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Anhedonia in pigs? Effects of social stress and restraint stress on sucrose preference

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HIGHLIGHTS

- Pigs were exposed to social mixing (Exp. 1) or restraint (Exp. 2) stress.
- Sucrose preferences (0.5 and 1%) relative to water were measured after stress.
- Stressed animals failed to prefer low sucrose concentrations (0.5%) after stress.
- Stress could reduce intake of low hedonic solutions in pigs reflecting anhedonia.

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ABSTRACT

The fact that consumption of normally palatable foods is affected by stress in both humans and rats suggests a means to assess hedonic reaction in non-verbal animals. However, little is known about anhedonia and stress in productive animals such as pigs. Thus we examined the separate effects of social stress and restraint stress in 42-day old pigs on the preference for dilute sucrose solutions over water. Pigs in the social stress group (SS) were mixed with unfamiliar animals from separate pens for two 20 minute periods (Experiment 1). Pigs in the restraint stress group (RS) were immobilized three times a day, for 3-min periods, on 3 consecutive days (Experiment 2). Consumption of dilute sucrose solutions and water was examined after these stress manipulations and in the unstressed control groups (CG). Pigs were tested in pairs (12 control and 12 experimental) with a choice between water and sucrose solutions (at either 0.5% or 1%) during 30 min sessions. In both experiments CG pigs showed higher intakes of 0.5% and 1% sucrose solutions over water. Neither SS nor RS pigs consumed more 0.5% sucrose than water, but both groups did consume more 1% sucrose than water. Both social stress and restraint stress reduced sucrose preference at low concentrations but not at higher concentrations suggesting that stress may limit food consumption in pigs unless a palatable feed is present. In addition, the results suggest that stress reduces the hedonic impact of dilute sucrose. Therefore, sucrose preference may be a useful test for the presence of stress and anhedonia in pigs.

1. Introduction

Sensory pleasure is an important determinant of behavior. Whether a sensation is pleasurable or not is determined both by the stimulus itself and by the state of the animal experiencing it. Therefore, a given food or solution could be perceived as pleasant or unpleasant according to the internal status of a mammal [1,2]. In this way, hedonic reactions during intake are affected not only by food or solution characteristics but also by an animal's physiological (e.g., nutrient status, sickness, and internal temperature) and psychological status (e.g. stress, depression, and anxiety). In the case of psychological status, it has been reported that chronic stress may reduce the ability to experience pleasure in response to typically positive stimuli thereby displaying an analogue of anhedonia [3]. In humans, a lowered ability to experience pleasure is observed in disorders such as depression where anhedonia could be regarded as a core symptom [4,5].

Non-human animals exposed to inescapable stress develop behavioral consequences consistent with anhedonia in humans. Unpredictable stressors have been shown to induce changes in a wide range of behavioral parameters, including feeding behavior [6,7]. In particular, decreased consumption of palatable solutions at low concentrations

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has been suggested as a reliable indicator of an anhedonic state [8]. This is based on the idea that the post-ingestive and oral effects of sucrose, which normally activate pleasure pathways and usually increase preference or acceptance, could be perceived in a different way if an animal is in an anhedonic state. A well-studied example comes from the "Chronic Mild Stress" (CMS) procedure, where mild and unpredictably rotating stressors are presented over several days [9]. The normal hedonic value of weak sucrose solutions relative to water does not appear to be reflected in the consumption responses of rats exposed to chronic stress, making this a useful tool for anhedonia evaluation [10]. It is also the case that total intake of these sweet solutions tends to decrease, and thus both preference (i.e. with a choice between sucrose and water) and acceptance (i.e. with only sucrose available) tests have been used [11,12] — although preference tests do have the advantage of potentially controlling for differences in thirst or overall feeding motivation.

