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# Effect of the ICV Injection of 5,7-di-Hydroxytryptamine on the Aggressive Behavior of Dominant and Submissive Pigeons (*Columba livia*)

M. ISON,<sup>1</sup> C. FACHINELLI AND E. L. RODRÍGUEZ ECHANDÍA

*Laboratorio de Psicología Experimental y Comparada, Universidad del Aconcagua-CRICYT  
and Laboratorio de Investigaciones Cerebrales (LINCE), Mendoza, Argentina*

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ISON, M., C. FACHINELLI AND E. L. RODRÍGUEZ ECHANDÍA. *Effect of the ICV injection of 5,7-di-hydroxytryptamine on the aggressive behavior of dominant and submissive pigeons* (*Columba livia*). PHARMACOL BIOCHEM BEHAV 53(4) 951-955, 1996. — The acute and chronic treatment with 5-HTP was reported to block the aggressive reaction induced by food competition in undernourished dominant pigeons. Such treatment was not effective, however, in submissive pigeons. In this report we describe the effect of the neurotoxic 5,7-DHT on aggression, defense, emotion, feeder control, and other behaviors in dominant and submissive male pigeons maintained at 80% of their normal weight. These were ranked through daily food competition trials. To obtain the prelesion scores dominant ( $n = 15$ ) and submissive pigeons ( $n = 16$ ) were submitted to a daily trial against a different intermediate subject (six trials). Then, the dominant and submissive groups were subcutaneously injected with Desipramine (25  $\mu\text{g}/\text{kg}$ ), anesthetized at a 60 min interval and injected with 5,7-DHT into the left lateral ventricle (25  $\mu\text{g}/25 \mu\text{l}$  0.9% NaCl). After a 30-day interval, both lesioned dominants and submissives were confronted to a different untreated intermediate subject over six daily sessions (postlesion scores). Differences between pre- and postlesion scores for all behavior studied were not found in dominant subjects. In submissive subjects, however, the postlesion scores of total aggression, defensive behavior, and emotional behavior were significantly higher than pre lesion scores. When brain 5-HT was assayed 60 days after injection about 34% depletion was found for both groups of pigeons. These findings suggest that the behavioral response to brain 5-HT denervation in pigeons is related to the behavioral characteristics of the subject previous to the lesion.

5,7-DHT    Aggressive behavior    Food competition    Undernourished pigeon

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THERE is evidence supporting that pharmacological treatments inducing an increased availability of serotonin (5-HT) at 5-HT synapses can decrease many aggressive reactions in rodents (3,15). Conversely, treatments that decrease 5-HT transmission may facilitate some aggressive behaviors (7,14).

In undernourished dominant pigeons, the acute treatment with the 5-HT precursor, 5-HTP has been shown to block the aggressive reaction induced by food competition (4,5). This treatment, however, was not effective to modify aggression in submissive pigeons (4,5).

The ICV administration of the neurotoxic 5,7-di-hydroxytryptamine (5,7-DHT), which is known to cause a selective

degeneration of 5-HT nerve terminals, has been reported to stimulate defensive aggression (8) as well as predation in rodents (8,9,14). To our knowledge, the effect of 5,7-DHT on the aggressive behavior of dominant and submissive pigeons has not been reported.

It was suggested that treatments decreasing 5-HT transmission would not induce aggression per se, but may facilitate the expression of aggressive and other impulses, already present in rats previously to treatment (6,18). If such were the case, it is conceivable that animals with different levels of spontaneous aggression respond to 5-HT manipulation in a different manner. In the present report we have investigated the effect

<sup>1</sup> Requests for reprints should be addressed to M. Ison, LINCE, Casilla Correo 425, 5500-Mendoza, Argentina.

of the ICV injection of 5,7-DHT on aggression, defense, emotion, maintenance, and feeder control in undernourished pigeons previously selected as dominants (high scores of aggression) and submissive subjects (low scores of aggression). These were exposed to food competition trials against an untreated pigeon of intermediate levels of aggression. The results suggest that the behavioral response to ICV 5,7-DHT injection is related to the behavioral characteristics of the subject previous to the 5-HT lesion.

