Acute and chronic stress in post-weaned pigs increases the detection threshold for sucrose solutions.

Anhedonia in nursery pigs. Effect of acute and chronic stress over the detection threshold for sucrose solutions.

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Abstract

Hedonism during intake is affected not only by food or solution characteristics but by animal's stress that has been reported to reduce the ability to experiment pleasure in front of palatable solutions in rats and humans. However, little information is known about anhedonia process in productive animals like pigs. To assess whether pigs could change their preferences for hedonic solutions due to stress situations we performed 3 consecutive experiments. Pigs (42d-old) were randomly allocated in two groups in each experiment: SG (stressed group) and CG (control group). SG pigs were exposed to an acute stress during 2 consecutive days by mixing animals of contiguous pens during 20 minutes (Experiment 1), subjected to movement restriction by immobilization for a 3-min period, 3 times a day for 3 consecutive days (Experiment 2) or were leave to their weaning process known to be a stressful factor itself. The ability to detect sucrose solutions in both groups were measured after the stress protocols of each experiment. Animals were tested in pairs (12 control pairs and 12 experimental pairs) to prefer water vs. sucrose (0.5 or 1%) solutions during 30 min. In experiments 1 and 2 control pigs showed higher intakes of 0.5% and 1% sucrose solutions over water during the choice tests. SG pigs did not show any difference between solutions intakes of sucrose 0.5% and water. However, at higher sucrose inclusion (1%) they clearly preferred sucrose. Results of experiment 3 were not consistent with anhedonia described in literature and stressed animals preferred both sucrose inclusions. Results shows that stress in pigs may increase their detection threshold to detect low concentrations of sucrose under specific stressful situations probably because of their impaired ability to experience pleasure as a result of a hedonic stimulus. However, preference for intense sweetness could be more pronounced in stressed animals due to their rewarding deficit.

Keywords: Anhedonia; Pigs; Sucrose; Stress

1. Introduction

Sensory pleasure has been pointed out as an important determinant of adapted behaviours. The pleasure or displeasure of a sensation is not stimulus bound but depends on internal signals. Therefore, a given food or solution could be perceived as pleasant or unpleasant according to the internal status of a mammal (Cabanac, 1971). In this way, hedonism during intake is affected not only by food or solution characteristics but by animal's physiological (hunger, satiety, sickness, internal temperature e.g.) and psychological status (stress, depression, anxiety e.g.). In the case of psychological status, it has been reported that chronic stress may reduce the ability to experiment pleasure in front of hedonic stimulus, process known as anhedonia. In humans, anhedonia could be regarded as a core symptom of depression and schizophrenia where a lowered ability to experience pleasure is observed (Ho and Sommers, 2013; Matthews et al., 1995). Non-human animals exposed to inescapable stress develop behavioral consequences that are similar to symptoms of human anhedonia. Unpredictable stressors have been shown to induce changes in a wide range of behavioral parameters, including feeding behavior (Willner, 1991; Grønli et al., 2005). Anhedonia, is claimed to be reflected in the animals' decreased consumption of palatable solutions (Willner et al., 1987). Post-ingestive and oral effects of sucrose, that innately active pleasure pathway and usually increase preference or acceptance of feeds and solutions, could be perceived in a different way. In rats, anhedonia may affect sucrose intake after protocols of "*Chronic Mild Stress"* (CMS), where mild, continuous and rotating stressors are exposed during several days (Koob and Zimmer, 2012). It has been shown that the hedonic value of weak sucrose inclusions in tap water is not detected by chronic stressed rats, being a useful tool for anhedonia evaluation (Grønli et al., 2004). Surprisingly, when sucrose incorporation is increased, stressed rats can consume significantly more than control animals. Something similar occurs in humans where stress could develop a greater palatable or comfort food intake associated with significantly decreased healthy eating (Groesz et al., 2012). An increase in carbohydrate "craving" is commonly observed in depressed humans and therefore presumably in "depressed" non-human animals too (Barr and Phillips, 1998). This effect may be due to decreases in hedonic intensity (Sampson et al., 1992).The intake or preference for sucrose solutions is the hedonic measure that has been most widely adopted to describe anhedonia. However, the validity of the CMS model has been questioned because a decrease in sucrose consumption is not consistently observed following the stress procedure among various laboratories (Bielajew et al., 2002; Forbes et al., 1996; Harris et al., 1997; Hatcher et al., 1997; Matthews et al., 1995; Nielsen et al., 2000; Strekalova et al., 2004).

It has been observed that chronic stress creates a disregulation of the neural substrates involved in normal hedonic behavior. There is a selective effect of CMS on the hedonic property or "liking" over sweet solution that is different from the desire or "wanting" to work for the same stimulus. Animals subjected to CMS appear to "want" the sucrose weak solution as much as non-stressed animals (Barr and Phillips, 1998). An example is the modulation of the mesolimbic DA system and doses of neuroleptics that inhibit lever-pressing for either food or water but leave consummatory responses intact (Blackburn et al., 1987; Salamone, 1996). Studies also showed that lesions of the 6-OHDA in the NAcc cause aphagia without affecting hedonic reactions to a sweet solution (Berridge et al., 1989).

Despite the large amount of information about anhedonia in humans and animals models like rats, little information exists about the anhedonia process in non-conventional species (Fureix et al., 2015) or productive animals like pigs. During the first days after weaning pigs have to cope with several stressors including animals mixing, new solid diets that could trigger neophobia, transportation, new environment, maternal separation etc. After this critical period, pigs suffer feeding behaviour problems and most weaned animals are reluctant to eat, leading to anorexia on the first days after weaning (Moeser et al., 2012; Pluske et al., 2007). Pigs like rats and humans present an innate and strong preference for sweet components like sucrose (Glaser et al., 2000; Hellekant and Danilova, 1999). These compounds have been used to enhance feed palatability and hedonism but also to facilitate the intake of neutral flavors by associative learning (Figueroa et al., 2011). Because stress is described to change hedonic perception of palatable compounds, it is logical to think that pigs could perceive sweet compounds in a different way during or after stress. This situation could change inclusion needs of palatable compounds to enhance animal's intake and also could be a useful tool to measure anhedonia (preference or acceptance for sucrose) to detect stressful situations that could affect welfare in these productive animals. The aim of the present study was to evaluate if pigs change their preferences for a hedonic sweet solutions due to acute or chronic stress situations.