Importantly, chronic stress appears to create a selective effect on hedonic responses or "liking" for sweet solutions that is different from the desire or "wanting" to work for the same stimulus. That is, animals subjected to CMS appear to want the dilute sucrose solution as much as non-stressed animals because the reduction in free consumption was not accompanied by a reduction in instrumental behavior directed to obtaining the same reward [13]. The separate hedonic and motivational effects appear to be reflected in separate neurobiological systems. For example, modulation of the mesolimbic DA system and doses of neuroleptics inhibit lever-pressing for either food or water but leave consummatory responses intact [14,15]. Studies also showed that lesions of the 6-OHDA in the nucleus accumbens cause a suppression in food consumption without affecting hedonic reactions to a sweet solution [16]. In contrast opioid modulations within sub-regions of the ventral palladium and nucleus accumbens appear to selectively influence hedonic responses [17,18].

Although effects of stress on dilute sucrose consumption are well characterized, stress is known to alter feeding responses in a bidirectional pattern such that intake may increase or decrease depending on the type and severity of stressor, or availability of highly palatable food [19]. For example, consumption of dilute sucrose solutions is typically reduced by stress manipulations, while stressed rats can consume significantly more concentrated sucrose than control animals [12,20,21]. Something similar may occur in humans where stress can result in greater palatable or comfort food intake associated with significantly decreased healthy eating [22,23]. An increase in carbohydrate "craving" is commonly observed in depressed humans and may perhaps be seen in "depressed" non-human animals in response to highly palatable compounds [13]. We will return to the implications of bidirectional effects of stress on food intake, and the selectivity of consumption changes, in the general discussion, but for the moment we would simply note that the experimental work reported here focused on dilute sucrose solutions: both for the pragmatic reason that consumption of such dilute solutions appears to have been most sensitive to stress effects in laboratory animals and because consumption of dilute sucrose solutions appears to be less sensitive to general motivational manipulations such as changes in food restriction than are more concentrated solutions (e.g. Spector et al. [48]).

While anhedonia in humans and rodents has been extensively studied, little information exists about anhedonia in species not conventionally used in the lab such as horses [24] or productive animals like pigs. In the period after weaning, production pigs have to cope with many stressors including mixing with unfamiliar animals, new solid diets that could trigger neophobia, transportation, new environments, and maternal separation. In this critical period many pigs suffer feeding behavior problems and most weaned animals are reluctant to eat, leading to weight loss in the first days after weaning [25,26]. Pigs, like rats and humans, present an innate and strong preference for sweet compounds like sucrose [27,28]. These compounds have been used to enhance feed palatability and to facilitate the intake of neutral flavors by associative learning [29]. Because stress is thought to change the hedonic perception of palatable compounds, pigs may well perceive sweet compounds in a different way during or after stress. Therefore stress could affect the level of palatable compounds needed to enhance animals' intake. Moreover, examining preference or acceptance for dilute sucrose could be a useful tool to measure anhedonia and to detect stressful situations that could affect welfare in these productive animals. Thus the present study examined whether the preference for dilute sweet solutions changes in pigs in response to either social mixing or physical restraint stressors.

2. Materials and methods

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).

2.1. Subjects

A total of 96 male and female pigs ([Large White × Landrace] × Pietrain) taken from two consecutive 240 animal weaning cycles were tested for their sucrose preferences after stress across the two experiments described here (48 pigs/experiment). All animals were individually identified at birth by using a plastic ear tag and stayed with their mother and littermates inside the farrowing crates and the corresponding area for piglets during the entire suckling period (28 days). Piglets were weaned at an average of 27 ± 2.3 days of age with a body weight of 7.2 ± 1.03 kg. 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 unflavored complete feed (pre-starter: 0–14 days, or starter: 15–35 days post-weaning). No general feed or water deprivation was applied to pigs in the experiment. However, their normal feed was removed for 1 h before the beginning of each test and it was returned to each pen 1 h after the end of the preference test in order to prevent feed consumption from interfering with fluid consumption. 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. During the second week of weaning, pigs were habituated to the future experimental conditions inside their own pens by offering them two equidistant control dishes with drinking water for 2 h (each morning from 9 to 11 am). After the experiments, 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. Social stress generated by mixing pigs during the nursery period and its effect on sucrose consumption and preference

