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CHANGES IN OPIOID RECEPTORS OF SOWS IN RELATION TO HOUSING, INACTIVITY AND STEREOTYPIES

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INTRODUCTION

Since the demonstration of specific opioid binding sites in the central nervous system and the isolation of the first endogenous opioid peptide in 1975[1] the field of opioid research has been intensively explored. The involvement of endogenous opioid peptides in responses to stressful stimuli (stress induced analgesia, stress induced changes in locomotor activity, aggressive and defensive behaviour, stereotyped behaviour etc.[2]) is one of the most promising research areas. Sows kept in confinement develop high levels of abnormal stereotyped behaviour. Cronin and Wiepkema[3] showed that the performance of stereotypies was associated with the release of endogenous opioids. Using the opioid antagonist naloxone they were able to disrupt the performance of the behaviour without changing exploratory behaviour. The idea that stereotypies are part of a coping strategy associated with the release of endorphin was put forward. It has also been suggested that inactivity and unresponsiveness may be part of a coping mechanism.[4]

There are three types of opioid receptors, mu, delta and kappa. The pharmacological effect of mu, delta and kappa agonists are quite different. Chronic treatment with opioid ligands causes an alteration in the number of cell surface receptors. Because modulation of receptor sites reflects a relatively long term change in the environment of the receptors, we decided to look at the relationships between housing conditions, level of activity, stereotypy incidence and the three types of opioid receptors.

MATERIAL AND METHODS

Seven cross breed (Landrace X Large White) tethered sows and 4 Landrace X Large White group-housed sows were used in this study. The sows were on two different commercial farms. They were culled from the breeding herd, mainly because of poor productivity.

The behaviour data consisted of direct observation and video recording.

Opioid receptors were measured using tritiated agonists in (a) brain homogenate (frontal cortex and caudatum) and (b) autoradiography (frontal cortex, hypothalamus, caudatum, adrenal glands and pituitary). [^{3}H] [D-Ala^2 , MePhe^4, glycol^5] enkephalin (Dagol, Amersham) was used as a mu receptor ligand. The concentrations of [^{3}H] Dagol for saturation studies varied from 0.25 to 12 nM. [^{3}H] [DPen^2, Dpen^5] enkephalin (DPDPE, Amersham) was used as a delta receptor ligand. The concentrations varied

from 0.4 to 16 nM of active ligand. [^{3}H] CI 977 (synthesized for Parke Davis by Amersham International) was used as the kappa receptor ligand. The concentrations of [^{3}H] CI 977 used varied from 0.05 to 3 nM.

RESULTS

There was a considerable variation among the sows in the B_{max} (maximum binding capacity) of the three subtypes of opioid receptors namely mu, delta and kappa in the two brain areas which were investigated. The three ligands used showd a consistently lower B_{max} value for group housed sows, except [^{3}H] Dagol B_{max} in the caudatum. Group-housed sows (n=4) had a significantly lower B_{max} for [^{3}H] Dagol (mu agonist) in the pig frontal cortex (p=.002) than tethered sows (n=7). (Figure 1).

The changes in B_{max} observed in the two experimental groups were accompanied by a change in the K_d (affinity constant) for [^{3}H] Dagol (mu agonist) in both brain areas. Group-housed sows had significantly lower K_d values than tethered sows, in the frontal cortex (0.58 and 1.52 respectively), conversely the K_d of [^{3}H] Dagol in the caudatum was significantly higher for group housed sows (2.25 and 0.81)

Changes in the affinity of the ligands were only observed for [^{3}H] Dagol.

The percentage of the time during which the sows were inactive was positively correlated with [^{3}H] Dagol binding to the frontal cortex in tethered sows (rs=.893: p=0.028), suggesting a relationship between level of activity and mu receptors.

The total level of stereotypy performed by each sow was calculated by adding the individual's scores for tongue-rolling and sham-chewing (Figure 2). Scores for tongue-rolling and sham-chewing were analysed separately as well in relation to opioid receptors.

[^{3}H] CI 977 binding to pig frontal cortex was negatively correlated with performance of stereotypes (tongue-rolling and sham-chewing), (rs=0.964 : p=0.0182). This suggests that stereotypes and kappa receptors in the pig frontal cortex are related (Figure 3).

