

## Differential dose effects of central CRF and effects of CRF astressin on pig behavior

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### Abstract

The elevation of central corticotropin releasing factor (CRF) causes an increase in behavioral activity, including increases in overall activity and oral/nasal/facial (ONF) chewing–rooting–rubbing behaviors in the pig and similar behaviors in other species. This study detailed changes in the frequency, duration and sequences of behaviors after central administration of vehicle or porcine CRF (pCRF at 0.5, 5.0, 50 and 150  $\mu\text{g}$ ). A sequential analysis described the complex behaviors induced in a dose-dependent fashion by central pCRF. The frequency and duration of ONF behaviors were significantly increased among pigs receiving 50  $\mu\text{g}$  of pCRF. For behaviors such as ONF, 50  $\mu\text{g}$  represented a breakpoint at which the frequency and duration of single behaviors increased. Pigs receiving 50  $\mu\text{g}$  of pCRF were considerably more active and exhibited more ONF behaviors than did pigs receiving lower doses. The highly sensitive sequential analysis revealed that very low doses of central pCRF induced subtle changes in sequences of behaviors. Low doses of central pCRF (0.5  $\mu\text{g}$ ) induced fear-related behavioral sequences that included ONF behaviors alternating with periods of inactivity. Central injection of astressin, a CRF receptor antagonist, blocked many, but not all, of CRF-induced behaviors. Compared with saline-injected control pigs, central pCRF increased general activity, ONF, fear-related freezing and sham chewing behaviors. When pCRF was given following astressin, fear-related freezing behaviors were not different compared with pigs receiving saline. However, pigs given astressin plus pCRF showed elevated sham chewing compared with saline-injected control pigs, as did pigs receiving intracerebroventricular (ICV) pCRF. These data indicate that central pCRF activates brain mechanisms associated with hyperactivity, ONF and fear-related behaviors, whereas other behaviors induced by pCRF may be nonspecifically mediated by CRF. Astressin antagonized some, but not all, pCRF-induced behaviors. This model represents the induction of hyperactivity and stereotyped behaviors, which may represent a new model for the study of mania or obsessive-compulsive behaviors.

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### 1. Introduction

Corticotropin releasing factor (CRF) plays a major role within the brain to coordinate the stress response, and it has been shown that CRF mediates various psychopathological states [1–4]. It has been suggested that CRF may also coordinate autonomic and behavioral responses in stress,

including feed intake, anxiety-like behaviors, arousal, learning and memory [2,5–9]. The role of CRF has been extensively studied in rodents, but less is known about the role of this particular neuropeptide in other species. The CRF system is a pivotal system in the response of the organism to stress, it is therefore important to understand how it works, especially at a time at which specific CRF antagonists are becoming available and are proposed as antistress or antianxiety drugs.

Direct administration of CRF into the central nervous system (CNS) produced a dose-dependent behavioral activation in rats [1,7], thus implicating its role in the

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behavioral response of animals. Behaviorally, CRF injections into the ventricles will induce anxiety-like responses in several animal tests of anxiety [10,11]. The availability of CRF antagonists is increasing, which provides a meaningful tool to use to elucidate the precise role of CRF and its receptor in regulating specific behaviors. Astressin, a novel 30-residue peptide CRF receptor antagonist, has been shown to inhibit CRF-induced effects in the brain and periphery [12,13]. Astressin binds both CRF<sub>1</sub> and CRF<sub>2</sub> receptors. It has been demonstrated that astressin can inhibit CRF- and stress-induced anxiety in mice [14] and reverse anxiogenic-like responses induced by social stress and central administration of CRF on the elevated plus-maze, but astressin did not inhibit CRF-induced locomotor activity in a familiar environment [15]. However, a CRF<sub>1</sub> specific antagonist, antalarmin, can block the motor-activating effects of CRF [16]. It seems likely that astressin may have a unique, but not complete, inhibitory effect on CRF-induced behaviors in rodents.

Behavioral responses of CRF in pigs have not been extensively studied. Parrott [17] was the first to investigate the behavioral and endocrine effects of CRF in the pig, finding that operant feeding was significantly reduced following an injection of ovine CRF. In addition, CRF has been reported to elicit hyperactive behavior and vocalization within minutes following an injection of porcine CRF [18,19]. The pigs' behavioral patterns when exposed to central CRF were indicative of anxiogenic behavior; pigs paced back and forth and repeated certain oral behaviors in stereotyped fashion. More recently, it has been reported that centrally administered CRF-like peptides have anxiogenic effects in the pig [20]. The current study aimed to (i) thoroughly investigate the effects of graded doses of central CRF on behavior and behavioral sequences in a familiar environment and (ii) document the effects of astressin and its ability to antagonize the behavioral responses induced by central CRF.

