of the ears. The ears would become lowered and held in an asymmetrical manner while facing different directions (Fig. 4b). Occasionally both ears would also be held completely backwards, horizontally, nearly aligning the neck (Fig. 4c).

Figure 4. (a) Pig during control trial with ears up and attentive. (b) Pig during Capsaicin trial with lowered and asymmetrical ears. (c) Pig during Capsaicin trial with ears held backwards.



Wrinkling of the snout could be seen in the pigs during noxious challenge (Fig. 5a). A varying number of lines were observed dorsal of the snout, due to contractions of *m. levator labii superioris*. Such wrinkling of the snout differs from the more relaxed and smooth appearance of the area seen otherwise (Fig. 5b), although wrinkling and movement of the snout may also be seen in pigs not exposed to noxious stimuli.

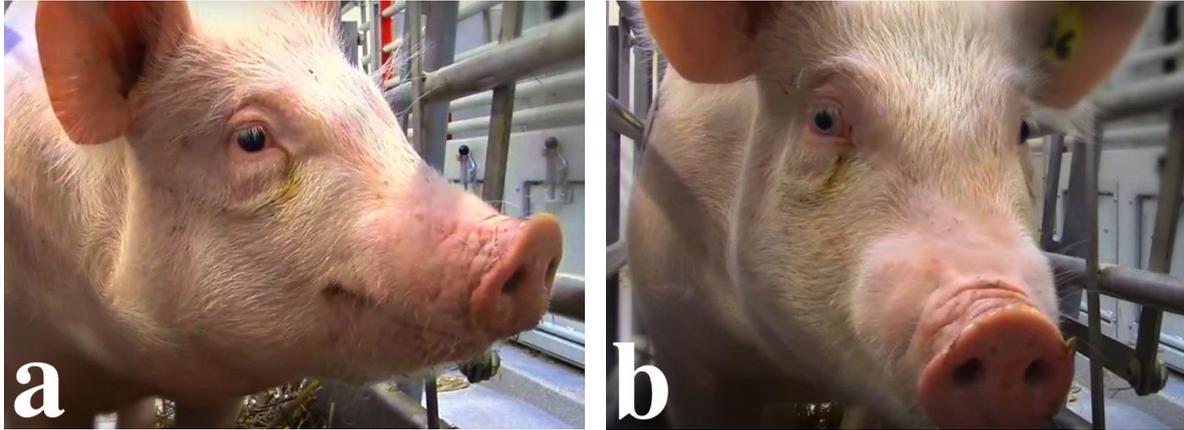


Figure 5. (a) Pig during control trial, with minor wrinkling of the snout. (b) Pig during Capsaicin trial, with increased wrinkling of the snout.

Occasionally identified as a pain face feature in the pigs, although subtle, was the tension of muscles along the lateral side of the head and around the mouth. This strained appearance might possibly be due to contraction of muscles such as *m. orbicularis oris*, *m. malaris*, *m. masseter* and *m. parotidoauricularis*.

### Blinded evaluation

A blinded evaluation was performed based on a total of 42 still images from the control trials, and a total of 84 still images from the Capsaicin trials. However, due to poor quality, not all images could be assessed regarding each pain face feature.

The blinded assessment of eye appearance, during which the area above the eyes was defined as either “angled” or “rounded”, revealed a significant correlation ( $P = 0.004$ ) between “angled eyes” and noxious stimuli (Table 3). However, the blinded evaluation and measurement of the eye angle did not reveal a significant difference ( $P = 0.06$ ).

Table 3. Number of images displaying “rounded” respectively “angled” eyes in pigs during control trials and Capsaicin trials

Eye appearance	Control (n=6)	Capsaicin (n=6)
Rounded eyes (number of images)	14	12
Angled eyes (number of images)	17	51

The evaluation of the number of wrinkles of the snout did not present any significant differences between the pigs during control trials and Capsaicin trials ( $P = 0.887$ ). Although not significant, the mean number of wrinkles increased during pain induction in two of the pigs, whereas in two other pigs the mean number of wrinkles decreased as compared to during control trials. In the remaining two pigs the mean number of wrinkles remained unchanged (Table 4).