When the individual scores for tongue-rolling and sham-chewing were analysed there was a negative correlation between [^{3}H] Dagol binding and [^{3}H] CI 977 binding to pig frontal cortex and performance of tongue-rolling (rs=0.778 p=.056 for both ligands). Sham chewing on the other hand showed a negative relationship with [^{3}H] CI 977 binding to pig frontal cortex (rs=0.929: p=0.022), and no significant relation with [^{3}H] Dagol binding to the same brain area.

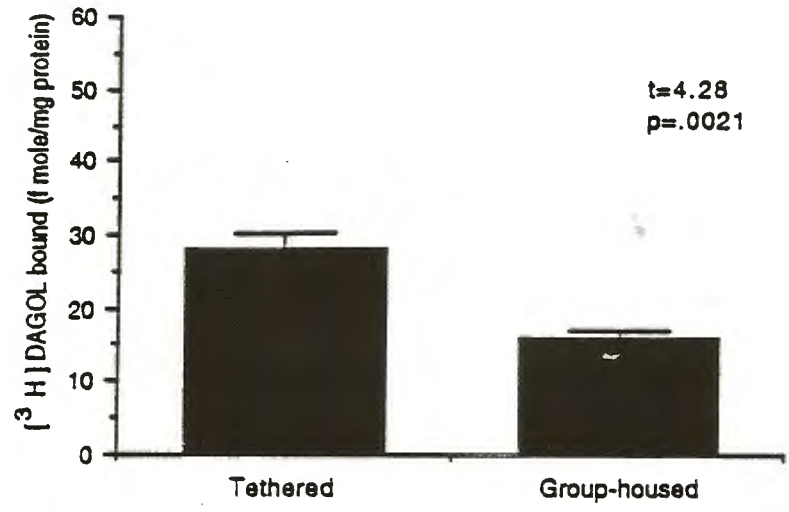

Fig. 1. Mean DAGOL(Mu agonist) binding to frontal cortex of tetherered or group-housed sows . Tethered sows were kept in stalls and held by a girth collar attached to a heavy gauge chain during pregnancy. Group-housed sows were kept in straw pens with access to individual stalls for feeding. Error bars represent 1 s.e.m.

