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Short-term Modulation of Domestic Pigeon (*Columbia livia* L.) Behaviour Induced by Intraventricular Administration of ACTH

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With 3 figures

Abstract

The behavioural responses induced in adult domestic pigeons by intraventricular injections of 0 to 6 IU of ACTH 1-39 are reported. The frequency of 10 different behaviour patterns was recorded for 90 min after administration of the peptide. The effect that was induced was complex, the frequency of some patterns increasing (yawning, headshaking, body shaking, wing-flapping), of some others decreasing (feeding, one-wing stretching, eye closing) or remaining unchanged (preening). The frequency of several patterns was maximal during the first 30 min following the injections but this was shown in some cases to be independent on the hormone administration.

Introduction

During the last decade, much attention has been paid to the behavioural role that various peptides and especially ACTH play in mammals (DE WIED 1977). Detailed studies have demonstrated that ACTH can modulate behavioural activities through complex changes of cerebral metabolism (DUNN and GISPEN 1977). ACTH appears to affect many aspects of behaviour, including agonistic behaviour (WEISS et al. 1969; LESHNER et al. 1973; MOYER and LESHNER 1976), learning behaviour (BOHUS 1979), and sexual behaviour (BERTOLINI et al. 1969; HAUN and HALTMEYER 1975), where the effects might be secondary to increased sex hormone levels.

Injected into the cerebral ventricles the hormone generally induces a specific behavioural syndrome characterized by frequent yawning and stretching, and by excessive grooming (FERRARI 1958; GESSA and FERRARI 1967; COLBERN et al. 1978; DUNN et al. 1979; JOLLES et al. 1979). The stimulation

of these patterns, particularly of grooming, is not specific to ACTH, as it also occurs after injections of MSH (GESSA and FERRARI 1967), peptides derived from ACTH (GISPEN et al. 1975) and fragments of lipotropin (GISPEN et al. 1976). All these compounds, however, share the amino acid sequence 4—10 of ACTH (BOHUS 1979).

The behavioural role of pituitary-adrenal axis hormones has been subjected to very few investigations in other vertebrates, especially in birds (imprinting behaviour of young duckling: MARTIN 1975, 1978; LANDSBERG and WEISS 1977; locomotor activity of American sparrows: MEIER et al. 1965; sexual behaviour of adult male ducks: DEVICHE 1976). The behavioural effects of intraventricular ACTH have been examined only in the pigeon by DELIUS et al. (1976). The authors observed that the frequency of yawning and headshaking increased shortly after the hormone injections. The experiment involved few subjects though, and effects on other behaviour patterns could have been easily overlooked.

In this paper, we confirm these results and show, moreover, that intraventricular administration of ACTH to pigeons modulates in a complex way a wider range of behavioural patterns.

Materials and Methods

Subjects: Adult pigeons of undetermined sex were purchased from a local breeder. Some pigeons had been previously used in other behavioural experiments but none of them had undergone any hormone treatments. At least two days before starting the experiment, each bird was allocated an individual cage, visually isolated from adjacent cages. All cages were in the same room, artificially lit with fluorescent tubes (12L:12D, with lights on at 8.00 h).

Surgery: Operations were carried out under anaesthesia (Equithesin), while the bird's head was held in a stereotaxic apparatus modified for the pigeon (KARTEN and HODOS 1967). A stainless steel cannula (22 gauge; total length ca. 15 mm) was inserted by percussion into one of the lateral forebrain ventricles. The procedure has been described in detail (DELIUS et al. 1976) and will therefore not be repeated here. It is sufficient to state that the correct positioning was checked histologically at the end of the experiment. The placement of the tips of the cannulas in all cases corresponded approximately to the coordinates A7, D12, L1 (KARTEN and HODOS 1967). After insertion, the cannula was anchored to the skull surface with dental cement, and closed with a stainless steel pin plug that could be removed later with minimum handling of subjects.

Behavioural observations: Behavioural recording was begun at least one day after the surgery. To avoid disturbing the subjects we employed a television system. The birds always remained in their home cages and the observations took place between 8.30 and 16.30 h.

Injections: Each pigeon received a total of 6 doses (0, 1, 2, 3, 4 and 6 IU) of ACTH (1-39) (Organon) in a random order. Each dose of ACTH was dissolved in 100 μ l of bird ringer's solution as formulated by LOCKWOOD (1961) but with half the glucose concentration to match the lesser glucose content of CSF as compared with plasma.

Infusion of 100 μ l of ringer alone served as the control dose.

No bird received more than one injection per day.

About 20 min before the injection, the plug of the cannula was removed and replaced by a long flexible polyethylene tube, which had been preloaded with the solution to be administered, and which was connected to a Hamilton syringe. The hormone could then be injected without handling the bird which could move freely in its cage. The liquid was infused slowly into the ventricles in the course of 1 min. Observations started within 2 min after the hormone administration.