Table 4. *Number of wrinkles (mean±SD) in each pig observed in still images from control trials and Capsaicin trials*

<b>Pig</b>	<b>Control (n=6) (Mean±SD)</b>	<b>Capsaicin (n=6) (Mean±SD)</b>
<b>1</b>	3.8±0.5	5.25±1.5
<b>2</b>	6.0±0.0	4.9±0.9
<b>3</b>	5.0±1.4	5.4±1.0
<b>4</b>	5.1±1.1	5.2±0.8
<b>5</b>	6.2±1.3	5.3±2.4
<b>6</b>	5.3±1.5	5.3±0.8

As a consequence of the continuous movement of the ears, the position of these was difficult to evaluate through merely a limited number of still images. Similarly, due to the subtleness of the previously observed muscle tension around the mouth and cheeks, this was also difficult to assess. Thus, these pain face features were not included in the blinded evaluation of still images.

## Other behaviours

An ethogram based on defined gross pain behaviour (Table 5) observed in the video recordings *without observer* was constructed. The pigs of the pilot study were excluded due to heavy editing of these films.

Table 5. Descriptions of the behaviours quantified in the video recordings as counts, frequency or proportion of time

Behaviour	Definitions of behaviour
<b>Total movement</b>	Sum of time sitting or standing, and moving head or rooting while lying down (proportion of time)
<b>Sitting or standing</b>	Standing with all four legs fully extended, or sitting with head up and both front legs fully extended (proportion of time)
<b>Head movement and/or rooting</b>	Moving head from side to side and rooting behaviour <i>while lying down</i> , or head up but not moving yet clearly attentive to the surroundings. Head up but clearly drowsy was not included (proportion of time)
<b>Ear movements</b>	
<b>Up and/or attentive</b>	Both ears in an erect position, or one/both ears facing forward or back clearly attentive to the surroundings. Ears not necessarily moving continuously (proportion of time)
<b>Back, lowered</b>	Both ears turned straight back, almost horizontally. Ears facing back but in an upright position was not included (proportion of time)
<b>Asymmetrical and/or low</b>	Ears not in an upright position, placed facing different directions but clearly not attentive to the surroundings, and/or lowering of the ears with increased distance between the apex of the ears (proportion of time)
<b>Eye blinking</b>	Movement of upper eyelid towards the lower eyelid (frequency)
<b>Other behaviours</b>	
<b>Tongue</b>	Sticking out tongue without any licking behaviour (counts)
<b>Licking or chewing</b>	Licking or chewing on something, licking around the mouth or chewing without anything in the mouth (counts)
<b>Yawning</b>	Opening of the mouth in a yawn (counts)
<b>Bruxism</b>	Excessive grinding (pressing together) of the teeth and/or clenching of the jaw producing a distinctive sound (proportion of time)

The statistical analysis of gross pain behaviour (Table 6) did not reveal any significant differences between the pigs when exposed respectively not exposed to Capsaicin, except for the ear movements described as “up and/or attentive”, which were significantly higher in pigs during control trials compared to pigs during noxious challenge. The two other ear movements, “back” respectively “asymmetrical and/or low”, were the only two behaviours in the ethogram that increased (although not significantly) in the pigs during Capsaicin trials

compared to during control trials. All remaining behaviours observed decreased (although not significantly) in the pigs during Capsaicin trials compared to during control trials.

Table 6. Comparison of behaviours quantified during 10 minutes in control trials and Capsaicin trials. Movements (sitting, standing and head movements), ear movements and bruxism are reported as a proportion of time (%). Eye blinking is reported as frequency during total time awake (per minute) and other behaviours (tongue, licking or chewing and yawning) is reported as counts