Experiments were conducted at the weanling unit of the pig facilities belonging to the Universitat Autònoma de Barcelona (UAB). Experimental procedures were approved by Ethical Committee on Animal Experimentation of the UAB. (CEAAH 1406).

2. Materials and methods

2.1 Subjects

A total of 864 entire male and female pigs ([Large White x Landrace] x Pietrain) were used during the total period and divided in three different experiments in consecutive weaning cycles (240 in experiment 1, 240 in experiment 2 and 384 in experiment 3). All animals were individually identified at birth by using a plastic ear tag and they stayed with their mother and littermates inside the farrowing crates (standard farrowing create, 0.5 m wide, 2.0 m large and 1.03 m high) and their corresponding area for piglets (total available area 4.63 m^2; 4.15 m^2 of complete slatted floor and 0.48m^2 of concrete heat area) during the entire suckling period (28d). The farrowing room was provided with controlled temperature; 22.4±2.05°C sow environment and 28.3±2.70°C piglet environment (HOBO U10, data logger, MA, USA) and automatic ventilation. Inside each crate, piglets had access to a heated area to provide a warmed resting area, which was also enriched with wood shavings, sawdust and drying material (Biosuper CONFORT +, Gratecap Services, La Rochelle, France). An unflavoured creep feed diet was offered ad-libitum from day 10 of birth onwards to all litters by using a pan feeder. Piglets were weaned at an average of 27±2.3 days of age with a body weight of 7.17±1.03kg. At weaning animals were moved to the weanling unit and distributed into weaning pens (10 pigs/pen; 24 total pens). The room was provided with automatic, forced ventilation and completely slatted floors. Each pen (3.2 m^2 in floor area) had a feeder with 3 feeding spaces and an independent water supply next to the feeder. Animals had ad-lib access to unflavoured complete feed (pre-starter; 0-14d or starter; 15-35d post-weaning) except 1 hr before and after each test session. Free access to fresh water was provided to all the animals for the entire experimental period and no environmental enrichments were applied during this period. At the second week after weaning pigs were adapted to future experimental conditions by offering them two equidistant control dishes with drinking water for 2 hours (each morning from 9-11am in each pen). After the experiment, pigs continued with the normal process of commercial pig production in the same experimental unit of the UAB.

2.2 Procedure

2.2.1 Experiment 1. Acute physical stress generated by mixing pigs during the nursery period and its effect over sucrose detection.

A total of 240 post-weaned pigs (42d-old) kept in 24 pens (10 pigs/pen) were randomly allocated into two groups: Acute stress group (AS) and Control group (CG). Pigs coming from AS group (12 pens) were exposed to a stress protocol during 2 consecutive days by mixing animals of contiguous pens during 20 minutes. The remaining animals (12 pens; CG) were maintained without disturbing them as a control group. After 15 minutes of the end of each stress protocol performed to the AS pens, both groups were tested in their ability to prefer low sucrose concentrations by performing a 30 minute preference test between sucrose (0.5 or 1%) and tap-water. Solutions preferences were measured in pigs' pairs. For this purpose, 2 pigs per pen were randomly selected and allocated in a testing pen (1.6 m^2 in floor area) inside the same nursery facility. Pigs were offered 2 equidistant dishes with 800 ml of the carbohydrate and water solutions. Sucrose concentrations (0.5 or 1%) were counterbalanced in each group across pens and between tests. In this way, half of pens in each group (6) were tested in the first day to prefer sucrose 0.5% or tap water and the remaining pens (6) to prefer sucrose 1% or tap water and in the reverse way the second testing day. The positions of sucrose and water solutions were also counterbalance (left/right) across pens so each solution appeared equally often on the left and on the right. Solution intakes of both dishes during the choice test were calculated after 30 minutes by measuring the difference between the solution volume in each dish at the beginning (800 ml) and end of each test. At a first sight, spillage was not visually important and, as a consequence, was not accounted for when measuring solution consumption. No feed or water deprivation was applied to pigs in the experiment. However, feed was removed for 1 hour before the beginning of each test and it was returned to each pen at the end of the choice test.

2.2.2 Experiment 2. Psychological stress performed by movement restriction during the nursery period and its effect over sucrose detection.

Another group of pigs (240) coming from the following weaning period of the same experimental farm were used in this experiment. As in experiment 1, animals were allocated in 24 pens (10 pigs/pen) inside the nursery facility. On week 3 after weaning (42d-old), pigs were separated into 2 groups: the psychological stress group (PS; 12 pens) and the control group (CG; 12 pens). Two animals in each PS pen were randomly selected and subjected to a psychological stress protocol. Each PS animal chosen was immobilized for a 3-min period, 3 times a day for 3 consecutive days. The immobilization was performed by placing the nursery pigs into an elevated plastic box (0.4 x 0.35m) with four openings to put each pig's legs, in this way the animal was totally immobilized with no option to move or scape. During the stress protocol and especially in the last sessions, pigs used to defecate and urinate the box due to stress. For this reason between each animal stressed, the plastic box was cleaned with water and drying with paper towels. After 15 minutes of the last stress session (3rd session of the 3rd day) performed to the PS pens, and following the same procedure than experiment 1, both groups were tested in two consecutive testing days in their ability to prefer low sucrose concentrations by performing a 30 minute preference test between sucrose (0.5 or 1%) and tap-water. Previously stressed pigs in the PS pens and 2 pigs randomly chosen in each CG pen were allocated in pen-pairs into a new pen inside the same nursery unit (testing pen). As in experiment 1, pigs-pair were offered 2 equidistant dishes with 800 ml of carbohydrate and water solutions. Sucrose concentrations (0.5 or 1%) and positions (left/right) were counterbalanced across pens and between testing days. Solution intakes of both dishes during the choice test were calculated after 30 minutes by measuring the difference between the dishes solution volume at the beginning (800 ml) and end of each test. Other experimental procedures were the same as the first experiment.