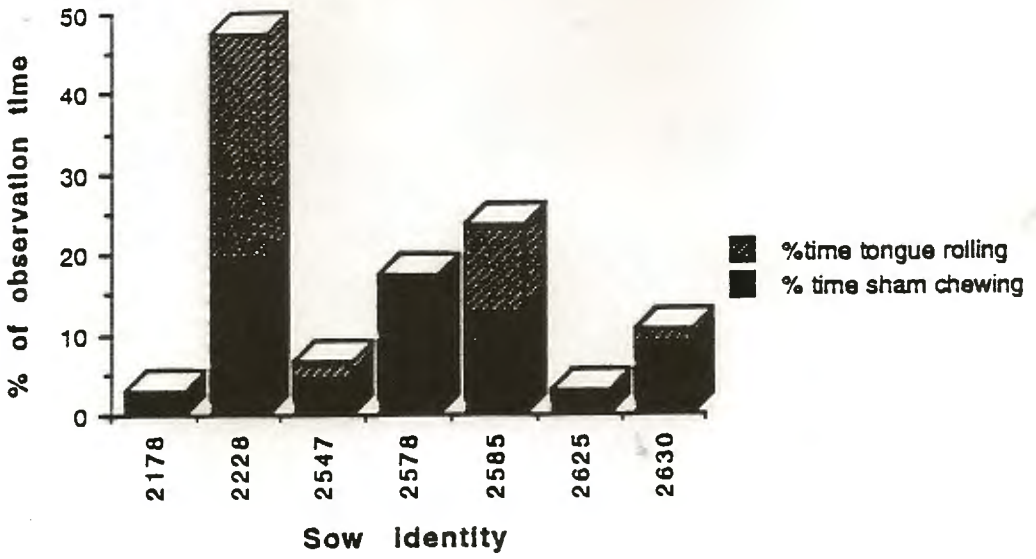

Fig. 2. Percentage of observation time during which individual sows show stereotypies .
Sham chew was scored when an animal opened and closed its mouth without having anything in it. Tongue-roll was scored when an animal performed repetitive rolling of the tongue outside the mouth. The figures are expressed as a percentage of instantaneous samples [24] collected by direct observation (n=145 samples per sow) and video recording (n=150 samples per sow) carried out 4 weeks before slaughter. Point samples of behaviour were collected every minute from a 24 hour video-tape during blocks of 30 minutes distributed as follows:1) 6:30-7:00, 2)8:30-9:00, 3)10:30-11:00, 4)14:30-15:00, 5)16:30-17:00. Samples were collected by direct observation, scanning every two minutes during periods in morning and afternoon on three days.

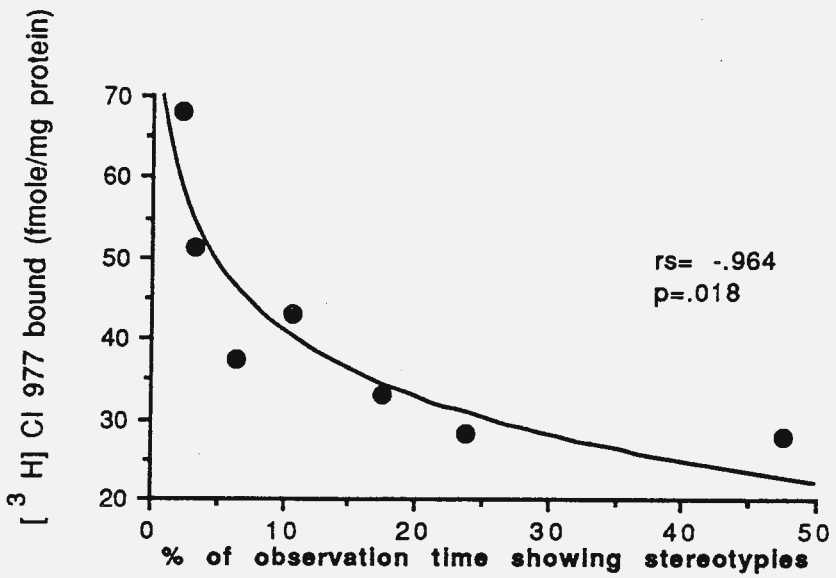

Fig 3 Cl 977 binding (Kappa agonist) in relation with time spending showing stereotyped behaviour. Sham-chew and tongue-roll frequencies were added from the total observation time.

^{3}H Cl 977 (Parke Davis) was used as the Kappa receptor ligand. The concentrations of ^{3}H Cl 977 used varied from .05 to 3 mMol. Non specific binding was defined for Mu, Delta and Kappa ligants as the amount of the radiolabelled agonist which remained bound in the presence of 10µM of naloxone

[^{3}H] DPDPE (delta agonist) binding was not related to any behaviour category analysed in the present study.

DISCUSSION

The behaviour data support previous work where a considerable variation among individuals in stereotypy performance in the same population was observed.[5,6]

Increase in mu receptors after restraint has been shown in rats.[7] Our data support such findings, tethered sows had significantly higher numbers of mu receptors in the frontal cortex, than group-housed sows.

Frischknecht, Siegfried and Waser[8] studied two different strains of mice which differ genetically in the number of opioid receptors. As a result of their characteristics in the test situation the strains were called the analgesic type and the locomotor type. Opioid agonists had strikingly different pharmacological and behaviour effects in the two strains. These results may help to explain the positive correlation of mu receptors and inactivity in pregnant tethered sows.

The relationship between kappa receptors and sham-chewing raises the question of the possible rewarding effect of this abnormal behaviour. Kappa agonists are not self administered by experimental animals (rats, monkeys) as mu agonists are, and kappa agonists do not cause dependence and withdrawal symptoms.[9,10] Tongue-rolling, on the other hand, seems to be related to mu receptors so, in that case, some reward may be associated with the behaviour.

CONCLUSIONS

Changes in brain opioid receptors are a sensitive indicator of the changes which occur during an animal's life-time and could be a useful indicator of welfare. Stereotypies and opioid receptors are related, and the type of opioid receptor involved differs according with the kind of stereotypy performed. These data support the previous suggestion that inactivity may be a coping strategy.

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