Behaviour patterns: The activity was recorded for 90 consecutive min. At the end of each 1-min period, the occurrence of 10 different behaviour patterns was recorded. The maximum possible score for each behaviour pattern was therefore 90. The patterns recorded were: feeding or pecking at food (Fee); drinking (Dri); preening (Pree); one-wing stretching (1WS); two-wing stretching (2WS); wing-flapping (WFl); yawning (Yaw); body shaking (BSh); headshaking (HSh); eye closing (EyC). All these patterns are highly stereotyped and can therefore be recognized easily. Their names are sufficiently descriptive as to make further details unnecessary. In addition, we recorded the mean arousal level (= Arou) using an index described by DELIUS et al. (1976) which takes into account the degree of fluffing of the plumage, the extension of the neck, the intensity of head movements, the body posture and the locomotor activity. It ranges from 1 (bird asleep) to 6 (bird excited).

Data analysis: The data collected for each bird, each dose and each pattern were grouped into 30-min periods. To minimize the variance ratios between the groups the scores underwent a transformation $\log(x+1)$. The data sets for each behaviour pattern were separately evaluated with a repeated measures analysis of variance program (BMDP2V, Los Angeles, Ca.). With this program the effects of two independent factors could be separated: dose (0 to 6 IU of ACTH) and time (P1 to P3, equivalent to the successive 30-min periods of observation) and the interactions between these two factors. Comparisons between sets of data were done using t-tests for paired samples. Two-tailed probabilities corresponding to $p \leq 0.05$ have been considered as significant, these corresponding to $0.05 < p < 0.10$ as borderline.

Results

The results of the analysis of variance are summarized in Table 1.

Table 1: Results of analysis of the variance performed for different behaviour patterns observed in pigeons during 90 min following intraventricular administration of 0, 1, 2, 3, 4 or 6 IU of ACTH 1-39. Abbreviations: Fee = feeding/pecking of food; Dri = drinking; Pree = preening; 1WS = one-wing stretching; 2WS = two-wing stretching; WFl = wing flapping; Yaw = yawning; BSh = body shaking; HSh = head shaking; EyC = eye closing; Arou = arousal level. Probabilities are indicated by: — = $p > 0.05$; x = $0.02 < p < 0.05$; xx = $0.01 < p < 0.02$; xxx = $0.001 < p < 0.01$; xxxx = $p < 0.001$

| Behavior pattern | Dose effect | Time effect | Interaction Dose x Time |
|------------------|-------------|-------------|-------------------------|
| Fee | xxx | - | - |
| Dri | - | - | - |
| Pree | - | xxxx | - |
| 1WS | xxx | - | - |
| 2WS | - | xxxx | x |
| WFl | x | - | - |
| Yaw | xxxx | xxx | - |
| BSh | xx | xxxx | - |
| HSh | xxx | xxxx | - |
| EyC | xxx | - | - |
| Arou | xx | xxxx | x |

The frequency of some patterns was affected by the ACTH injections (e.g. Fee, 1WS, WFl), by the time progression (e.g. Pree) or by both factors (e.g. Yaw, BSh, HSh). The influence of these factors will be presented separately.

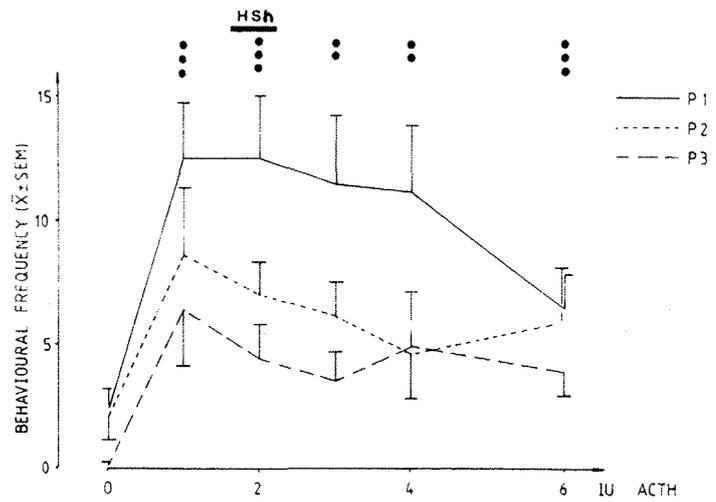


Fig. 1: Mean frequencies ($\bar{x} \pm S.E.M.$; $N = 8$ for each point) of headshaking observed in pigeons during 3 successive 30-min periods (P1, P2 and P3) following the intraventricular injection of different doses of ACTH 1-39. The probabilities ($\bullet\bullet = 0.01 < p < 0.02$; $\bullet\bullet\bullet = 0.001 < p < 0.01$) refer to the comparison with the zero dose scores and refer to the totals over the 90-min observations

Influence of time: The frequency of several patterns varied considerably from one observation period to the next. The results for HSh are presented in Fig. 1 as an example of this effect.