2.2.3 Experiment 3. Stress at weaning. Pre and Post weaning evaluation of piglet's ability to detect and consume sucrose solutions.

After the evaluation of the effect of acute (Exp. 1) and psychological (Exp. 2) stress over sucrose detection in nursery pigs, the objective of this third experiment was to elucidate if the weaning process itself could be a stressful factor that may alter the recognition of this carbohydrate at low concentrations. A total of twelve litters (10 piglets/sow) were tested in two consecutive days before weaning (26-27d) to detect low sucrose concentrations by performing a 30 minute preference test between sucrose (0.5

or 1%) and tap-water (Pre-W group). In this group, differently to experiments 1 and 2, solutions preferences were measured in suckling litters as the experimental unit.

For these purpose litters were randomly selected from a universe of 35 sows available and tested inside their lactating piglets crate area. A total of 240 piglets coming from the remaining not tested litters (23) were weaned at day 28 (Post-W group). As in experiments 1 and 2, animals were allocated in 24 pens (10 pigs/pen) inside the nursery facility. One day after weaning (29d-old) 12 pens were randomly selected to detect low sucrose concentrations by performing a 30 minute preference test between sucrose (0.5 or 1%) vs. tap-water in two consecutive testing days. Pens were offered 2 equidistant dishes with 800 ml of sucrose (0.5 or 1%) and water solutions. As in nursery pigs a pre-training period (1 week before weaning) was performed to habituate both groups of piglets (Pre and Post-W group) to experimental conditions by offering them two equidistant control dishes with drinking water for 2 hours (each morning from 9-11am in each pen). Sucrose concentrations (0.5 or 1%) were counterbalanced in each group across litters (before weaning) or pens (after weaning) and between tests. The positions of sucrose and water solutions were also counterbalance (left/right) across pens so each solution appeared equally often on the left and on the right. Solution intake was calculated using the same procedures of experiment 1 and 2. As in previous experiments spillage was not visually important and was not accounted for when measuring solution consumption. No feed or water deprivation was applied to pigs in the experiment. Creep feed and pre-starter feed was removed for 1 hour before the beginning of each test in the Pre-w and Post-w groups respectively and it was returned to each litter or nursery pen at the end of the choice test.

3. Statistical analysis

Solutions consumption during the choice test after the stress protocol was analyzed with ANOVA by using mixed linear models with the MIXED procedure of the statistical package SAS® (SAS Inst. Inc., Cary NC); taking into account the effects of the group (control vs. Acute stress in experiment 1; control vs. psychological stress in experiment 2 and pre-weaning vs post-weaning in experiment 3), solution consumed (sucrose 5 g/kg, sucrose 10g/kg or water) and the interaction between the group and solution consumed as the main factors. Pig's pairs during the choice test were also included as a repeated measure specifying the covariance structure of the residual matrix as completely general (unstructured). Before ANOVA analysis normality and homoscedasticity of the dataset were analyzed by using the UNOVARIATE and GLM procedures with the Shapiro-Wilk and O'Brien's Test, respectively for each factor. As no significant p-values were obtained for any of the specific factors, the original hypothesis for normality and homogeneity of variance were accepted ($P>0.10$). The mean values are presented as LSMeans considering a significance level of 5% adjusted by Tukey. Percent preference for sucrose in each test was calculated as sucrose solution intake divided by total intake x 100.

4. Results

4.1 Experiment 1. Acute physical stress generated by mixing pigs during the nursery period and its effect over sucrose detection.

Total consumption was not different between CG and SG pigs pairs (105 vs. 129 mL; SEM 28.82; P=0.427 respectively) during the choice test between sucrose 0.5% and water solutions. When solutions of sucrose 1% and water were compared, CG and SG pairs showed again similar amounts of total consumption (138 vs. 191mL; SEM 34.14; P=0.129 respectively).

The intake of sucrose (0.5 or 1%) and water solutions by the CG and SG pigs pairs during the choice tests are summarized in **Figure 1**. Water intake tended to be higher in stressed pairs (57 vs. 132mL; SEM 30.48; P=0.099) during the choice test between sucrose 0.5% and water solutions. However, no differences were detected between sucrose consumption of both groups (153 vs. 126mL; SEM 38.07; P=0.88 for CG and SG respectively) during this test. When 1% sucrose solutions were compared with water, no differences were detected between water intake (69 vs. 124mL; SEM 23.68; P=0.123) or sucrose intake (206 vs. 258mL; SEM 56.47; P=0.789) between groups. Control pigs showed higher intakes of 0.5% and 1% sucrose solutions over water during the choice tests (153 vs. 57mL; SEM 26.78; P=0.008 and 206 vs. 69 mL; SEM 37.67; P=0.008 respectively). On the other hand, SG pigs did not show any difference between solutions intakes of sucrose 0.5% and water (126 vs. 132mL; SEM 26.78; P=0.996). However, at higher sucrose inclusion (1%) they clearly preferred sucrose (258 vs. 124mL SEM 37.67; P=0.009).

4.2 Experiment 2. Psychological stress performed by movement restriction during the nursery period and its effect over sucrose detection.