## 2. Methods and materials

### 2.1. Animals and housing

Female half-sibling crossbred piglets were selected at 3 weeks of age and housed individually in stainless steel metabolism crates (2×1 m) under a light–dark cycle (12-h light:12-h dark; 0600 h:1800 h) in a vivarium maintained at 27 °C. Pigs could freely move and turn around throughout the entire experimental period. For video-recording purposes, red lights were placed in the room and remained on 24 h a day. All animals were meal fed (0.46 kg/day) an early wean commercial diet (Moormans Frosty Coats) at 0700 h, and water was available *ad libitum*. Daily human–animal interaction occurred at 0700 h, which consisted of touching the animals, giving the animals treats of maple syrup and getting the animals accustomed

to the investigators so that stress could be avoided later in the protocol when minimal handling was required. Twelve pigs were used in Experiment 1 and three pigs in Experiment 2. In Experiment 1, each pig was evaluated with at least two of the five dose treatments, while in Experiment 2, each pig experienced each treatment. Due to the intense, stressful experience associated with the intracerebroventricular (ICV) administration of porcine CRF (pCRF), a minimum number of animals were used. The behavioral effects of ICV CRF lasted 6 to 8 h. The day after CRF treatment, the pigs seem less active, and by the following day, their behavior is apparently normal. No negative health effects were observed for pigs given acute ICV CRF in our previous work [19] or in this study. All procedures were approved by and in compliance with the Institutional Animal Care and Use Committee at Texas Tech University.

### 2.2. Cannulation of the lateral ventricles

Pigs were allowed to acclimate at least 7 days to their new housing environment before surgery. Animals were fasted 24 h prior to surgery. Surgery was conducted under aseptic conditions. The intracerebroventricular (ICV) cannulation procedure was performed as previously described [19]. Prior to intubation, pigs were anesthetized. Throughout the surgical procedure, the pigs were deeply anesthetized with Halothane (2%) and maintained on oxygen (2 l/min). The animal's head was oriented such that the forehead rose at a 30° angle to the horizontal plane in a modified sheep–pig stereotaxic unit. A small circular incision was made on top of the head with a trephine, and the skin and underlying periosteum were removed. The raw edges of the skin were cauterized to prevent excessive bleeding. A 2-mm hole was made in the skull (coordinates from bregma: +5 mm anterior and +5 mm lateral). An 18-gauge sterile needle was used to penetrate the dura mater before the 30-mm stainless steel guide cannula (20 gauge) was inserted into the brain. An injection cannula (22 gauge) was inserted and lowered until negative pressure was achieved and saline freely flowed through the cannula and into the lateral ventricle. Once the appropriate depth was established, based on negative pressure, the proper length of the guide and injection cannulas were selected for each pig. The actual depth or length of the guide cannula ranged from 12 to 37 mm, and the injection cannula projected 1 to 3 mm beyond the guide cannula. In all cases, the 12-mm depth (from the base of the skull to the dorsal aspect of the ventricle) entered the ventricle. Pigs of this age are variable in size of body, head and skull, which accounted for the variable cannula length. The guide cannula was anchored in place to the skull using four sterile screws and dental acrylic. Over several days, antibiotics were administered to each pig. Analgesic (Banamine, 1.1 mg/kg) was administered immediately following surgery and every 24 h for 72 h. Cannula placement was confirmed at necropsy.

### 2.3. Peptide injection

Porcine CRF (pCRF; American Peptide, Sunnyvale, CA, USA) and Astressin (CRF receptor antagonist; Sigma, St. Louis, MO, USA) were dissolved in sterile saline. ICV injections were 200  $\mu$ l in volume and injected slowly over time (3–5 min) using the appropriate length injection cannula for that particular animal. Animals were injected in their home cages without restraint.

### 2.4. Central administration of CRF and antagonist

On the day of each experiment, pigs were randomly assigned either a hormone or control treatment. Each pig served as its own control. As an additional control, saline-injected animals served as controls for vehicle and time-of-day effects. Pigs were habituated to ICV injections by daily handling and manipulation of cannula for 10 to 14 days prior to experiments. Throughout the entire experimental period, pigs moved freely throughout their cages. At least 48 h elapsed between treatments on individual pigs.