<b>Behaviour</b>	<b>Control (n=4) (Mean±SD)</b>	<b>Capsaicin (n=4) (Mean±SD)</b>	<b>Paired difference (Mean±SD)</b>	<b>p-value</b>
<b>Total movement</b>	42±11%	28±13%	15±15%	0.15
<b>Sitting or standing</b>	17±10%	7±13%	10±12%	0.19
<b>Head movement and/or rooting</b>	26±11%	21±12%	5±18%	0.64
<b>Ear movements</b>				
<b>Up and/or attentive</b>	74±9%	36±18%	39±17%	0.02
<b>Back</b>	4±4%	7±12%	-3±8%	0.55
<b>Asymmetrical and/or low</b>	22±8%	58±28%	-36±25%	0.06
<b>Eye blinking</b>	25±6	22±4	3±4	0.21
<b>Other behaviours</b>				
<b>Tongue</b>	3±3	0.3±0.5*	3±3	0.18
<b>Licking or chewing</b>	7±5	6±2	1±7	0.83
<b>Yawning</b>	0	0	0	-
<b>Bruxism</b>	0.6±1%*	0±0%	0.6±1%*	0.39

Figures are rounded to nearest whole number (\*rounded to nearest tenth due to very low values).

The proportion of time (%) calculated regarding various ear movements of the pigs is based on the total time the head of each pig was within frame. Thus, the total time out of frame was subtracted from the 10 minutes of observational time for the analysis of “ear movements.” Similarly, the frequency of eye blinking is based on the total time within frame and awake. Hence, the total time out of frame as well as the total time drowsy, with eyes closed, was subtracted for the analysis of “eye blinking.”

## DISCUSSION

### Facial expressions of pain

The findings of the present study suggest that also in pigs, pain can be deciphered through the readings of facial expressions. Although the changes in facial expressions observed in the pigs were sometimes very subtle, they seem to be similar to many of the findings in several other species. Alike the findings in previous studies (Gleerup et al., 2015; Kunz and Lautenbacher, 2014; Sotocinal et al., 2011), the pain face features detected in the pigs were not all present simultaneously at any one time. The observations made by the two external assessors, experienced in analysing facial expressions, displayed great resemblances and were made independently of each other. The observations were however not made through blinded evaluations, which may constitute a bias.

The tension above and the contraction of muscles around the eyes seen in the pigs has also been observed in humans, interpreted as “narrowed eyes” and “furrowed brows” (Kunz and Lautenbacher, 2014), as well as in horses, cows, rodents and rabbits, referred to as “orbital tightening” (Gleerup et al., 2014; Keating et al., 2012; Langford et al., 2010; Sotocinal et al., 2011). In horses, an “angled appearance” of the upper eyelid has been described (Gleerup et al., 2015), and resembling changes could be detected in the pigs during pain induction ( $P = 0.004$ ), as compared to the soft and rounded appearance above the eyes seen in pigs not exposed to noxious stimulus. Anatomical similarities between the species exist and the facial muscle, *m. levator anguli*, responsible for this angular appearance in horses has a similar anatomy in pigs (Dyce et al., 2010, p. 755; Fig. 1). The measurement of the eye angle did however not reveal a significant difference between the pigs during control and Capsaicin trials ( $P = 0.06$ ). In particular, the measurement of the eye angle in the still images was difficult to perform though, as the images were taken from varying views and angles, making a correct comparison between the pigs problematic. Thus, these results exhibit some uncertainties. To enable a more reliable analysis of the eyes, all images should be taken from the same angle, preferably perpendicular to the pig’s face. Furthermore, facial colouring may impede correct assessment of the eye area, and in methodological studies, this should be avoided for an easier and more reliable analysis. The tense stare identified as a pain-related feature in humans, horses and cows was not observed in the pigs of this study.