Results of Experiment 2 are summarized in **Figure 2.** As in Experiment 1, total consumption was not different between CG and PS pigs pairs (31 vs. 35 mL; SEM 6.50; P=0.501 respectively) during the choice test between sucrose 0.5% and water solutions. During the choice test between sucrose 1% and water, CG and PS pairs also showed similar amounts of total consumption (74 vs. 94mL; SEM 15.20; P=0.206 respectively). Water intake was higher in stressed pairs (10 vs. 40mL; SEM 7.61; P=0.012) during the choice test between sucrose 0.5% and water solutions. In terms of sucrose consumption, no differences were found between CG and PS pairs during this test (51 vs. 30mL; SEM 10.69; P=0.256 respectively). When 1% sucrose solutions were compared with water no differences were detected between water intake (48 vs. 58mL; SEM 22.42; P=0.969) or sucrose intake 99 vs. 130mL; SEM 18.61; P=0.389) between CG and PS animals.

In the same way as Acute physical stress performed during experiment 1, CG pairs showed higher intakes of 0.5% and 1% sucrose solutions over water during the choice tests (51 vs. 10mL; SEM 10.97; P=0.015 and 99 vs. 48mL; SEM 17.14; P=0.013 respectively). However, PS pigs pairs did not show any difference between solutions intakes of sucrose 0.5% and water (30 vs. 40mL; SEM 7.03; P=0.533). Nevertheless, at higher sucrose inclusion (1%) they clearly preferred sucrose (130 vs. 58mL SEM 21.31; P=0.027).

4.3 Experiment 3. Stress at weaning. Pre and Post weaning evaluation of piglet's ability to detect and consume sucrose solutions.

Total consumption was different between Pre-W and Post-W groups (164 vs. 281 mL; SEM 38.22; P=0.006 respectively) during the choice test between sucrose 0.5% and water solutions. Post-W group also presented a higher total intake when solutions of sucrose 1% and water were compared (220 vs. 375mL; SEM 49.54; P=0.005).

The intake of sucrose (0.5 or 1%) and water solutions by Pre-W and Post-W groups during the choice tests are summarized in **Figure 3**. Water intake was higher in Post-W than Pre-W animals (215 vs. 133mL; SEM 28.76; P=0.045) during the choice test between sucrose 0.5% and water solutions. Post-W pigs also presented a tendency to show higher intakes of sucrose than Pre-W pigs (348 vs. 194mL; SEM 65.46; P=0.056) during this test. When 1% sucrose solutions were compared with water, no group differences were detected between water intake (159 vs. 266mL; SEM 47.93; P=0.143). Nevertheless, sucrose intake was higher in Post-W animals (281 vs. 484mL; SEM 66.50; P=0.027).

Pre-W pigs failed to show intake differences between sucrose 0.5% solutions and water during the choice test (133 vs. 194mL; SEM 33.09; P=0.282). However, when the inclusion of sucrose was higher (1%) they showed a preference for sucrose solutions over water (281 vs. 159mL; SEM 42.55; P=0.041). On the other hand, Post-W pigs showed higher intakes of 0.5% and 1% sucrose solutions over water during the choice tests (348 vs. 215mL; SEM 33.09; P=0.003 and 484 vs. 266mL SEM 66.50; P=0.027 respectively).

DISCUSSION

Reward-related deficits experienced by stress involve loss of pleasure (anhedonia) in front of several activities including food intake. This study evaluated if nursery pigs could change their ability to prefer sweet solutions, known to be highly hedonic and palatable for these animals (Glaser et al., 2000), due to different kinds of stress. Results showed that acute stress (experiment 1), created by a brief animal mixing process (20 min.) and chronic stress (experiment 2) performed by movement restriction may increase pigs detection threshold to detect low concentrations of sucrose (5 g/kg) probably because of their impaired ability to experience pleasure when these hedonic ingredients are present at these inclusion levels.

Like rats and humans, anhedonia measures in pigs by using sucrose preferences seems to be a valid way to estimate specific stressful factors (Ho and Sommers, 2013; Koob and Zimmer, 2012). However, in experiment 3 when animals were tested to prefer sucrose before and after a weaning process, stressed animals (Post-W) did not show results according to anhedonia behaviours as previous experiments. Post-W pigs showed higher intakes of 1% sucrose but also for 0.5% sucrose solutions over water during the choice tests. This situation could reflect an extra energy craving created by the decrease on feed intake that animals suffer during this critical period (Dunshea et al., 2003; Moeser et al., 2012; Pluske et al., 1997, 2007) situation that could change pleasure perception because of internal needs or alliestesia (Cabanac, 1971; Guzmán-Pino et al., 2014). This behaviour did not happened on experiment 1 and 2 where feed intake seems to be not affected by the stress protocols performed. Stressor factors after weaning also could increase water intake (Bhatnagar et al., 2006; Brooks et al., 1984; Fraser 1978; Kashiha et al., 2013). This could explain why Post-W animals presented higher total intakes than Pre-W pigs in this experiment. In addition, Pre-W pigs (non-stressed animals) only presented preference for sucrose over water with the higher inclusion during experiment 3. Specific behaviours of pigs during lactation may also cause intake differences with Post-W animals elucidating the incapacity to prefer sucrose at low inclusion levels. Suckling animals are used to present low consumption of solutions or solid feed that contain palatable dairy products that even could overcome inclusions of 140g/kg (Graham et al., 1981). This situation could be produce because the highly hedonic value that maternal milk have for piglets and although it presents only around 50g/kg of lactose (Klobasa et al., 1987), with a lower sweetness power in pigs than sucrose; 1:5 ratio (Glaser et al., 2000), milk could increase its hedonic value because of previous associative learning between its flavours and maternal protection or its post-ingestive positive effects (Clouard et al., 2012; Mennella and Beauchamp, 1999). Moreover, milk also provides hydration to young animals and other of their components like fat (50g/Kg) may contribute to their palatability. All of these situations may have affected the total intake of Pre-W animals during the choice test between sweet solutions and water during experiment 3. Piglets probably redirect their appetitive behavior to milk, a most palatable source of nutrients and water, to satisfy their nutrient and water needs. Furthermore, a contrast effect between milk (highly hedonic fluid) and solutions tested in this experiment could change palatability perception, like in rats, of the lower sucrose inclusion (0.5%) decreasing its intake and creating no differences on its consumption relative to water (Dwyer et al., 2011; Flaherty and Largen, 1975).