A total of 240 post-weaned pigs taken from a single weaning cycle (42 days-old) were kept in 24 pens (10 pigs/pen). Two animals of a similar weight from each pen were selected and allocated so as to form two equal body weight groups (10.52 and 10.55 kg respectively, SEM: 0.35 kg; $F(1, 22) < 0.01$, $P = 0.957$): the social stress group (SS) and control group (CG). Pigs in the SS group (12 pens) were exposed to a stress protocol over two consecutive days by mixing the animals of each pen ($n = 10$) with the animals of their adjacent pen ($n = 10$) for 20 min. The remaining animals (12 pens; CG) were maintained without mixing. Fifteen minutes after the end of each stress period, both groups were tested by performing a 30 minute preference tests between sucrose (0.5 or 1%) and tap-water. Pigs were tested in pairs because individual testing produces more vocalizations, attempts to escape and a longer latency for the first solution contact observed [30–33]. Thus, two pigs per pen were selected and allocated to 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 sucrose and water solutions. All animals performed the preference choice with both concentrations. Half of pens in each group (6) were tested on the first day with 0.5% sucrose and tap water and the remaining pens (6) with 1% sucrose and tap water with the assignment of test solutions reversed on the second testing day. The positions of sucrose and water solutions were also counterbalanced (left/right) across pens so each solution appeared equally often on the left and on the right. Intakes from both dishes during the choice tests were calculated by measuring the difference between the solution volume in each dish at the beginning (800 ml) and end of each test with a plastic graduated cylinder. No significant spillage was apparent on visual inspection. The experimental unit was the pig pair with the consumption measure reflecting the total amount consumed by both pigs in the pair.

2.2.2. Experiment 2. Restraint stress performed by movement restriction during the nursery period and its effect on sucrose consumption and preference

A second group of pigs (240) coming from the following weaning period of the same experimental farm was used in Experiment 2. As in Experiment 1, animals were allocated in 24 pens (10 pigs/pen) inside the nursery facility. On week 3 after weaning (42 days-old), pigs were separated into 2 equal body weight groups (10.63 and 10.64 kg, SEM: 0.34 kg; $F(1, 22) < 0.01$, $P = 0.994$): the restraint stress group (RS; 12 pens) and the control group (CG; 12 pens). Two animals in each RS pen were randomly selected and subjected to a physical stress protocol. Each RS animal chosen was immobilized for a 3-min period, 3 times a day for 3 consecutive days. The interval between the three stress procedures within a day was approximately 1 h and the stress protocol started each morning at 10 am. Immobilization was performed by placing the pigs into an elevated plastic box (0.4×0.35 m) with an opening for each of the pig's legs thus providing no option to move or escape. During the stress protocol (especially in the latter sessions) pigs were seen to defecate and urinate in the box. For this reason the plastic box was cleaned with water and dried with paper towels after each use. The first test session was performed 15 min after the last stress session (3rd session of the 3rd day), and the second test was performed the following day. The control group animals remained in their pens while the restrain stress was applied to the experimental group animals. The test procedure and counterbalancing was the same as in Experiment 1.

2.3. Statistical analysis

Solution consumption during the preference tests was analyzed with ANOVA by using mixed linear models with the MIXED procedure of the statistical package SAS® (SAS Inst. Inc., Cary NC). Data was analyzed in each experiment taking into account the effects of sucrose concentration (0.5 or 1%), group (control vs. social stress in Experiment 1 and control vs. restraint stress in Experiment 2), solution consumed (sucrose or water) and the interaction between these main factors. Pig pairs during the choice test were included as a repeated measure specifying the covariance structure of the residual matrix as completely general (unstructured).

As will be detailed later on, overall levels of consumption differed between the social stress and restraint stress experiments. Therefore, in order to allow a direct comparison of the two stress effects, a combined analysis was performed by using the preference ratios for sucrose. Ratios were calculated as consumption of the sucrose solution divided by the consumption of the sucrose solution and water added together. Data also was analyzed with ANOVA by using the same procedure of SAS® taking into account the effects of sucrose concentration (0.5 or 1%), group (control vs. stress), kind of stress (social or restraint stress) and the interaction between these main factors. For this measure, values above 0.5 reflect a preference for the sucrose solution, values below 0.5 reflect avoidance of the sucrose solution and values around 0.5 reflect no preference between the sucrose solution and water.