#### METHODS

The procedures described below had been previously approved by the Committee for Animal Welfare of the Medical School.

The subjects were feral male mature domestic pigeons (*Columba livia*) weighing 350–400 g ( $n = 65$ ). They were captured in the university campus and kept individually in  $50 \times 40 \times 40$  cm cages that were visually isolated from one another. The animal room was maintained at constant temperature ( $24 \pm 3^\circ\text{C}$ ) and lighting (lights on from 0800 to 1900 h). The amount of food provided (mixture of grains) was calculated to maintain subjects at 80% of their normal body weight. Water was provided ad lib.

#### Interaction Chamber

This was a box of  $1.5 \times 1.5$  (base)  $\times$  2.0 (height) m, which had four legs 40 cm high and the floor covered with chaff. The lateral and back walls were made with green polyethylene. The front wall was a removable wire grid allowing both accesses to the chamber and direct observation of animal

behavior. To induce fighting, a feeder was placed at the center of the arena. This was a 25 cm high pyramidal structure with a short lateral arm bearing a single 2.0 cm hole through which just one animal could freely eat a mixture of grains. Dominance for owning the feeder rapidly develops in pairs of undernourished pigeons exposed to food competition trials [see (4)].

#### Ranking Method

Once the desired body weight was reached the pigeons were exposed in pairs to food competition interactions. In all cases, daily 20-min trials were performed from Monday to Friday and the pre- and posttrial body weight of each member was recorded. The observers were 2 m away from the front of the observation chamber. The time spent in each component of aggressive behavior, defensive behavior, emotional behavior, feeder control behavior, eating behavior, and other behaviors was recorded in a PC through a specially designed soft. The recorded behaviors are illustrated by Table 1.

In order to estimate the spontaneous aggression levels of the captured birds, each of them was daily ranked in the interaction chamber in the presence of another bird, who was a different one for each day, i.e., six interactions. Pigeons showing a mean total aggression time higher than 200 sec/20 min over the six test sessions were arbitrarily classified as dominant subjects ( $n = 15$ ). Pigeons with the shortest aggression time (less than 50 sec/20 min) were classified as submissive members ( $n = 16$ ). Pigeons with a total aggression time of 90 to 150 sec/20 min were classified as intermediate subjects ( $n = 27$ ).

TABLE 1  
STRUCTURE OF BEHAVIORS SELECTED FOR RECORDING

1. Aggressive behavior	4. Eating behavior
R: pursuing	K: eating at the feeder
E: hooking	I: eating out of the feeder
P: biting to prevent opponent's access to food	W: body weight gain
A: wing beating	
V: aggressive vocalization close to feeder together with horizontal movements of head and body causing wing tremor in opponent or running away	5. Feeder control behavior
Z: threatening	Y: eating attempts
4: biting in areas far from food, after chasing	M: pushing the opponent out of the feeder
6: aggressive vocalizations in areas far from food	G: wing covering the feeder
	{: walking around the feeder
2. Defensive behavior	6. Other behaviors
H: running away	J: bloating (bristling the feathers)
D: defensive fighting (mainly when beating in response to an attack of the opponent)	U: autocleaning
	Q: immobility
3. Emotional behavior	N: locomotion
B: watching	F: wing flapping
O: wing tremor	/: defecation
-: fear vocalizations (short vocalizations accompanied by expiration and immobility after an aggressive attack of the opponent)	
.: immobility in a corner to prevent an attack	
8: intents to leave the observation chamber (flying up to the top of the chamber)	

### Pretreatment Food Competition Trials

When ranking was completed, each dominant and each submissive pigeon was retested through a daily food competition trial against one different intermediate subject over six daily sessions. Pretreatment scores are expressed as means  $\pm$  SEM/six trials. During the pretreatment trials the intermediates were invariably dominant when exposed to submissive partners, and they behaved as submissives when confronted to dominant ones.