Taking out results of experiment 3 that not fit with anhedonia literature, the clear preferences for 1% sucrose solutions over water that stressed pigs showed during all experiments are similar to results in other mammals that demonstrate that stress mechanisms create anhedonia and a decrease on solution intake only with low inclusions of sweet sources (Grønli et al., 2004, 2005; Ho and Sommers, 2013; Matthews et al., 1995; Willner, 1991; Willner et al., 1987). In the present experiments, when inclusions were increased, stressed animals were capable to detect and prefer sweet solutions even more than control not stressed animals, probably associated with a great desire and tolerance for sweet compounds. It seems that pigs like other mammals (Koob and Zimmer, 2012) probably suffer deficits in their hedonic capacity after being exposed to stress but their preferences for intense sweetness could be more pronounced due to this rewarding deficit that could increase craving for palatable compounds (Torres and Nowson, 2007). This increase in sweet carbohydrate craving is observed in depressed humans and therefore presumably in depressed non-human animals like rats and, probably as these results suggests, in pigs (Barr and Phillips, 1998; Groesz et al., 2012). Similar results were observed by Menella et al. (xxxx) where she observed that chronic stress or depression in kids may change their ability to feel pleasure by using low sucrose inclusions in water to reduce thermic stress but the same kids showed a greater tolerance than control kids to extremely elevate (> 30%) sucrose inclusion in water. Preferences for sweet carbohydrates as sucrose in this case may be related to the physiological and analgesic properties of sugars (Blass and Hoffmeyer, 1991; Blass and Watt, 1999; Slater et al., 2010; Taddio et al., 2009). Under productive conditions analgesic properties of sugars could also work by using them previously to stressful conditions associated with pain like castration or post-weaning injuries because of mixing. This could be an opportunity to use sweet compounds to increase pig's welfare without changing routine procedures.

However, the validity of anhedonia and the stress models related has been questioned because a decrease in sucrose consumption is not consistently observed after stress procedures among laboratories and experiments [Grønli et al., 2005; Harris et al., 1997; Matthews et al., 1995; Willner, 1997]**.** Sweet preferences and acceptance may change due to several factors like racial and age differences in some mammals like humans (Schiffman et al., 2000; Pepino and Mennella, 2005) so it's expected that also could change between pigs breeds or different productive stages in these animals. In addition, not all sweet compounds could work to estimate anhedonia or to decrease stress levels in pigs (Glaser et al., 2000; Harris et al., 1997). All of these factors have to be clarified before trying to develop specific anhedonia measures or use sweet compounds to reduce or measure stress. Furthermore, palatability measures could fit even better with anhedonia and hedonism in general than preferences or acceptance during the intake. Measures performed in rats and humans like lick cluster size (Dwyer, 2008; Spector et al., 1998) and taste reactivity test (Berridge, 2000; Grill and Norgren 1978) could be develop in productive animals like pigs to estimate in a better way intake pleasure and anhedonia (Clouard et al., 2014).

Investigating anhedonia in productive animals is methodologically challenging, but potentially important for welfare and nutrition fields. New animal models could be used to improved knowledge in anhedonia field but also to understand and improve specific animal's conditions. Last experiments in non- conventional species like horses (Fureix et al., 2015) showed that sucrose intake could be used to determinate differences between animals with or with-out depression-like conditions with most withdrawn horses eating the least sucrose. However, because of procedures in that experiment used solid blocks of sucrose it was not possible to see detection or intake differences relative to sucrose inclusion levels that used to appear in previous anhedonia experiments. The present study in pigs could be the beginning of anhedonia research area in productive animals, improving their productive conditions as well as welfare.

CONCLUSION

We measured intake of sucrose at two inclusion levels relative to water to estimate anhedonia in pigs, to our knowledge ,research never previously applied in this productive animals. Stress procedures that usually occur after the weaning process may create several problems that influence feeding behaviour of animals decreasing feed intake and affecting animal's growth and health (Maniam and Morris, 2012; Pluske et al., 1997). The change on pig's ability to prefer palatable compounds as sucrose because of stress observed represent an extra problematic factor to post-weaning problems and its need to be attended. Present results suggest that by reducing stress, pigs may increase the preference for diets with low inclusion of palatable compounds. This could have repercussions on feed formulation enabling to reduce inclusion amounts of specifics additives like palatable flavours or milk derivatives that are associated with higher costs and also could help to increase the total feed intake without changing the formulation especially during the first weeks after weaning.

ACKNOWLEDGEMENTS

The present study was supported by the Spanish Government (MCI, project PET2008_0002). We want to thank the Chilean research fellowships “Becas Chile”, and Sergio Guzmán, Rosa Franco and Alexandra for their collaboration during the experiments.