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 tests, respectively for each factor. As no significant P-values were obtained for any of the specific factors, the original hypotheses for normality and homogeneity of variance were accepted ($P > 0.10$). Mean values are presented as LSMeans, with simple effect comparisons between means with a significance level of 5% (adjusted by Tukey).

3. Results

3.1. Experiment 1. Social stress generated by mixing pigs during the nursery period and its effect on sucrose consumption and preference

The intake of sucrose (0.5 or 1%) and water solutions by the CG and SS pig pairs and the sucrose solution preferences expressed as percentages are summarized in Fig. 1. Inspection of this figure suggests that control animals preferentially consumed sucrose over water at both 0.5 and 1% concentrations, while pigs subject to social mixing stress showed a preference for sucrose over water only at 1% concentration but not 0.5% concentration. This suggestion is broadly in line with the results of the ANOVA analysis. There was a main effect of concentration [$F(1, 22) = 5.39$, $P = 0.030$] whereby consumption was higher overall

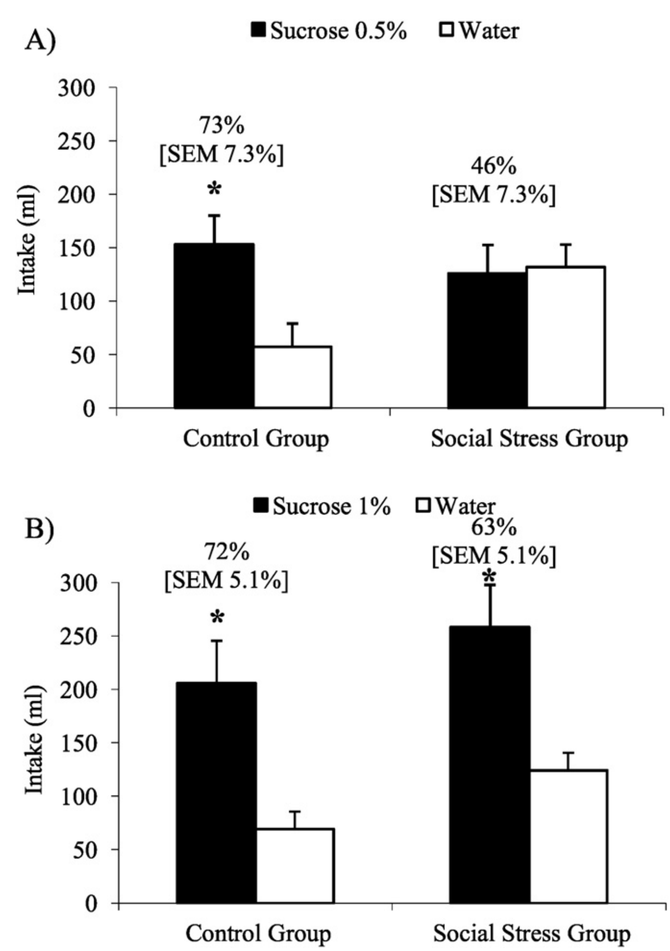

Fig. 1. Experiment 1. Mean (+SEM) solution intake measured in volume (ml) of nursery pig pairs offered 0.5% sucrose vs. water (A) or 1% sucrose vs. water (B) during 30 min. Choice tests after being exposed (social mixing stress group) or not (control group) to social mixing stress. Numbers above the bars indicate the average value of the corresponding percentage preference for the sucrose solutions. Asterisks indicate that intake is significantly different between sucrose and water for each group and sucrose concentration (*$P < 0.05$). Error bars: ±1 SEM.

on the 1% than 0.5% test session. This appears to have been largely due to differences in sucrose rather than water consumption with higher consumption of 1% sucrose than 0.5% sucrose and little difference in water consumption across tests as reflected in the solution by concentration interaction [F(1, 22) = 8.96, P = 0.007]. There was no overall difference between stress and control animals in terms of total consumption [F(1, 22) = 2.55, P = 0.125] and no interaction between the experimental group and the consumption in each test [F(1, 22) = 0.56, P = 0.463].