### 5,7-DHT Injections

When pretreatment scoring was completed, some dominants ( $n = 9$ )—and some submissives ( $n = 10$ )—selected at azar, were subcutaneously injected with desipramine (25.0 mg/kg) dissolved in 1 ml of 0.9 percent NaCl. After a time interval of 60 min, the pigeons were anesthetized with an intraperitoneal injection (3 ml) of tribromoethanol (250 mg/kg), placed in the stereotaxic apparatus and injected into the left lateral ventricle with 5,7-DHT (25  $\mu$ g, dissolved in 25  $\mu$ l 0.9% NaCl with 7.5  $\mu$ g of citric acid added). After injection, animals were returned to their home cages and maintained undisturbed up to the initiation of the postinjection food competition trials (30 days). Some dominant ( $n = 6$ ) and submissive ( $n = 6$ ) pigeons were injected into the lateral ventricle with 25  $\mu$ l of a 0.9% NaCl containing 7.5  $\mu$ g of citric acid. These were maintained undisturbed, until brain 5-HT assay was performed (day 60 after injection).

### Posttreatment Food Competition Trials

Before starting the posttreatment trials, food was restricted to maintain pigeons at 80% of their normal body weight.

Each lesioned dominant and each lesioned submissive subject were submitted to a daily food competition trial (total six trials) against a different intact intermediate pigeon as described above. The observers were blind to the animal condition.

Results are expressed as 1) the mean  $\pm$  SEM total time spent in each behavior/six sessions and 2) structure of behaviors (mean  $\pm$  SEM time spent in each component of the six behaviors illustrated by Table 1).

### HPLC Determination of Brain 5-HT

The saline-injected controls and some 5,7-DHT-injected dominant ( $n = 6$ ) and submissive ( $n = 6$ ) pigeons were selected for brain 5-HT assay 2 months after ICV injections. These were killed by decapitation, the brain was removed, quickly weighed, frozen, and stored at  $-80^{\circ}\text{C}$  until assay. The frozen brains were homogenized with an ultrasonic cell disruptor in 4 ml of ice-chilled 0.2 M perchloric acid containing 0.1 mM EDTA. After centrifugation at  $25,000 \times g$  for 30 min at  $4^{\circ}\text{C}$  the clear supernatants were recentrifuged. This supernatant (400  $\mu$ l) was then transferred to a test tube containing 400  $\mu$ l Tris-HCl (pH 8.6) and 25 mg of activated alumina and shaken for 30 min. Tubes were then centrifuged for 5 min and 50  $\mu$ l of the clear supernatant were injected into the HPLC system. The HPLC system was a 2150 LKB HPLC pump provided with an LKB 2143 electrochemical detector. The mobile phase was 0.05 M sodium phosphate monobasic and phosphoric acid, up to pH 3.2, containing 20% (vol/vol) acetonitrile and 0.1 mM EDTA. The column temperature was  $15\text{--}20^{\circ}\text{C}$ , and the flow rate was 1 ml/min. The oxidizing po-

tential was maintained at 0.5 V. The recorder was an LKB 2221 integrator.

### Statistics

Data were analyzed by the one-way analysis of variance (ANOVA) and the Tukey's multiple range test. Results are expressed as means  $\pm$  SEM. A level of probability of less than 0.05 was considered significant.

## RESULTS

### Pre- and Postlesion Behavior of Dominant Subjects

In prelesion trials the intermediate pigeons behaved as submissive members when confronted with the dominant subjects.

Differences between pre- and postlesion scores of total time spent in aggression ( $369.28 \pm 25.18$  vs.  $327.53 \pm 51.01$  s), running away ( $42.00 \pm 10.72$  vs.  $29.86 \pm 7.19$ ), maintenance ( $150.48 \pm 38.46$  vs.  $201.99 \pm 37.95$ ), and eating behavior ( $575.80 \pm 38.78$  vs.  $608.05 \pm 33.72$ ), were not found in the dominant group. Consistently, differences in the structure of each behavior did not reach significance (results not shown). The lesion did not change the scores of feeder control behavior ( $25.94 \pm 10.06$  vs.  $17.86 \pm 9.36$ ) and neither did intratest body weight gain ( $27.96 \pm 2.90$  vs.  $26.95 \pm 2.07$  g). Therefore, dominance on the intact intermediate subjects was maintained after 5-HT lesion.