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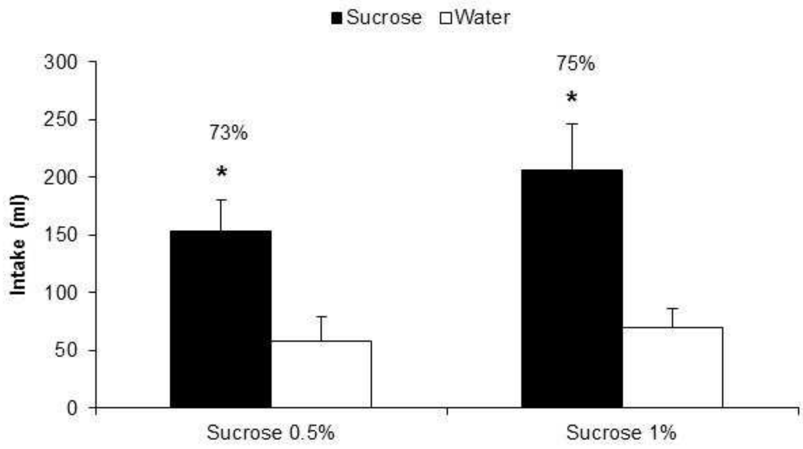
Figure Captions

A)



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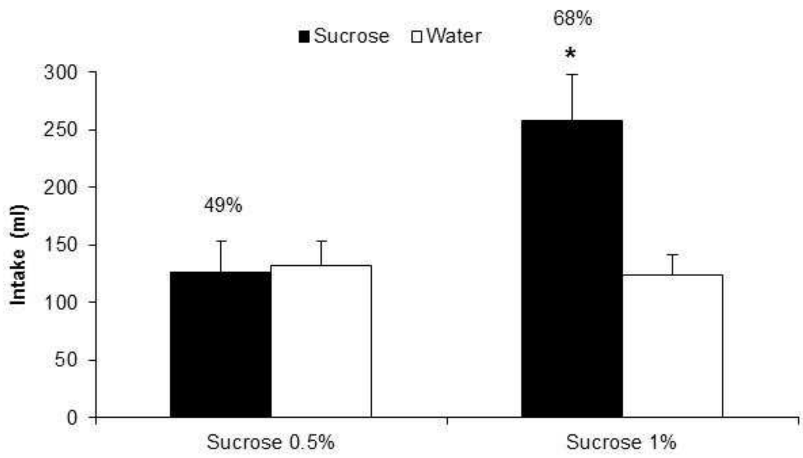
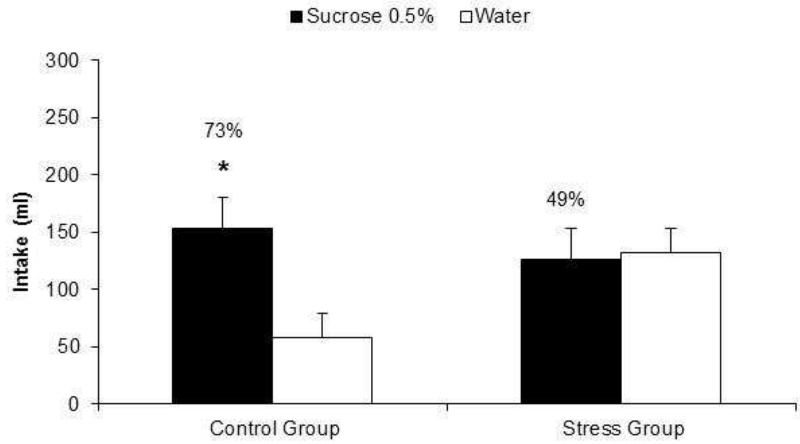


Figure 1. Experiment 1. Means (+SEM) solution intake of control (A) and stressed (B) nursery pig's pairs offered sucrose or water solutions after 30 min. choice test. Animals of stress group (B) were mixed during 15 minutes with aliens (next door) nursery pigs before the choice test. Numbers in the top of bars indicate the average value of the corresponding percentage of preference for the sucrose solutions. Asterisks indicate that intake is significantly different between sucrose and water solutions for each sucrose concentration (5 or 10 g/kg) (*P<0.05).

A)



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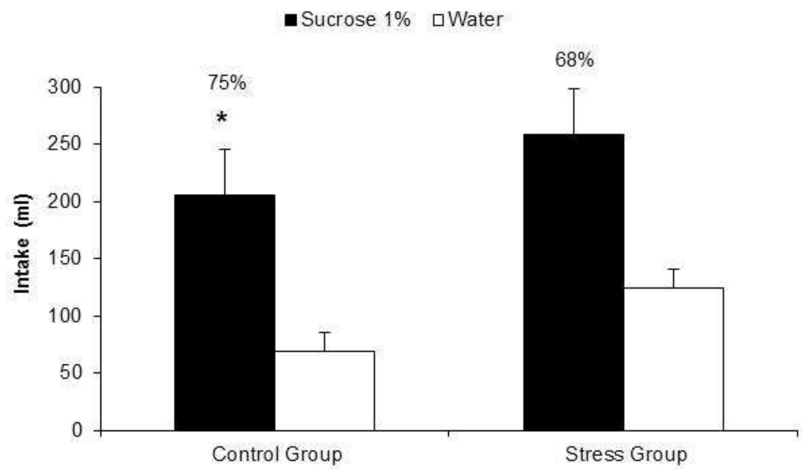
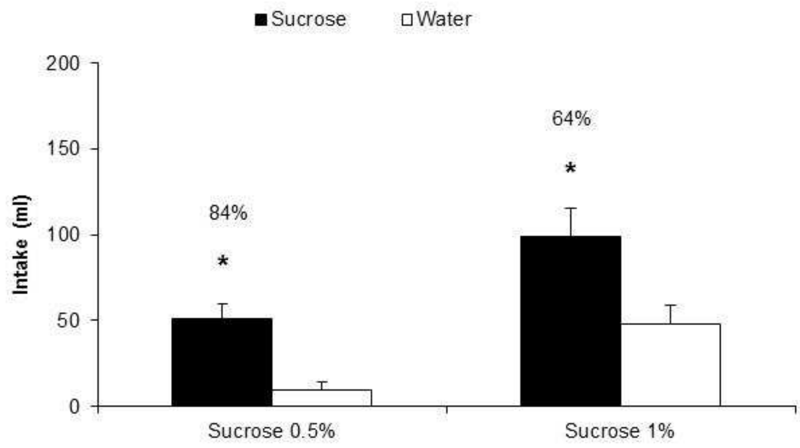