Although no interaction was observed between group (stressed or control) and the type of solution consumed (sucrose or water) [F(1, 22) = 2.22, P = 0.151], and no significant three way interaction between group, solution, and concentration [F(1, 22) = 2.70, P = 0.115], the simple effect comparisons do suggest that the preferences for sucrose over water were not the same between stress and control groups. Control pigs showed higher intakes of 0.5% and 1% sucrose solutions over water during the choice tests (t (22) = 3.58, P = 0.029 and t (22) = 3.63, P = 0.027 respectively). On the other hand, SS pigs did not show any difference between solution intakes of sucrose 0.5% and water (t (22) = 0.22, P = 1.000). Nevertheless, at the higher sucrose concentration (1%) they clearly presented a higher intake of sucrose solutions relative to water (t (22) = 3.56, P = 0.031).

3.2. Experiment 2. Stress performed by movement restriction during the nursery period and its effect over sucrose detection

The intake of sucrose (0.5 or 1%) and water solutions by the CG and RS pig pairs and the sucrose solution preferences expressed as percentages are summarized in Fig. 2. As in Experiment 1, control animals presented higher consumption of sucrose than water at both 0.5 and 1% concentrations, while pigs subjected to restraint stress showed (as with social stress) preference for sucrose over water only at the 1% concentration. The results of the ANOVA analysis show that there was a main effect of concentration [F(1, 22) = 6.60, P = 0.017] whereby consumption was higher overall on the 1% than 0.5% test session. Once again this may be explained by the differences in sucrose rather than water consumption with higher consumption of 1% sucrose than 0.5% across tests as reflected in the solution by concentration interaction [F(1, 22) = 11.62, P = 0.002].

The groups differed in their overall consumption [F(1, 22) = 11.10, P = 0.003] with RS pigs displaying higher intakes than control pigs. There was an interaction between the experimental group and the sucrose concentration [F(1, 22) = 6.24, P = 0.021] where control and RS pigs showed a similar consumption when sucrose was tested at 0.5% but not when it was tested at 1% where RS pigs presented a higher total consumption. No interaction was observed between the group (stressed or control) and the type of solution consumed (sucrose or water) [F(1, 22) = 0.95, P = 0.341].

There was a three way interaction between group, solution, and concentration [F(1, 22) = 6.75, P = 0.016]. Control pigs showed higher intakes of 0.5% sucrose over water (t (22) = 3.50, P = 0.035) although this difference was not significant for 1% sucrose over water (t (22) = 2.83, P = 0.136). On the other hand, SS pigs did not show any difference between intakes of sucrose 0.5% and water (t (22) = 1.34, P = 0.873). Nevertheless, at the higher sucrose concentration (1%) they clearly presented a higher intake of sucrose relative to water (t (22) = 4.74, P = 0.002).

3.3. Comparison of social and restraint stress effects using sucrose preferences

There was a main effect of concentration on sucrose preference [F(1, 46) = 9.31, P = 0.004] where pigs presented a generally higher preference for 1% sucrose over water than for 0.5% sucrose. There was also a main effect of stress [F(1, 46) = 0.61, P = 0.001] whereby control animals presented higher sucrose preference than stressed animals (t (46) = 3.49, P = 0.001). Critically, there was an interaction between