### Pre- and Postlesion Behavior of Submissive Subjects

In prelesion trials the intermediate pigeons behaved as dominant members when paired with submissive subjects.

In the submissive group differences between pre- and postlesion scores of total time spent in aggressive behavior were significant (Fig. 1) due to the higher postlesion scores (Tukey's test:  $p < 0.05$ ).

In addition to this enhancement in total aggression, the 5,7-DHT lesion (Fig. 1) also raised the scores of emotional

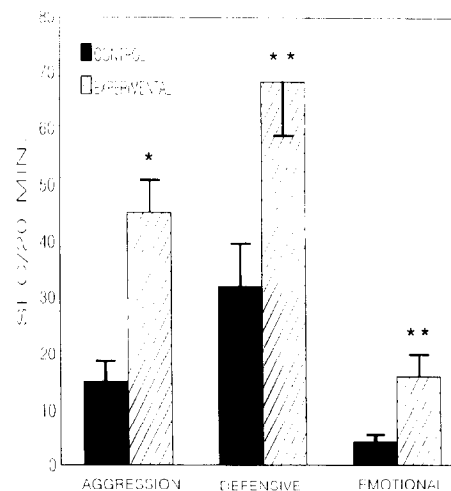


FIG. 1. Scores of total time spent in aggression, defense, and emotional behavior by submissive pigeons. Control: prelesion scores. Experimental: postlesion scores (means  $\pm$  SEM/six trials). \* $p < 0.05$ ; \*\* $p < 0.01$  (Tukey's multiple range test).

behavior (Tukey's test,  $p < 0.01$ ) and defensive behavior ( $p < 0.01$ ) in the submissive group. The structure of these behaviors (Fig. 2) showed a significant increase in the scores of biting and wing beating (aggression), running away, and defensive fighting (defensive behavior), fear vocalizations, and intents to leave the arena (emotional behavior).

Differences between pre- and postlesion scores of total time spent in feeder control behavior and eating behavior were not found. This also was the case for scores of intratest body weight gain (results not shown). However, eating attempts scores, a component of feeder control behavior, increased significantly after 5,7-DHT injection (Fig. 2).

As to the components grouped as other behaviors in Table 1, a significant decrease in the scores of immobility time was found after lesion (C:328.36  $\pm$  49.67; lesioned: 201.73  $\pm$  32.73;  $p < 0.05$ ). The other components of other behaviors remained unchanged.

In spite of the increased aggressive behavior and defensive behavior of the lesioned submissives, it is of interest that their intact intermediate opponents maintained dominance and kept the feeder under control as they did in pre lesion trials.

#### Effect of 5,7-DHT Injection on Brain 5-HT

Differences between dominant and submissive pigeons in the pre- and postlesion brain content of 5-HT were not significant; the results were, therefore, pooled. The mean level of 5-HT in the whole brain of saline-injected controls was 2.53  $\pm$  0.20  $\mu$ g/mg. Two months after 5,7-DHT injection the brain content of 5-HT was significantly lower than controls (1.66  $\pm$  0.13, Tukey's test:  $p < 0.05$ ). The treatment caused, therefore, a 34.39% reduction of brain 5-HT at this postlesional time.

#### DISCUSSION

We have reported elsewhere that the acute administration of the 5-HT precursor, 5-HTP decreases the aggressive reac-

tion of dominant pigeons submitted to food competition interactions (4). It is known that the acute treatment with 5-HT precursors, as well as with 5-HT uptake blockers, can stop firing of 5-HT neurons in minutes (1,2). It remained unclear, therefore, if the antiaggressive effect of the acute 5-HTP administration was due to increased or decreased 5-HT function. However, we have reported recently that a daily injection of 5-HTP during 16 days (chronic treatment) was also effective to decrease total aggression of dominant pigeons in food competition trials (5). Because the chronic pharmacological stimulation of the 5-HT system is known to enhance 5-HT function (1,2), it was speculated that the 5-HT system plays an inhibitory role on the expression of food competition-induced aggressive behavior in dominant pigeons. This was not the case, however, for submissive pigeons. Neither acute nor chronic 5-HTP exposure was followed by a decrease of aggressive behavior in submissive subjects (4,5). We had speculated earlier (4) that the lack of aggression decreases in submissives after 5-HTP might be ascribed to their initially low aggression levels.