Figure 1. Experiment 1. Means (+SEM) solution intake of nursery pig's pairs offered 0.5% sucrose vs water solutions (A) or 1% sucrose vs water solutions (B) after 30 min. choice test. Animals of stress group (B) were mixed during 15 minutes with aliens (next door) nursery pigs before the choice test. Numbers in the top of bars indicate the average value of the corresponding percentage of preference for the sucrose solutions. Asterisks indicate that intake is significantly different between sucrose and water solutions for each sucrose concentration (5 or 10 g/kg) (*P<0.05).

A)



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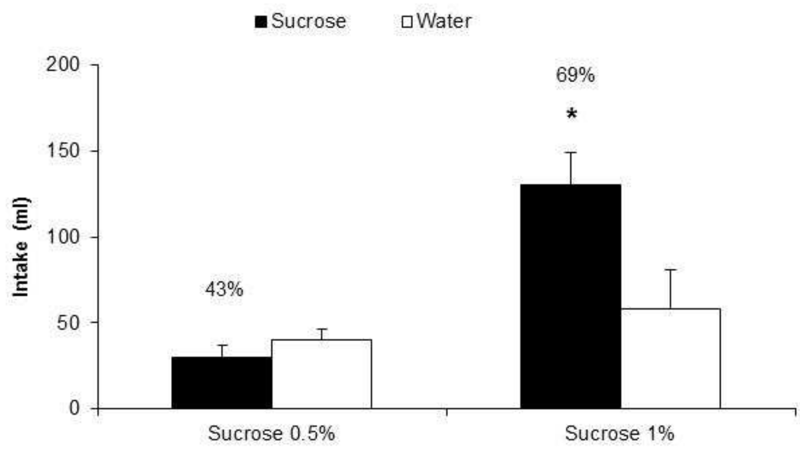
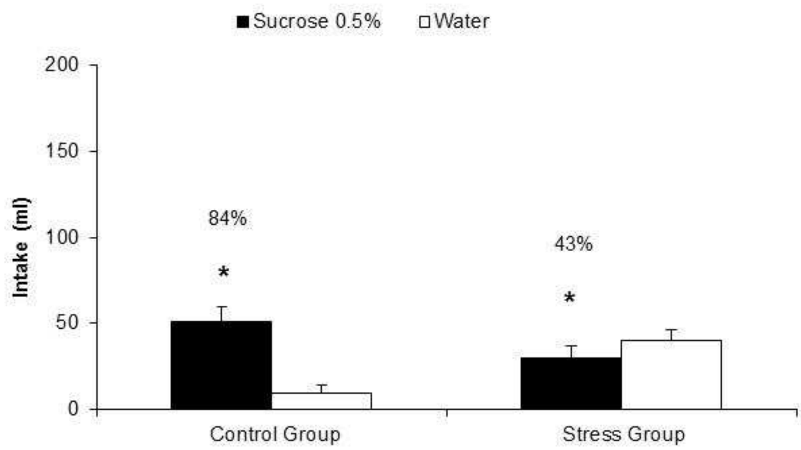


Figure 2. Experiment 2. Means (+SEM) solution intake of control (A) and stressed (B) pig's pairs offered sucrose or water solutions after 30 min. choice test. Animals of stress group (B) were psychological stressed by immobilisation (3min/ 3times d/3d) during the nursery period before the choice test. Numbers in the top of bars indicate the average value of the corresponding percentage of preference for the sucrose solutions. Asterisks indicate that intake is significantly different between sucrose and water solutions for each sucrose concentration (5 or 10 g/kg) (*P<0.05).

A)



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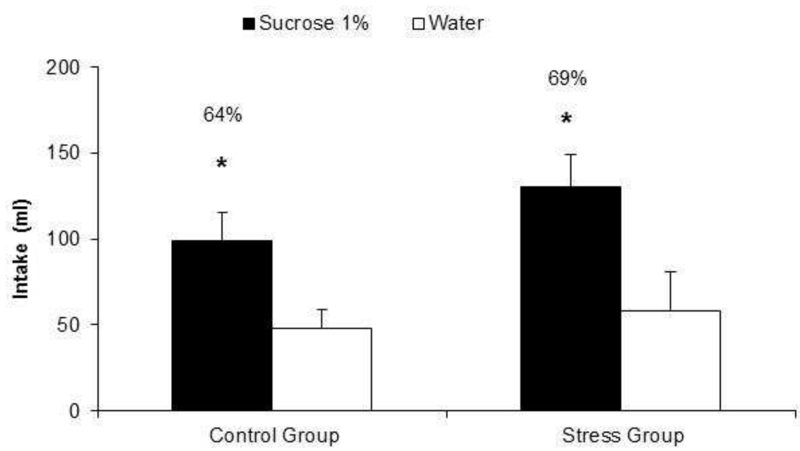
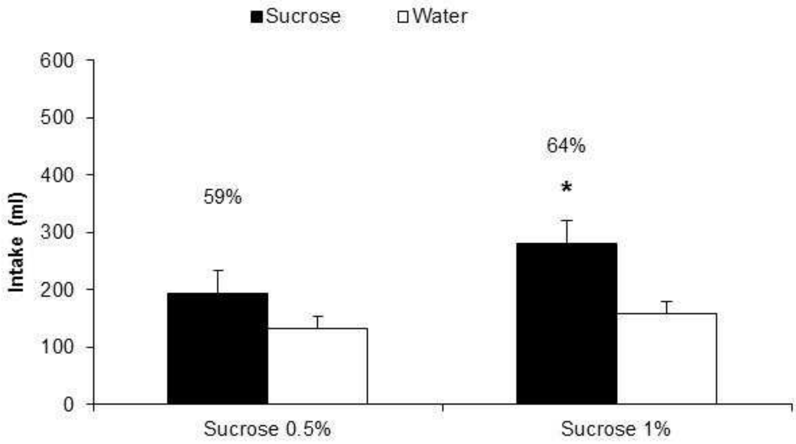