All treatments were administered at 0800 h. In Experiment 1, 12 pigs were injected via ICV cannula with either vehicle or pCRF (0.5, 5.0, 50 or 150  $\mu$ g). In addition, each pig had a pretreatment control behavior sample collected. In Experiment 2, pigs were injected with the antagonist (100  $\mu$ g astressin) 30 min prior to the administration of pCRF (50  $\mu$ g). A total of three pigs was evaluated under three conditions: following an ICV injection of physiological saline, following ICV pCRF and ICV pCRF when pretreated with astressin. Pigs were videotaped 24 h before and after treatments were administered. Behavioral reactions were focused on the 6-h period immediately following the administration of treatments.

### 2.5. Behavior recordings

Each pig was videotaped at 30 frames/s in their home cages for 6 h following the central injection of treatments. Behavioral frequencies were averaged over 2-h periods for the entire 6-h observation period. Two trained observers (with interobserver correlations exceeding 0.9) watched the tapes at 30 frames/s or in slow motion (depending on the pigs' activity level); however, only one observer watched the videos from a given experiment. Data were entered into the Observer, a computerized behavioral analysis program (Noldus, Leesburg, VA, USA), which summarized the frequency, duration and sequences of behaviors. One can calculate the duration per bout by dividing the duration by the frequency. In this study, this information provided no additional information.

All behaviors were mutually exclusive. The observers were blind to treatments. Only one observer was used per experiment to evaluate all pigs and treatment groups. Observers were trained to record consistent data from stock tapes in the laboratory.

Feeding, drinking, standing, walking, sitting, lying (still or moving) and oral/nasal/facial (ONF) behaviors were recorded. Oral/nasal/facial behaviors included rooting (pressing snout against the floor or penning materials), chewing (open mouth on bars or penning material) and licking/sniffing [tongue on penning material, typically accompanied by moving the rooting disk (tip of the snout) indicating sniffing]. Another behavior noted for certain pigs was described as "fit". A fit resembled convulsions or rapid movements, including twisting of the body, lying down rapidly, then standing and jumping. In the astressin study (Experiment 2), the behavioral catalog was expanded by separately recording ONF as root/lick and sham/chew; furthermore, stand/walk was subdivided into walk, freeze and rear/escape. All behaviors were described in detail in a previous publication [19].

### 2.6. Statistical analysis

Analysis of variance was performed on durations of behaviors using general linear model procedures, and means were separated with the predicted difference test (PROC GLM of SAS software; SAS Institute, Cary, NC, USA), with the exception of the dose–response study. The mixed models procedure (PROC MIXED) of SAS was used to analyze the data for the dose–response study to accommodate the missing samples of certain pigs for certain doses (each dose was represented by two or three pigs). Behavioral durations were normally distributed. Each pig served as its own control; thus, the experimental unit used was the pig. However, in the analysis of behavioral sequences, the experimental unit used was the individual behaviors, not the pig. Control data were collected at the same time of day as treatments and after a saline ICV injection. In addition, control and treated pigs were examined simultaneously on a given day, which controlled for day effects. The error term used to test treatment effects was the treatment-by-pig effect. The error term used to test for treatment-by-time interactions was treatment-by-pig-by-time (residual error). The antagonist data were compared with data from the same pig's control data (negative and positive controls). Behavioral durations expressed as percentage of time were compared using Student's *t*-tests comparing treatment with control values. Simple linear regression using polynomial equations was used to describe the relationship between the dose of pCRF and behavioral measures. Data were analyzed using the regression procedures within Microsoft Excel (Redmond, WA, USA), and for the analysis of variance, SAS software was used (SAS Institute). Polynomial lines were presented to describe the relationships over doses of peptide.

In addition to the parametric analyses described above, behavioral sequences were analyzed using a number of approaches, with individual behaviors as the experimental unit. Markov-chain analyses were performed using the sequences of mutually exclusive behaviors. A lag-sequential

analysis was performed according to the methods described by Fagen and Young [21]. In this analysis, the data were examined using a one- and two-behavior lag in the sequential analysis. The lag analyses were consistent with the single transition data set. It was not possible to perform continuous-time semi-Markov analysis due to a separation of data into duration and sequential data sets during original data collection. To compare behavioral sequences among treatments, sequence data were compared using chi-square analysis [19]. In this technique, the sequences that fell out of the Markov-chain analyses as significantly greater than expected by random chance were compared with control values.