The frequency of HSh decreased significantly from P1 over P2 to P3 (separate two-way analysis of variance yielded $p \leq 0.05$ for both the P1/P2 and the P2/P3 comparisons). A similar kind of decrement was observed for BSh, Yaw and Pree. In the two latter cases, however, the decrease from P2 to P3 was not significant. The results pertaining to Pree are presented in Fig. 2.

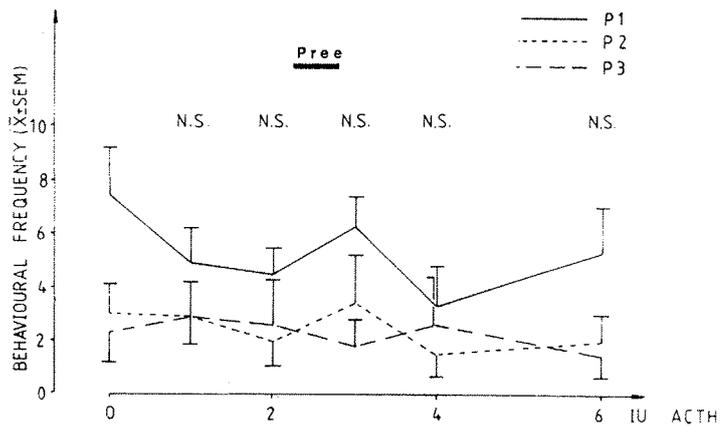


Fig. 2: Mean frequencies ($\bar{x} \pm S.E.M.$) of preening observed in pigeons following the intraventricular administration of different doses of ACTH 1-39. N.S. = $p > 0.05$. See Fig. 1 for further details

There was also an overall time effect on Arou and on the frequency of 2WS. This consisted in a decrease in the case of Arou, and an increase in the case of 2WS but was complex as it also involved an interaction with the ACTH dose injected (see Table 1).

The frequency of five patterns (Fee, Dri, 1WS, WFl and EyC) did not vary in a significant way in the course of the observation period (Table 1).

Dose effects: ACTH administration strongly affected the frequency of most behaviour patterns (see Table 1). However, the details differed very much from one pattern to another. The frequency of some patterns clearly increased following an ACTH infusion. A typical example (HSh) is shown in Fig. 1: all doses of ACTH as compared with injections of Ringer's alone increased the frequency of this pattern. A similar picture was obtained for Yaw (significant effect for each dose of ACTH, except 4 IU) and for WFl (significant increase for 1 and 2 IU, but not for 3, 4 or 6 IU of ACTH). Arou and the frequency of 2WS likewise increased following ACTH treatment. This result, however, ought to be interpreted with care, as for both behaviour patterns there was a significant interaction with the time factor. Strikingly, the frequency of Pree was not influenced by any dosage of ACTH (Fig. 2).

Finally, the frequency of 3 patterns (Fee, 1WS, EyC) decreased following ACTH treatment. This kind of change is exemplified in Fig. 3 (1WS). Because there was no time progression effect on the frequency of 1WS, as Fee and EyC (Table 1), Fig. 3 presents the mean frequencies over 90 min of observation, rather than for each 30-min period separately. The depressing effect of ACTH injections on 1WS was significant for each hormone dose, except for 3 IU where it was borderline. A generally similar picture was obtained for Fee (significant decrease for 1 and 2 IU of ACTH) and for EyC (significant decrease for 1, 2 and 6 IU of ACTH; borderline for 3 and 4 IU of ACTH).

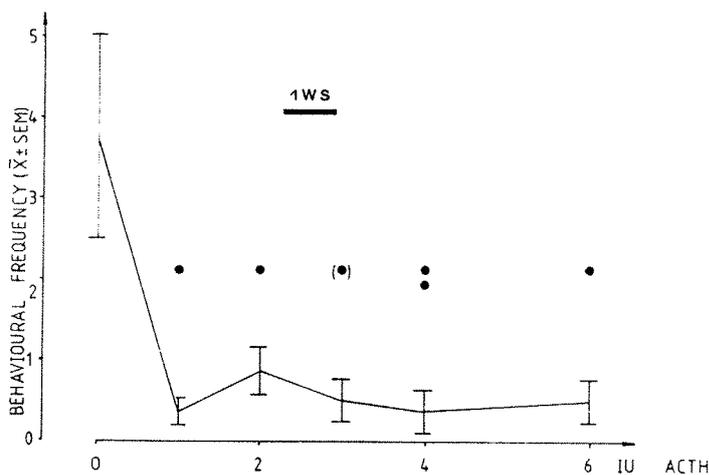


Fig. 3: Mean frequencies ($\bar{x} \pm \text{S.E.M.}$) of one-wing stretching observed in pigeons during 90-min sessions following the intraventricular injection of different doses of ACTH 1-39. Probabilities are symbolized by: (•) = $0.05 < p < 0.10$; • = $0.02 < p < 0.05$; •• = $0.01 < p < 0.02$. See Fig. 1 for further details