Figure 2. Experiment 2. Means (+SEM) solution intake of pig's pairs offered 0.5% sucrose vs water solutions (A) or 1% sucrose vs water solutions (B) after 30 min. choice test. Animals of stress group (B) were psychological stressed by immobilisation (3min/ 3times d/3d) during the nursery period before the choice test. Numbers in the top of bars indicate the average value of the corresponding percentage of preference for the sucrose solutions. Asterisks indicate that intake is significantly different between sucrose and water solutions for each sucrose concentration (5 or 10 g/kg) (*P<0.05).

A)



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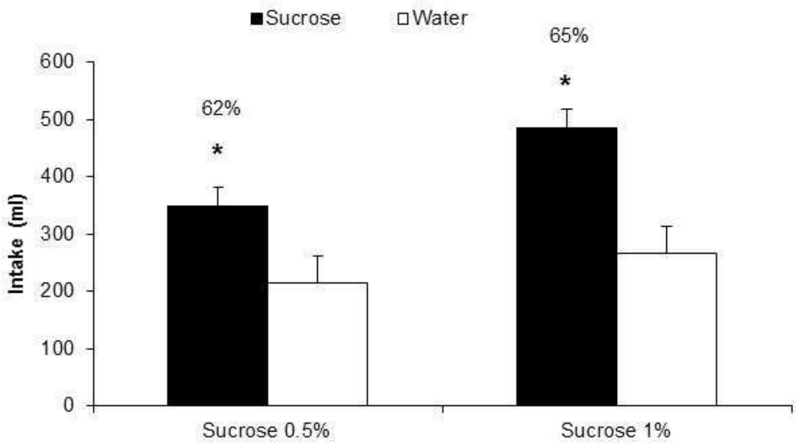
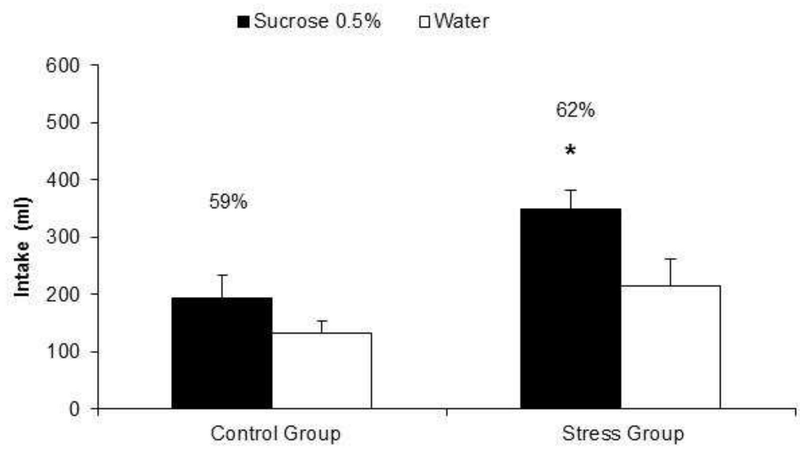


Figure 3. Experiment 3. Means (+SEM) solution intake of control (A) and stressed (B) pigs offered sucrose or water solutions after 30 min. choice test. Animals of control group were tested during the last 2 days of lactation and animals from stress group (B) were tested the first and the second day after weaning. Numbers in the top of bars indicate the average value of the corresponding percentage of preference for the sucrose solutions. Asterisks indicate that intake is significantly different between sucrose and water solutions for each sucrose concentration (5 or 10 g/kg) (*P<0.05).

A)



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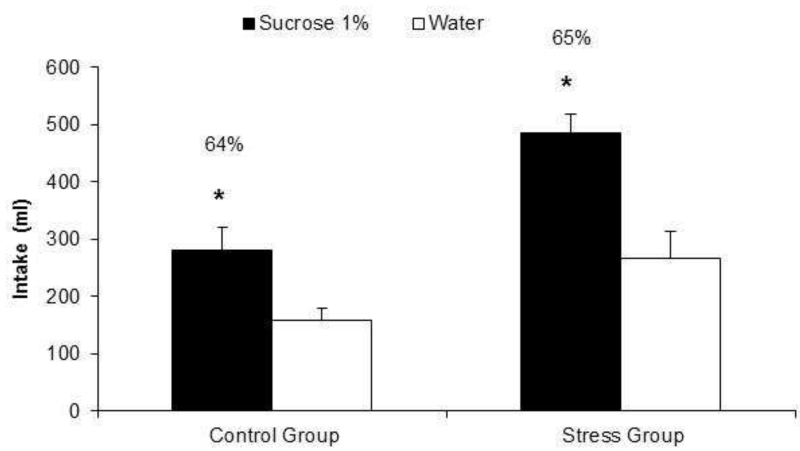

Figure 3. Experiment 3. Means (+SEM) solution intake of pigs offered 0.5% sucrose vs water solutions (A) or 1% sucrose vs water solutions (B) after 30 min. choice test. Animals of control group were tested during the last 2 days of lactation and animals from stress group (B) were tested the first and the second day after weaning. Numbers in the top of bars indicate the average value of the corresponding percentage of preference for the sucrose solutions. Asterisks indicate that intake is significantly different between sucrose and water solutions for each sucrose concentration (5 or 10 g/kg) (*P<0.05).