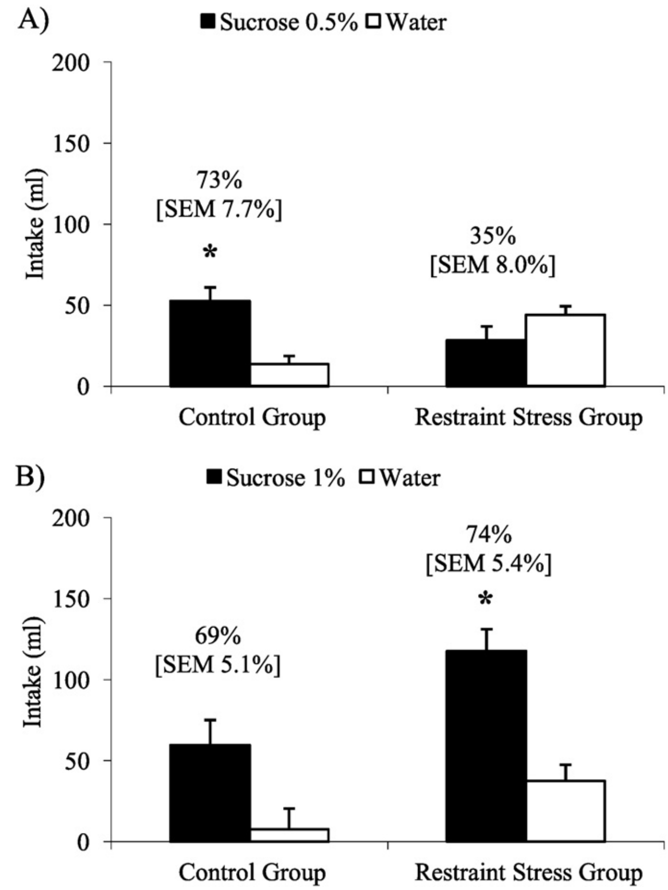

Fig. 2. Experiment 2. Mean (+SEM) solution intake measured in volume (ml) of pig pairs offered 0.5% sucrose vs. water (A) or 1% sucrose vs. water (B) during 30 min. Choice tests after being exposed (restraint stress group) or not (control group) to restraint stress. Numbers above the bars indicate the average value of the corresponding percentage preference for the sucrose solutions. Asterisks indicate that intake is significantly different between sucrose and water for each group and sucrose concentration (*P < 0.05). Error bars: ±1 SEM.

stress and concentration [F(1, 46) = 12.1, P = 0.001] where control animals presented a higher preference than stressed animals for 0.5% sucrose relative to water (t (46) = 4.20, P < 0.001) but not for 1% sucrose relative to water (t (46) = 0.38, P = 0.981). In relation with the type of stress (social mixing vs. restraint) there was no effect of the type of stress on preference for sucrose [F(1, 46) = 0.01, P = 0.922], nor was there an interaction between type of stress and sucrose concentration [F(1, 46) = 1.35, P = 0.252] or between type of stress and group [F(1, 46) = 0.02, P = 0.895]. The three way interaction between type of stress, group, and concentration was not significant [F(1, 11) = 2.00, P = 0.163].

A comparison of the preference ratios for each condition and test session against the neutral point of 50% reveals the following for the social stress experiment: There was a clear preference for sucrose at 0.5% (t (11) = 4.54, P < 0.001) and 1% (t (11) = 6.37, P < 0.001) in control animals; stressed animals did not prefer 0.5% sucrose over water (t (11) = −0.71, P = 0.491), but did present clear preferences for 1% sucrose over water (t (11) = 2.51, P = 0.029). This reinforces the idea that – despite the absence of the 3-way interaction in the analysis of raw consumption – sucrose preference did differ between the mixing stress group and their control group at the lower, but not higher, sucrose concentration. For the restraint stress experiment, control animals showed a trend to prefer (over 50%) sucrose solutions relative to water when 0.5% sucrose was examined (t (10) = 1.83, P = 0.097) and a clear preference (over 50%) for sucrose when sucrose was tested at 1% (t (11) = 3.07, P = 0.011). Pigs exposed to restraint stress did not prefer 0.5%

sucrose over water (t (9) = −1.68, P = 0.127). However, when sucrose was tested at 1% pigs presented clear preferences for the sucrose solutions (t (11) = 4.54, P < 0.001) — similar to the intake results and in the same direction than data coming from Experiment 1. These preference ratio results reinforce the interpretation of the raw consumption data. Control animals displayed preferential consumption of sucrose over water regardless of the sucrose concentration. In contrast, for both social stress and restraint stress, there was evidence of preferential consumption of sucrose over water only when 1% sucrose was examined and not when 0.5% sucrose was examined.