The results are summarized in Table 2. The behaviour patterns have been grouped according to the way their frequency was affected by the ACTH treatment (increase, decrease, no effect) and by the time factor. The direction of the variation (increase or decrease from at least one 30-min period of observation to another) has moreover been indicated. Two patterns (2WS, Arou) are bracketed to indicate a significant interaction between the dose and the time factors (Table 1).

Table 2: Behavioural modifications observed during 90 min following intraventricular injection of different doses of ACTH to pigeons. The table was built by taking into account the influence of 2 factors: (a) ACTH injections and (b) time of observation, divided into 30-min periods. For (b) factor, the general direction of the variation, when significant, was indicated by an arrow: \uparrow (or \downarrow) = increase (or decrease) from one period to another. See Table 1 and text for further explanations

| Influence of ACTH administration | | | | | |
|----------------------------------|-----|-------------|-----|-------------------|-----|
| Increase | | Decrease | | No effect | |
| Time effect | | Time effect | | Time effect | |
| Yes | No | Yes | No | Yes | No |
| (2WS \uparrow) | WFL | | Fee | Pree \downarrow | Dri |
| Yaw \downarrow | | | 1WS | | |
| BSh \downarrow | | | EyC | | |
| HSh \downarrow | | | | | |
| (Arou \downarrow) | | | | | |

Discussion

Our results show that the intraventricular administration of ACTH to pigeons induces modifications in the frequency of a wide range of behaviour patterns. The results obtained by DELIUS et al. (1976) on the same species are thus confirmed and expanded.

As compared with the doses of hormone employed in mammalian studies, where as little as 0.12 IU ACTH/kg body weight yielded behavioural effects (FERRARI et al. 1963), the doses we used are rather high. It must however be pointed out that in the rat, for example, very high doses (20 μ g) as well as quite small doses (0.01 μ g), of ACTH 1—24 induce a similar behavioural alteration, i.e. an increase in grooming (GISPEN et al. 1975).

The responses of the pigeon to brain infusion of ACTH appear as very complex ones. Indeed, ACTH treatment altered the frequency of some, but not all, behavioural patterns recorded. Moreover, not all patterns were modified in the same way: the frequency of some of them (e.g. headshaking, yawning) was strongly increased, while the occurrence of other patterns was on the contrary depressed (e.g. one-wing stretching, feeding, eye-closing) or remained unaffected. The behavioural changes induced by ACTH seem therefore to be very specific.

DELIUS et al. (1976) have discussed the relationship that the ACTH-induced behaviour might bear with the normal behaviour of pigeons and DUNN et al. (1979) have done the same concerning rats. Both argue that the

ACTH syndrome has similarities with the behavioural response that these animals show after the incidence of mildly stressful events. In the light of the more detailed description of the hormone-induced response we present in this paper it would seem, at least in the case of the pigeon, that the similarity needs to be better documented before it can be seriously considered. Further theorizing may also have to take into account the neuro-transmitter role that ACTH seems to have in vertebrates (KRIEGER and LIOTTA 1979).

In this study, several behaviour patterns were clearly affected by the progression of time (Table 1, 2; Figs. 1, 2): the frequency of 4 out of 6 patterns which were stimulated by ACTH treatment decreased from the first 30-min period of observation to the two subsequent periods. Because this effect was not dose dependent (except Arou, Table 1) it may not be directly caused by the ACTH administration. Rather, it may reflect the influence of handling the bird prior to observation or some related factors which were superimposed on the ACTH effect but which were of short duration. This hypothesis finds some support in the observation that the frequency of preening, while not affected by the peptide infusion, was nevertheless influenced by the time factor (Fig. 2).

None of the three behaviour patterns depressed by the ACTH treatment (Table 2) showed a significant time effect. This holds for each hormone dose, including the zero one as there was no interaction between the factors. These patterns are thus different from those stimulated by the hormone which were all influenced by the time progression (except wing-flapping).

It should finally be observed that in the present work, intraventricular infusion of ACTH enhanced the occurrence of yawning, but failed to affect preening activities (Fig. 2; Table 1). This observation suggests that in the pigeon, yawning and preening are controlled by partially distinct nervous mechanisms. Such an hypothesis agrees with DELIUS' results (1967), showing that although electrical stimulation of brain loci in gulls (*Larus* sp.) yielding preening also tended to produce yawning, the correlation was less than perfect.

Further neuroethological investigation should provide complementary informations on this subject.

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