### 3. Results

Studies were conducted to determine pCRF dose–response relationship for stereotyped oral/nasal/facial behaviors (ONF) behaviors in pigs. The effects of ICV administration of saline or various doses of pCRF on mean behavioral frequencies and durations are shown in Table 1. Lying and ONF behaviors were influenced by ICV pCRF (Table 1), but no differences were observed for maintenance behaviors (e.g., walk, drink, or stand; Table 1). Pigs' behavioral responses to pCRF for lying and ONF behaviors are shown in Fig. 1. The central administration of 50 µg of pCRF increased the duration of ONF behaviors compared with 0, 0.5 and 5 µg of pCRF [ $t(1,7)=2.74, 2.54$  and  $2.60$ , respectively,  $P<0.05$ ]. Pigs receiving 50 µg of pCRF were active (nonresting) during the entire observation period. Activity included alternating walking and standing while engaged in vigorous ONF behaviors, which one could subjectively describe as 'appearing agitated' based on their behavioral profile. Instead of simply rooting/licking the

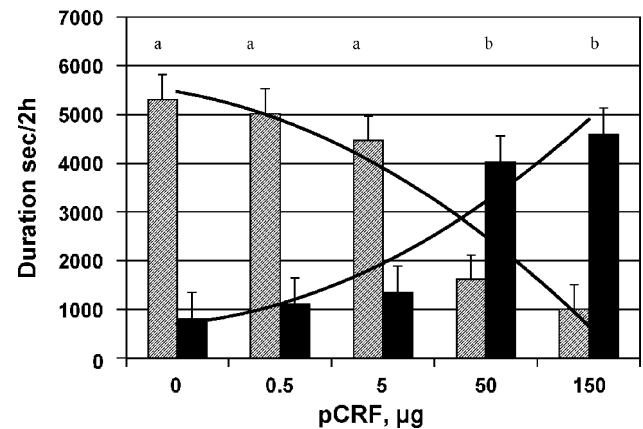


Fig. 1. Data are presented as duration of lying and ONF behaviors (in s; mean±S.E.M.). Striped bars represent lying down behavior and solid bars represent ONF behaviors. Pigs received ICV injection of vehicle or pCRF (0.5, 5.0, 50 or 150 µg). Plotting the duration of lying and ONF behaviors over time and using a second-order polynomial regression trend line, pigs receiving at least 50 µg of ICV pCRF were considerably more active and exhibited more ONF behaviors than did pigs receiving lower doses of pCRF (lying,  $y=209.7x^2+54.7x+5628.4$ ,  $R^2=0.924$ ; ONF,  $y=213.1x^2-228.9x+718.8$ ,  $R^2=0.912$ ). Analysis of variance revealed a significant overall effect ( $P<0.05$ ), and means differed where superscripts differ (a and b;  $P<0.05$ ).

ground and lying down, high doses of pCRF evoked ONF behaviors, which were followed by walking behavior. This increase in locomotor activity and apparent agitation may be indicative of an "anxiety" state in pigs. In addition, there was an affect of pCRF on lying behavior. Fifty micrograms of central pCRF caused a decrease in the duration of lying compared with other treatments [ $t(1,7)=2.4$ ,  $P<0.05$ ]. During these active bouts, pigs were always engaged in other activities, which appeared to be indicative of 'agitation' and a hyperactive state. Lower doses of pCRF had no effect on pig behavior (Table 1).

Table 1

Effects of central administration of saline or various doses of pCRF on mean behavioral frequencies (least squares mean±S.E.) averaged per 2-h period taken over a 6-h period

Measure	Dose of porcine CRF (µg)					SE	Dose P-value
	0	0.5	5.0	50	150		
<i>Frequency (number/2 h)</i>							
Walk	23.9	22.3	63.8	104.7	66.3	29.2	0.31
Lying	16.4	27.8	10.6	42.7	36.0	12.1	0.32
Drink	2.2	4.8	12.0	12.6	14.0	7.8	0.79
Fit	3.0 <sup>a</sup>	5.0 <sup>a</sup>	8.0 <sup>a</sup>	24.9 <sup>b</sup>	6.5 <sup>a</sup>	6.1	0.14
Oral/Nasal/Facial	46.2 <sup>c</sup>	44.3 <sup>c</sup>	78.6 <sup>c,d</sup>	179.7 <sup>d</sup>	134.0 <sup>c,d</sup>	33.8	0.23
Stand	40.6	30.7	83.7	110.5	86.2	35.6	0.52
<i>Duration (s/2 h)</i>							
Walk	131.5	140.0	268.3	448.2	357.9	118.1	0.33
Lying	5310 <sup>a</sup>	5026 <sup>a</sup>	4469 <sup>a</sup>	1615 <sup>b</sup>	3436 <sup>a,b</sup>	963	0.12
Drink	40.0	140.8	161.3	85.1	93.6	88.0	0.87
Fit	392.4	126.2	300.1	629.1	192.5	367.9	0.86
Oral/Nasal/Facial	799 <sup>a</sup>	1102 <sup>a</sup>	1350 <sup>a</sup>	4026 <sup>b</sup>	2637 <sup>a,b</sup>	833	0.10
Stand	527.1	198.1	385.2	361.3	477.8	186.0	0.80