4. Discussion

This study evaluated whether different types of stress would reduce the typical preference for sweet solutions in pigs [27]. Experiment 1 examined the effects of social stress (20 min interaction with unfamiliar animals) while Experiment 2 examined the effects of restraint (nine 3-min periods over three days). Non-stressed control pigs clearly showed preferential consumption of both 0.5% and 1% sucrose over water. In contrast, pigs subject to either social or restraint stress did not consume more 0.5% sucrose than water, but did preferentially consume 1% sucrose over water. Because absolute levels of consumption across control and stressed animals (for both sucrose and water) differed between the two experiments, an analysis of the preference ratios for sucrose over water was used to facilitate the comparison between stress manipulations. This analysis indicated that there was a difference between the social and restraint stress groups on one hand, and their respective non-stressed control groups on the other, in terms of the preference for 0.5% sucrose, but not for 1% sucrose. Moreover, the difference between stress and control groups was equivalent for both social and restraint stress manipulations. These results provide important information regarding both the use of sucrose consumption as a welfare assessment tool in the context of production systems and for the possibility that stress produces an analogue of anhedonia in pigs.

The present study provides clear evidence that both social stress and restraint stress reduce the preference for dilute sucrose solutions in weaned pigs. These results parallel rodent laboratory studies where reductions in dilute sucrose consumption and preference are well established as sensitive outcome measures of stress [6,7]. The parallels between the prior rodent and current pig studies strongly suggest that sucrose consumption and preference will be a sensitive means to assess the presence of stress effects in pigs. However, it should be noted that, the reliability of rodent stress models has been questioned because a decrease in sucrose consumption is not consistently observed after stress procedures in all laboratories and experiments [5,34,35]. Although stress has been widely described to affect consumption by several authors [3,6,11,12,20,36,37] there could be boundary conditions determining when the stress effects are apparent. For example, the fact that the current study was in the nursery period raises the possibility that the effects of the experimental stressors examined here could have been potentiated by testing against the background of this generally stressful period.

Another such possible boundary condition is suggested by the fact that the effects of stress have been shown to be bidirectional — including either a decrease or increase in food intake [38,39]. The bidirectional stress-induced changes in food intake are multifactorial and may be influenced by the type of stressor, severity of the stress being applied to an organism and palatability of the offered food or solution [19]. For example, the intensity and timing of stressors could differentially activate the HPA axis liberating different amounts of glucocorticoids and/or presenting different post-stress recovery periods for glucocorticoids to return to their baseline. Indeed, in rats there is evidence that different stressors can have markedly different levels of suppression of food intake [20]. That said, we did not find differences in sucrose preference between the two stress protocols examined here. This might reflect that fact that the design of the current experiments means that both social mixing stress and restraint stress would have allowed for some contribution of both acute and chronic stress effects. In Experiment 1, the first test was performed directly after the first mixing stress experience, and should reflect acute effects, while the second test followed an additional mixing stress exposure, and so could have reflected both acute and chronic effects. In Experiment 2, the first test was performed directly after the third day of restraint stress treatments, and so could have reflected both acute and chronic effects, while the second test followed one day later with no additional stress experience, and so should reflect only chronic effects. A preliminary analysis of both experiments including a factor of test order revealed no main effect of this factor (or interactions with other factors). However, the power of such an analysis is small given the sub-group size (n = 6), and so it is not possible to draw any firm conclusions about the relative impact of acute and chronic effects of either the mixing or restrain stressors.