The wrinkling of the snout in pigs may correspond to nose wrinkling commonly observed in humans during pain (Kunz and Lautenbacher, 2014) as well as the tension of muscles resulting in wrinkles and puckers above the nostrils in cows (Gleerup et al., 2014). Rodents and rabbits have displayed a forward movement of the whiskers, and changes in the appearance of the nose and cheeks, which despite the anatomical differences between pigs and rodents, further indicates that this area is important for the interpretation of facial pain expressions. However, the blinded evaluation of the still images did not show any significant increase in the number of wrinkles displayed by the pigs exposed to Capsaicin compared to the pigs during the control recordings. Many of the images were impossible to assess due to the head positioning of the pigs. Furthermore, wrinkles were sometimes created by the pigs pressing their snout against the interior of the weighing crate. Suitable images for the purpose, clearly displaying the snout, should thus be used for such an assessment. Short video sequences may furthermore be more appropriate rather than a number of still images, as the

pigs commonly wiggle their snout, although the number of wrinkles may on the other hand be rather difficult to assess using motion pictures too. Dilated nostrils as seen in horses and cows during pain could not be seen in pigs, due to anatomical reasons. Corresponding facial behavioural changes in pigs could perhaps be represented by the wiggling movement of the snout, however not analysed thoroughly in this study.

In horses and cows a typical “outward rotation and lowering of the ears” have been observed (Gleerup et al., 2014), similar to the “outward and forward rotation” in rodents and rabbits (Keating et al., 2012; Langford et al., 2010). Changes in ear position in pigs during pain might likewise possibly be present, but due to the anatomical differences and the frequent normal ear movements seen in pigs, such evaluation was problematic. When comparing the proportion of time in which the pigs displayed ears in an upright position or an attentive fashion, the statistical analysis did reveal a significant decrease in the pigs when exposed to Capsaicin compared to when not ( $P = 0.02$ ). However, the quantification of the time spent with the ears in various positions was very difficult. Despite the definitions constructed prior to the assessments of the films, it was challenging to clearly differentiate the various ear movements and positions. It was especially hard to distinguish the “lowering and asymmetrical positioning” of the ears in the pigs, as a consequence of pain induction, from the very much similar appearance of the ears seen in drowsy or sleeping pigs. In addition, one pig had ears that normally would be described as low, impossible to clearly separate from the lowering of the ears as during pain induction. There were no other significant differences regarding the positioning of the ears between the trials.

Tension of certain facial muscles around the mouth and cheeks have been reported in humans and other primates, horses, cows, rodents and rabbits (Gleerup et al., 2014; Hadjistavropoulos et al., 2002; Keating et al., 2012; Langford et al., 2010). Although very subtle, similar changes could possibly be detected in some of the pigs in this study. Supportingly, significant changes in cheek tension have recently been described in piglets during surgical pain (unpublished data, Lonardi et al., 2013). The contraction of certain facial muscles, such as *M. malaris* and *M. orbicularis oris*, could perhaps be responsible for such an appearance in pigs.

Although the evaluation of pain based on facial expressions has frequently been referred to as a more objective method compared to many other pain assessment tools, largely because of the consistency of the pain-related facial expressions (Leach et al., 2011; Leach et al., 2012), it does however involve a certain amount of subjective ratings. Facial behavioural changes such as the positioning of the ears or a strained appearance due to the contraction of certain muscles may be difficult to detect, especially when such changes are subtle. The frequency and intensity of certain facial expressions associated with pain may thus be dependent on the individual observer. An automatic detection system of facial expressions has been investigated in humans (Lucey et al., 2009). Such a system would reduce the impact of the individual beholder during the assessment of facial expressions, and would thus be desirable also in other species.

## **Other behaviours**

The statistical analysis following the quantification of certain gross pain behaviour observed, revealed no significant differences between the pigs when exposed and not exposed to

Capsaicin. The duration of 10 consecutive minutes that each video sequence comprised were chosen to avoid the latter part of the films, during which the pigs would typically become drowsy. To further avoid a bias, the latter part of the film was excluded as the effects of the Capsaicin were expected to have diminished, due to the transient effects of the crème. Moreover, in the study by Gleerup et al. (2015), topical Capsaicin induced behavioural changes in the horses after two minutes. The quantification of gross pain behaviour was however not done through a blinded evaluation, which may constitute a bias.