Least-squares means within a row with uncommon subscripts differ: <sup>a,b</sup> $P<0.05$ ; <sup>c,d</sup> $P<0.08$ .

N=12 pigs (each pig received at least two doses of pCRF).

Table 2  
Sequences of oral/nasal/facial (ONF) behavioral frequencies with other behaviors for control or pCRF-treated pigs

		Dose of pCRF ( $\mu\text{g}$ ) or no injection					
		None	0	0.5	5	50	150
Number of pigs per dose		3	2	2	3	3	2
Behavior 1	Behavior 2						
Lying	ONF	130*	132	354*	82*	376*	513*
ONF	Lying	176*	192	345*	128*	428*	546
Stand	ONF	120*	426	321*	952*	1376	1062*
ONF	Stand	78*	366*	282*	534	862	888
ONF	Walk	100	141*	174*	596*	1192*	693*

Chi-square for table=1010.5;  $P<0.001$ .

$N=3$  pigs per treatment.

\* Represents a behavioral sequence that contributes significantly (individual cell chi-square value of over 6.63,  $P<0.01$ ) and is either greater or less than the expected value. ONF behaviors are associated with chewing and rooting.

Plotting the durations of lying and ONF behaviors over time and using a second-order polynomial regression trend line revealed that animals receiving 50  $\mu\text{g}$  of ICV pCRF were considerably more active and exhibited more ONF behaviors than did pigs receiving lower doses of pCRF (lying,  $y=209.7x^2 + 54.7x + 5628.4$ ,  $R^2=0.924$ ; ONF,  $y=213.1x^2 - 228.9x + 718.8$ ,  $R^2=0.912$ ; Fig. 1). In fact, behavioral activation, measured as the frequency and duration of single behaviors, peaked at 50  $\mu\text{g}$  of pCRF (Table 1). There were no behavioral differences observed for 0, 0.5 and 5.0  $\mu\text{g}$  of pCRF. A clear break point was found in the activation of ONF between 5 and 50  $\mu\text{g}$  pCRF when single behaviors were examined (Table 1).

Vocalization was observed (but not quantified) for pigs receiving 50 and 150  $\mu\text{g}$  of pCRF. The highest dose of pCRF seemed to evoke a more desperate seeming and longer sustained vocalizations than 50  $\mu\text{g}$ , whereas the lower doses (0.5 and 5.0  $\mu\text{g}$ ) of pCRF did not induce vocalization.

Sequences of locomotor (walking) activity increased, and maintenance behaviors decreased in ICV pCRF-injected pigs, whereas the sequence of behaviors in saline-injected pigs remained constant and simple. Behavioral sequences for ONF behaviors are shown in Table 2. The chi-square analysis discriminated between randomly increased behavioral sequences and sequences that might be changed due to treatment effects. Oral/nasal/facial (ONF) sequence frequencies were increased by central pCRF in a dose-dependent fashion (Table 2). Compared with saline, differences in the frequencies of ONF behavioral sequences were evident at a dose as low as 0.5  $\mu\text{g}$  of pCRF and continued to increase as the dose of pCRF increased (Table 2). In addition, it appears that as the dose of central pCRF increases, the repertoire of non-ONF behaviors became less complex, but the frequency of the ONF behavioral sequences increase with increasing doses of pCRF (Table 2).