In the present work, the 5-HT denervation caused by the ICV injection of 5,7-DHT clearly increased total aggression, as well as emotional behavior and defensive behavior in submissive pigeons. This treatment did not affect such behaviors in dominant subjects. We feel tempted to offer the suggestion that 5,7-DHT cannot increase aggression any further in spontaneously dominants because their levels are initially high. The 5-HT denervation, therefore, was effective to increase aggressiveness, and an aggressive-type defensive behavior, i.e., defensive fighting, selectively in subjects showing low basal levels of aggression. The effect of 5-HT denervation in intermediate pigeons was out of the scope of this study and remains to be analyzed.

It seems improbable that differences in the behavioral response to 5,7-DHT of dominant and submissive subjects can be explained by differences in sensitivity to the neurotoxin. In fact, dominant and submissive pigeons showed similar basal contents of brain 5-HT as well as similar 5-HT depletion in response to the 5,7-DHT injection.

It is known that 5,7-DHT and other neurotoxins placed into the dorsal and medial raphe region cause both supersensitivity to 5-HT agonists and increased binding of 5-HT<sub>1</sub> receptor subtypes in cerebral cortex and total brain of rats (17,19). It might be that dominant pigeons can develop higher 5-HT<sub>1</sub> supersensitivity than submissive pigeons in response to 5-HT denervation. However, the fact that 5-HT<sub>2</sub> receptors have been reported to be involved in the antiaggressive effects of 5-HT stimulation (4) is against this speculation. The 5-HT<sub>2</sub> receptor subtype are known to remain unchanged after 5-HT denervation (16).

In the present report, the brain turnover of 5-HT was not investigated. Therefore, differences in 5-HT function between dominant and submissive pigeons can not be ruled out. In fact, it has been reported that killer rats show lower central 5-HT turnover than nonkiller rats (10). This might be due to increased activity of the 5-HT<sub>1a</sub> receptor subtype (13). If such were the case also for dominant pigeons, it might be speculated that a further reduction of 5-HT function cannot affect the expression of aggression and other behaviors in spontaneously aggressive pigeons. However, a similar decrease in 5-HT function in submissive pigeons may be effective to increase aggression because they might have a higher basal 5-HT turnover.

The significant increases in defensive behavior, emotional behavior, and eating attempts scores as well as the decreases

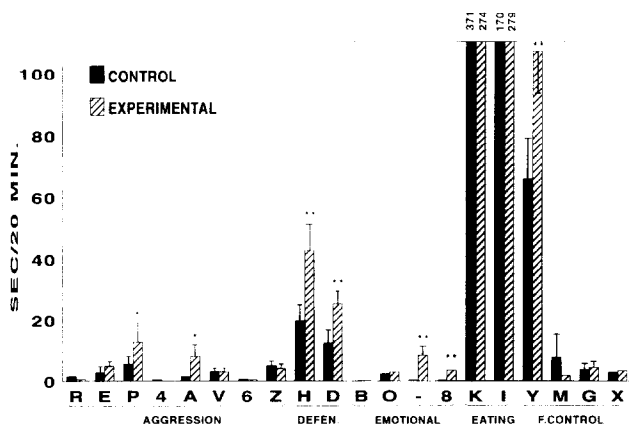


FIG. 2. Structure of aggressive behavior, defensive behavior, emotional behavior, eating behavior, and feeder control behavior of submissive pigeons (means  $\pm$  SEM/six trials). Control: prelesion scores. Experimental: postlesion scores. For explanation of columns, see Table 1. \*\* $p < 0.01$ ; \* $p < 0.05$  (Tukey's multiple range test). Nonasterisk on columns: no statistically significant differences.

in the scores of immobility time seem to suggest that the 5-HT lesion has removed an endogenous behavioral inhibition rather than inducing a specific increase in aggressive behaviors.

Whatever the case, the behavioral response increases after 5-HT denervation, found exclusively in submissive pigeons, may contribute to the understanding of the individual differences in behavioral response to both brain lesions and neuroactive drugs in laboratory animals and humans.

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