Previous studies regarding pain-related behavioural changes have merely been based on surgical castration, and the evaluations have thus mainly focused on *e.g.* walking patterns and postural changes (Kluivers-Poodt et al., 2013; Lonardi et al., 2015). Pain assessment in pigs through behavioural changes such as eye blinking frequency, chewing, or licking, would constitute a method in which the signs of pain would be fewer, located to the relatively small area of the face, and remain the same despite the location and type of pain (Leach et al., 2011; Leach et al., 2012), in accordance with the advantages associated with the analysis of facial pain expressions. Normally, pigs are however extremely curious animals, which tend to examine their surroundings through rooting and chewing, and there was no significant differences regarding such behaviours between the pigs when exposed to Capsaicin and when not. A hypothetical change in such behaviours during pain may be difficult to detect, as pigs likely tend to suppress signs of pain if possible. In the present study, the pigs did however seem to move around somewhat less during pain induction, which although not a significant finding, may be in accordance with the stoic nature of the species.

The treatment schedule was designed so that the control trials were always conducted first, and the trials with Capsaicin were conducted last, to avoid an association between the sensation of pain and the weighing crate during the control trials. Furthermore, the video recordings were conducted following three weeks of acclimatisation and training, and the pigs were very well acquainted with the weighing crate prior to the trials. The manure removal system did occasionally cause some background noise, as did the other pigs in their pens, which the pigs however were also very well accustomed to.

### **Capsaicin as a noxious stimulus**

A prerequisite for the specific study of pain sensation is that other factors, which may obscure the assessment of pain in particular, can be excluded. Most studies regarding pain assessment in pigs have been based on the procedure of surgical castration, as it is frequently performed and well known to be painful for the piglets (Hay et al., 2003; Kluivers-Poodt et al., 2013; Lonardi et al., 2015; Prunier et al., 2005; Sutherland et al., 2011). However, there are limitations to such studies when the objective is to evaluate pain in particular.

Although the acute pain accompanying surgical castration or tail docking is in no way to be doubted, the procedures in addition also induce an inflammatory reaction (Lonardi et al., 2015; Prunier et al., 2005). This may distort both the physiological and behavioural measures considered as specific pain indicators. The inflammatory reaction following the tissue damage is associated with a wide range of physiological effects such as fever and decreased demeanour, that may obscure gross behavioural changes related to pain (Zachary and McGavin, 2012). Behaviours such as prostration and an increased latency to move, as well as the decreased occurrence of other behaviours considered not related to pain such as “playing”

and “suckling”, may thus not merely be due to the pain sensation, but partly a result of the inflammatory reaction following surgical castration.

In addition, surgical castration in itself as well as the handling of individual piglets is very stressful to the pigs. It leads to the activation of the sympathetic nervous system, which may further distort the expression of pain – both behavioural and physiological measures (Binder et al., 2004). The sympathetic activation and the subsequent release of stress hormones such as noradrenaline, may reduce the sensation of pain and the expression of it, known as “stress-induced analgesia” (Binder et al., 2004). Furthermore, physiological indicators such as plasma cortisol will also be affected by such an activation of the sympathetic nervous system.

In this study, topical Capsaicin was chosen as a noxious stimulus, as it constitutes a previously utilised and standardised method for moderate pain induction in *e.g.* humans (Sharma et al., 2013) and horses (Gleerup et al., 2015). An advantage of topical Capsaicin is that it allows for the study of pain more specifically, as several other factors that may be misinterpreted as pain reactions have been reduced. It induces a *local* cutaneous neurogenic inflammation, which should not affect any behavioural parameters in the same way as a general inflammatory reaction or infection might do. In addition, the crème induces a transient burning pain sensation within minutes, enabling the occurrence of pain to be confined to a certain time period. The transient effects of Capsaicin were additionally advantageous in this study, since the pigs could thus be used as their own controls.

As the similarities between human and porcine skin are numerous (Simon and Maibach, 2000), it may be presumed that the effects of topical Capsaicin will be similar in these two species. However, in a study by Di Giminiani et al. (2014), various Capsaicin concentrations up to 20% did not seem to elicit an evident pain reaction. Likewise, the efficiency of the crème as a noxious stimulus in the present study may be questioned. The gross pain behaviour observed did not significantly increase following the administration of Capsaicin, which may indicate that it induced no, or merely a slight or inconsistent, pain sensation in the pigs. However, as pain assessment in pigs have previously been concluded to be difficult, and as even substantial pain may cause only subtle signs of discomfort, the magnitude of pain induced by Capsaicin should perhaps not solely be based on the presence or absence of gross pain behaviour.