Perhaps more importantly, the current studies suggest that stress manipulations only influenced sucrose consumption and preference at the most dilute, 0.5%, concentration, but not at 1%. The clear preference for 1% sucrose over water displayed by stressed pigs is similar to results in rats where stress produces a decrease in low but not high concentrations of sucrose [40–42]. However, it should be noted that the point at which increases in sucrose concentration begin to attenuate differences in consumption between stress and control animals appears to be somewhat higher in rats than in pigs. For example, Willner et al. [41] report that, although suppression of sucrose consumption following chronic mild stress was greatest at 0.7%, it was still apparent at 2.1 and 7.6%, and only at 13.6% and 34% were no effects observed. Even though the current data clearly exemplifies the value of using sucrose consumption and preference as a means of investigating stress and other welfare challenges in pigs, the differences between previous rodent studies and the current experiments with pigs do suggest that further work may be needed to optimize the methodology for use in production contexts.

In addition, the current data also have implications for the practice of using sweet and other palatable compounds to enhance feed consumption and to overcome the challenges produced by stressful or painful procedures associated with the production systems such as castration, early age weaning or vaccination with circovirus [29]. Because stressed animals only showed increases in consumption at the higher concentration tested, it implies that stress will affect the level of palatable compounds needed to enhance animals' intake. That is, higher levels of inclusion of palatable compounds may be needed in order to motivate additional consumption than would be expected from studies of non-stressed animals.

Thus far, we have examined the changes in sucrose consumption and preference largely in terms of their functional sensitivity to stress without a direct consideration of why stress might impact on the response to sucrose. However, in both humans and rodents, the reduction in responses to normally pleasurable stimuli has been taken as a reliable indicator of the presence of anhedonia — a reduction in hedonic state [4,9,19]. Moreover, the fact that “withdrawn”, under-responsive riding horses eat less sucrose than more typically responsive animals [24] suggests that sucrose intake could be used to detect animals with depression-like conditions outside “typical” lab species. In this light, the fact that we observed reductions in sucrose preference in response to stress is consistent with the idea that the stress procedures have reduced the hedonic value of sucrose to the point where 0.5% sucrose no longer elicits a larger hedonic response than water. That is, the reduction in dilute sucrose consumption may reflect the production of stress-induced anhedonia.

However, it should be acknowledged that it is also possible that the absence of a preference for 0.5% sucrose is related to sensory dysfunctions produced by stress such that the presence of this dilute solution is simply not detected. This possibility is a reminder that multiple processes can contribute to an end-point measure of consumption and preference. As a result, consumption and preference are sensitive, but not always selective, assays of hedonic state. In rodents, the potential lack of specificity of total intake measures has been addressed by the examination of the fine details of consumption behaviors. For example, the taste reactivity test [43,44] is based on the observation that orofacial behaviors can be classified into "aversive" (e.g. gapes) and "appetitive" (e.g. tongue protrusions) patterns while the analysis of licking microstructure has revealed that the mean size of licking clusters is directly related to solution palatability [45,46]. Critically, these assays can separate hedonic from non-hedonic influences on consumption — for example pairing sucrose with electric shock will suppress consumption but not produce a change from appetitive to aversive taste reactivity patterns [47] while concentrated sucrose solutions are consumed in small volumes but with large lick cluster sizes [45,48]. Indeed, we have preliminary evidence that consumption patterns in pigs also appear to be related to sucrose concentration (Frias et al., in preparation). Therefore, there is the potential for future studies to develop assays, based on the existing rodent measures, which may directly assess hedonic reactions for production animals such as pigs.

5. Conclusion

Investigating anhedonia and behavioral assays of stress in productive animals is methodologically challenging, but potentially important for welfare and nutrition fields. Here, we report that both restraint stress and social stress reduced consumption of, and preference for, dilute sucrose solutions in pigs. Although an entirely novel approach in production animals such as pigs, changes in the consumption of weakly palatable solutions as a result of stress have been attributed to hedonic changes in other mammals [8,10,36] suggesting that stress may have induced an analogue of the anhedonic state seen in human depression. But regardless of whether changes in sucrose consumption and preference are mediated by the induction of anhedonia, the present results provide a clear demonstration that preference for dilute sucrose is highly sensitive to stress effects in pigs. Taken alongside the rodent literature, these results establish the generality of sucrose preference as a test of stress effects. In short, the sucrose preference test appears to be a general assay of stress or low welfare that has great potential value in production animals such as pigs as well as in the experimental laboratory.

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