The behavioral effects of the nonspecific CRF receptor antagonist, astressin, are shown in Table 3. Astressin

Table 3

Effects of the control, 50  $\mu\text{g}$  pCRF or 100  $\mu\text{g}$  of astressin+50  $\mu\text{g}$  pCRF on the percentage of time that pigs spent engaged in behaviors for 6 h after ICV administration of pCRF or saline (control)

Behavior	Control		ICV pCRF		Astressin+pCRF		Student's <i>t</i> values (when significant)		
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Control vs. pCRF	Control vs. astressin+pCRF	pCRF vs. astressin+pCRF
Feed	2.5	0.5	11.7	7.6	1.5	1.0			
Root/lick <sup>a</sup>	3.5	2.4	43.4	1.1	4.6	1.0	23.1**		37.9**
Head shake	0.0	0.0	0.3	0.2	0.2	0.02		18.8**	
Sham chew <sup>a</sup>	0.0	0.0	6.0	1.6	1.8	0.7	7.4*	5.3*	
Drink	0.5	0.5	0.4	0.08	1.4	0.1			-10.6**
Walk	9.7	1.7	18.6	2.6	3.9	0.9		-4.5*	8.5*
Freeze	0.0	0.0	18.5	6.7	2.9	2.2	5.5*		
Rear/escape	0.0	0.0	0.9	0.3	0.2	0.1	*7.0		
Run	0.0	0.0	1.4	0.4	0.0	0.0	*7.5		7.5*
Sit	0.0	0.0	0.0	0.0	0.03	0.03			
Lie down	83.8	0.8	0.0	0.0	83.5	1.6	-217.3**		-106.0**

Astressin was injected centrally 30 min prior to pCRF.

$N=3$  pigs per treatment.

<sup>a</sup> Components of ONF behaviors.

\*  $P<0.05$ .

\*\*  $P<0.01$ .

blocked many of the major behavioral effects induced by ICV pCRF. Specifically, astressin blocked pCRF-induced increases in general activity, as reflected by lying down behaviors and ONF rooting, licking and chewing behaviors (Table 3). Sham chewing in pigs receiving astressin prior to pCRF was significantly higher than in pigs receiving saline (Table 3). The duration of fear-related (freezing, escape attempts) behaviors expressed by pigs receiving astressin plus pCRF was not different from that of the control or pCRF-injected pigs. Central pCRF increased fear-related behaviors compared with controls but were not different from pigs receiving astressin plus pCRF (Table 3). In addition, running was increased among pCRF-injected pigs compared with control pigs and pigs receiving astressin plus pCRF (Table 3).

#### 4. Discussion

Animal management and husbandry practices have intensified in recent decades, resulting in the potential for stressors related to boredom and lack of stimulation. Older production systems induced different stress responses (e.g., thermal stressors). The objective assessment of specific behaviors expressed by animals (and humans) experiencing stress will contribute to a better understanding of animal (and human) welfare. The CRF system is a pivotal system in the response of organisms to stress; therefore, it is important to understand CRF-behavior mechanisms. We examined the effects of various doses of pCRF on behavior and the effect of CRF antagonist on CRF-induced behavior. Despite these large differences among means, overall, the dose–response effects on behavior were not significant. This nonsignificant omnibus finding is most likely due to the low animal number, coupled with the large number of doses tested. The biological significance of these findings was consistent across Experiments 1 and 2, such that 50  $\mu\text{g}$  of ICV pCRF consistently increased general activity and induced ONF behaviors in pigs (Tables 1 and 3).

Central injections of 50  $\mu\text{g}$  of pCRF increased general activity and induced ONF behaviors in pigs in a dose-dependent fashion (Table 1). Pigs receiving higher doses of pCRF were constantly walking and standing while engaged in vigorous ONF behaviors and appeared agitated, as indicated by their behavioral profile. Instead of simply rooting/licking the ground and lying down, high doses of pCRF caused ONF behaviors, which were followed by locomotion (walking); this behavioral profile appears to be indicative of an anxiety-like state in pigs. Central pCRF increased lying bouts while decreasing the duration of lying (Fig. 1). During these lying bouts, pigs were always engaged in other activities, which appeared to be indicative of agitation and a hyperactive state. Lower doses of pCRF had no effect on single behaviors of the pigs. Sequences of locomotor activity increased, and maintenance behaviors decreased with higher doses of pCRF, whereas the sequence

of behaviors in saline-injected pigs remained complex (Table 2). In addition, vocalization was observed, but not quantified, in the pigs receiving 50 and 150  $\mu\text{g}$  of pCRF. The highest dose of pCRF seemed (subjectively) to evoke a more desperate and longer sustaining vocalization than 50  $\mu\text{g}$  did, whereas the lower doses of pCRF (less than 50  $\mu\text{g}$ ) did not induce vocalization. These results are consistent with findings that central administration of pCRF induces hyperactivity, vocalization [19] and vigorous oral–nasal activity [20] in pigs.