Plausible reasons for the varying responses to Capsaicin and other topical substances applied to porcine skin have been considered in previous studies, including race, age and size of the pigs (Di Giminiani et al., 2014; Simon and Maibach, 2000). The effects of topical Capsaicin rely on its ability to reach cutaneous sensory neurons of the skin, and thus its ability to penetrate the physical barrier that the skin constitutes. Differences in the cutaneous anatomy between the equine, porcine and human skin may thus explain why Capsaicin induces a gross behavioural pain reaction in horses and humans, but seem not to be as efficient in pigs. However, the similarities between human and porcine skin has been described as profound, which is precisely why pigs are commonly used as an animal model for human dermatology research. Although the equine skin exhibits some distinct features in comparison with porcine and human skin (Wong et al., 2005), the diverse reactions following application of topical Capsaicin 10% in horses and pigs could not be explained. While the

same concentration was used in both this study and the study by Glerup et al. (2015), it is however possible that the actual amount of crème applied during the trials were not equal between the studies, which might contribute to the varying effects of Capsaicin. When the same crème used on the pigs was applied to human skin of two different persons, there was no substantial pain reaction or local erythema experienced by any of them.

In the present study, some pigs scarcely displayed rubbing and scratching behaviour, whereas other pigs did not. This is consistent with the results obtained by Di Giminiani et al. (2014). Moreover, Di Giminiani et al. (2014) reported significant thermal hyperalgesia following topical Capsaicin application in a group of smaller pigs ( $27\pm 5$  kg) but not in a group of larger pigs ( $57\pm 3$  kg). Likewise, Simon and Maibach (2000) suggest topical penetration to be greater in smaller pigs, and thus the age and weight of the pigs in our study ( $44,8\pm 3,3$  kg) may contribute to the seemingly lacking effect of Capsaicin. Further, the effects of various concentrations and formulations of topical Capsaicin need to be investigated, in order to find the most suitable concentration for the purpose.

The histological examination of skin biopsies from two of the pigs did not reveal any indications of a marked cutaneous inflammation or hyperaemia, although the latter could clearly be seen macroscopically in association with the application of Capsaicin. However, the time elapsed from the application of Capsaicin to the collection of biopsies may have allowed for substantial regression of the hyperaemia, as the effects of Capsaicin are transient. It is also possible that the vasodilatation may have subsided post mortem. Furthermore, the macroscopically visible hyperaemia, does not necessarily verify that pain was effectively induced. As presented by Di Giordano et al. (2012), the pain perception associated with the exposure to Capsaicin, depends largely on the involvement of the TRPV1 receptor. Although it has been suggested that the activation of the TRPV1 receptor may also contribute to the vasodilatation following the administration of Capsaicin (Ohta et al., 2005), this may not solely depend on the activation of the TRPV1 receptor in particular. Hence, despite deficient pain induction following the exposure of topical Capsaicin, macroscopically visible hyperaemia might be present.

## CONCLUSION

The findings in this study suggest that also in pigs, a pain face can be identified. This porcine pain face seems to comprise an angled appearance of the area above the eyes, wrinkling of the snout, and tension of muscles around the mouth and along the lateral side of the head, as well as lowered ears held in an asymmetrical manner or completely backwards. These features were sometimes rather subtle however, and the method may thus be difficult to apply in a clinical situation. Moreover, the effectiveness of the crème as a noxious stimulus may be questioned. Further studies are required to evaluate and develop the porcine pain face, which may then possibly constitute a valuable additional pain assessment tool in pigs.

Reactions to this study have occasionally included doubt that pigs would feel, and even less so, express pain as well as mere questioning of *why* such a study would at all be desirable. This is largely what makes this study desirable indeed. To alleviate pain, one must first be able to detect its presence and recognise the signs of it – also in pigs.

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