Stress induced by central pCRF seems to activate brain mechanisms associated with hyperactivity and ONF behaviors. The behavioral profile induced by central pCRF in this study was not generally influenced by animal–human interactions because individuals were not present during the experiment. However, some minor effects of handling were observed, not on behavioral frequencies or duration, but on the more sensitive sequences of behavior (Table 2). Pigs given ICV saline, compared with nontreated pigs, show more ONF and standing in sequence. One explanation for this effect is that injection of ICV saline may increase central CRF through a handling effect.

Others [20] have speculated that the presence of a person may have influenced the behavioral scores in their study due to a potential effect of animal–human interactions. Although it appears that the increase in locomotor activity and the behavioral profile induced by central pCRF in a familiar environment is indicative of an emotional state that is similar to “anxiety” [10,20], we are uncertain whether these behaviors are actually equivalent to the anxiety states reported in rodents because these studies describe the emotional state of pigs in response to pCRF in a familiar environment [19,20], not pigs exposed to a defined experimental paradigm of anxiety often used in rodent studies. However, these findings strongly support the notion that central pCRF induces a behavioral profile that includes hyperactivity–agitation, fear (freeze) and ONF behaviors, which may be indicative of an anxiety state in pigs. If CRF modulates or coordinates responses to environmental stress, then the CRF-induced behavioral profile is less similar to the confined sow that shows oral-based stereotyped behaviors and is more similar to a state of hyperactivity. This type of hyperactive behavior has been observed in young pigs that have been isolated from other pigs or their mother. In this situation, the pig vocalizes and runs back and forth in an apparent frantic search for its mother or littermates; this fear–stress–hyperactive state is associated with acute stress.

The sequential analyses (Fig. 2) shows a progressive change in behavioral sequences with increasing dose of pCRF. Under control situations, or when given saline ICV, pigs express ONF behaviors in sequence with lying down. They commonly performed ONF and then lie down. Beginning at a dose as low as 0.5  $\mu\text{g}$  pCRF, pigs begin expressing the sequence of stand then ONF. At 5  $\mu\text{g}$  pCRF, they add to these sequences the sequence ONF–walk. This represents an increase in activity. At 50  $\mu\text{g}$  pCRF, they do

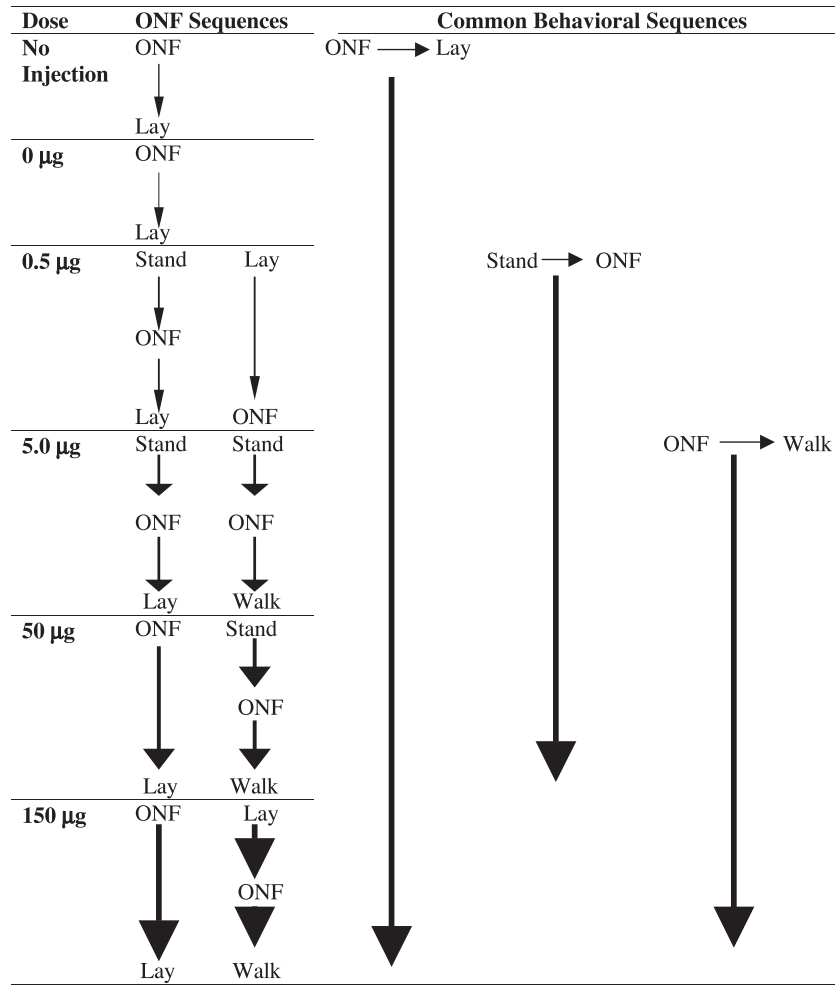


Fig. 2. Graphic representation of oral/nasal/ facial (ONF) behavioral sequences of pigs receiving no injection, ICV saline or various doses of ICV pCRF. Greater line width represents a greater frequency of behavioral sequences. Each behavioral sequence is expressed at a greater frequency ( $P < 0.01$ ) than expected due to random chance (by chi-square analysis). All behavioral sequences shown by these pigs are quantitatively presented in Table 2.

not add sequences, but they express ONF in sequence with lying, standing and walking at a higher rate. At 50 μg ICV pCRF, the pigs are very active, and (not shown in Fig. 2) they vocalized nearly continuously. At 150 μg ICV pCRF, the pigs added lying–ONF in the sequence, and they lost the sequence stand–ONF. The large increase in activity may have made the pigs tired, resulting in the need to lie down more at 150 μg ICV pCRF than at lower doses. It is clear that with increasing doses of ICV pCRF, pigs expressed ONF in sequence with more behaviors. Thus, elevated central pCRF not only increased the frequency and duration of ONF behaviors, but CRF caused the expression of new sequences of ONF and other behaviors (stand, walk and lie). Central pCRF at increasing concentrations lead to an increase in hyperactivity associated with oral and locomotor behaviors.

Central injection of the CRF antagonist, astressin (which binds both CRF<sub>1</sub> and CRF<sub>2</sub> receptors), given 30 min prior to central pCRF, blocked the major behavioral effects of central pCRF (Table 3). Specifically, compared with pigs receiving ICV saline, astressin blocked the increase in

general activity and ONF behaviors but not fear-related behaviors or sham chewing induced by pCRF. Our findings confirm that astressin has potent antagonist effects in the pig brain, although the degree to which astressin blocks CRF<sub>1</sub> and CRF<sub>2</sub> receptors remain to be determined.

In rodents, the local administration of astressin into the brain attenuated CRF- and stress-induced anxiety [14] and blocked motor activation following CRF [6]. A critical role for CRF<sub>1</sub> in CRF-induced locomotor activity has been shown to be necessary for CRF-related locomotor behaviors [16,22]. In rats, pretreatment with a CRF<sub>1</sub> specific antagonist, antalarmin, blocked the motor activating effects of CRF and anxiety behavior in the elevated plus-maze [16]. Thus, CRF<sub>1</sub> is likely to be responsible for CRF-induced locomotor activity in rats, and we speculate that the same is true for pigs. The ability of astressin to attenuate pCRF-induced locomotor activation may indicate that the CRF receptor and/or mechanism of the CRF system are different for locomotor behaviors than for sham chewing behaviors in the pig.

The results of this work contribute to our understanding of issues related to pig husbandry. Pigs in a confined setting

display ONF behaviors via mechanisms not well understood. Some argue that some ONF behaviors are indicators that the pigs are experiencing stress [23], while others have not associated ONF behaviors of confined adult pigs with stress [24]. Because astressin can antagonize CRF-activated ONF behaviors, we may use ONF to test hypotheses related to the mechanisms of ONF behaviors of pigs in commercial settings. Such information may improve our understanding of mechanisms of behaviors of importance in issues of animal welfare.

In summary, these findings suggest that central pCRF induces hyperactivity (constant movement), ONF (rooting, chewing, rubbing, etc.) and fear-related (freezing) behaviors in pigs. At higher doses of pCRF, pigs do not show their usual resting behaviors; instead, they engage in intense ONF (including chewing available substrates and sham chewing) and have frequent changes in behavioral sequences. This emotional state and the increase in locomotor activity in a familiar environment, induced by pCRF, appear to be similar to anxiety (such as freezing and repeated changes in behavioral sequences) described among rodents. In addition, astressin antagonized most, but not all, of the behavioral effects of central pCRF. Elements of fear, including freeze and rear/escape, induced by pCRF were not different from astressin plus pCRF, while resting (lying down) and root/lick were antagonized by astressin, implying that the CRF receptors may differentially regulate pCRF-induced behaviors. Thus, astressin antagonized some, but not all, pCRF-induced behaviors. This model represents the induction of hyperactivity and stereotyped behaviors, which may represent a new model for the study of mania or obsessive-compulsive behaviors.

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