

Effects of sodium valproate and carbamazepine on food competition aggression in pigeons

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Abstract

Valproate and carbamazepine (CAR) have been proposed as adjunct alternatives for the control of aggression in psychiatric patients, although no definite conclusions have been reached. We examined the effects of these drugs on food competition offensive aggression and other behaviors in high- and low-aggression food-restricted pigeons. These were divided into pairs containing previously ranked high-aggression (N = 10 pairs) and low-aggression females (N = 10 pairs). In Experiment 1, a pigeon in each pair of high- and low-aggression subjects was treated daily with an oral dose of sodium valproate (50 mg kg⁻¹ mL saline⁻¹) for 15 days. The other animal received the vehicle. On days 1, 7, and 15, food competition trials (10 min) were performed 60 min after treatment. In Experiment 2, one pigeon in each pair was treated daily with an oral dose of CAR (20 mg kg⁻¹ mL saline⁻¹) for 15 days. Each pair was submitted to a food competition trial on days 1, 7, and 15 of treatment. Valproate (15 days of treatment) selectively decreased the time spent in offensive aggression (control: 102.7 ± 9.3 vs valproate: 32.7 ± 9.2 s; P < 0.001, ANOVA-2-TAU) of high-aggression pigeons. This was also the case for 7 and 15 days of CAR treatment (control: 131.5 ± 8.9 vs CAR: 60.4 ± 5.3, P < 0.01, and control: 122.7 ± 7.1 vs CAR: 39.1 ± 5.2; P < 0.001, ANOVA-2-TAU, respectively). Thus, the two anticonvulsive drugs have a similar effect on food competition aggression in pigeons.

Key words

- Valproate
- Carbamazepine
- Aggressive behavior
- Food competition
- Pigeons

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Introduction

Some anticonvulsive drugs such as sodium valproate and carbamazepine have been proposed as adjunct alternatives for the control of agitation, wandering, and violence in psychiatric patients. Partially positive results have been obtained with these drugs regarding aggressive and impulsive behaviors in personality disorders (see Ref. 1), dementia

(2), schizophrenia (3), acquired brain injury (4,5), and other disorders. However, while the antiaggressive effects of valproate and carbamazepine in humans are promising, in the absence of controlled data no definitive conclusions can be reached (6).

The understanding of the effects of drugs on aggressive behavior requires consideration of biological variability, including genetic and interindividual differences. The food competi-

tion interaction test performed with food-restricted pigeons is a useful animal model for the study of offensive and defensive social aggression (7). Using this test we have shown that the behavioral response of male and female pigeons to some psychoactive drugs is related to their natural aggressiveness exhibited prior to the treatments (8-10). To our knowledge, however, no analysis has been reported of the effect of valproate and carbamazepine on this type of aggressive behavior in pigeons. In this species, valproate has been used mainly for studies on learning (11), matching-to-sample performance (12), and drug discrimination properties (13). In addition, we are not aware of the use of carbamazepine in pigeons.

The purpose of the present study was to examine the effects of acute, subchronic and chronic sodium valproate (Experiment 1), and carbamazepine (Experiment 2) on the aggressive and general behavior responses of food-restricted high- and low-aggression female pigeons exposed to food competition interactions.

Material and Methods

Subjects

The subjects were mature wild-female domestic pigeons (*Columba livia*) weighing 350-400 g. They were captured on the University Campus during the winter season (short daylight hours and low sex hormone levels in pigeons) and reared singly in 50 x 40 x 40-cm cages that were visually isolated from one another for 30 days. The animal room was kept at constant temperature ($24 \pm 3^\circ\text{C}$) and lighting (lights on from 8:00 to 19:00 h). Food (a mixture of grains) and water were available *ad libitum*. On day 31, pigeons were weighed (basal weight). Thereafter, the food given was calculated to maintain subjects at 80% of basal weight. Water was provided *ad libitum*. All experimental treatments were approved by the Animal

Welfare Committee of the National University of Cuyo Medical School.

Interaction cage

The dimensions of the interaction cage were: 2.0 x 2.0 (base) x 2.0 (height) m, with four 35-cm high legs. The floor was covered with chaff. The inner walls were painted white. The front wall had a 1.35 x 0.41-m dark glass window allowing direct observation of animal behavior without visual disturbance. To induce fighting, a feeder was placed at the center of the arena. The feeder was a 25-cm high pyramid with a short lateral arm bearing a single 2.0-cm hole through which just one animal could freely eat a mixture of grains. Previous reports have shown that dominance for feeder control rapidly develops in pairs of food-restricted pigeons exposed to food competition interactions (7). In order to habituate the pigeons to the interaction cage, all animals were individually submitted to a daily 20-min exposure to the arena 15 days before ranking.

Ranking method

All agonistic interactions involving a real or potential risk to the opponent pigeon were considered to be offensive behavior (see Table 1). In order to estimate the spontaneous aggression levels of the captured birds, each one was ranked daily in the interaction cage (5-min trials) for 6 consecutive days in the presence of another bird which was a different one for each day. The experienced observers were 0.30 m away from the front of the interaction cage.

Females showing a mean time spent in offensive aggression exceeding 60 s/5 min over the six-test sessions were arbitrarily ranked as "high-aggression subjects". Pigeons with the shortest offensive aggression time (less than 10 s/5 min) were ranked as "low-aggression subjects". A total of 40 pairs

of similar offensive aggression scores were obtained.

Experiment 1. Sodium valproate treatment

The literature indicates that the valproate doses used in pigeons range from 20 to 120 mg/kg (11-13). In preliminary dose-response experiments we found that acute oral doses of 20-50 mg/kg did not impair the general performance of pigeons, as was the case after larger doses (80-100 mg/kg), causing a dose-related blockade of eating behavior. In the experiments reported here, one pigeon in each pair of high-aggression (N = 10 pairs) and low-aggression subjects (N = 10 pairs) was treated daily with an oral dose of sodium

valproate (50 mg kg⁻¹ mL⁻¹) for 15 days. The other member of the pair received 1 mL of vehicle. Each pair was exposed to a food competition trial (10 min) 60 min after the oral dose of valproate on day 1 (acute treatment), day 7 (subchronic treatment) and day 15 (chronic treatment). The time during which the animals exhibited offensive behavior, emotional behavior, feeder control behavior, and eating behavior was measured by a well-trained observer who was blind to the treatment conditions. Time was recorded on a PC using software designed by us. The specific behaviors recorded for each category are listed in Table 1. Offensive aggression is reported as: a) total time spent/10 min in all forms of these behaviors, and b)

Table 1. Structure of the pigeon behaviors selected for recording.

1. Offensive behavior: time spent (s/10 min) in agonistic interactions involving a real or potential risk to the opponent pigeon.
 - Pursuing: to follow in order to attack or cause running away in the opponent.
 - Hooking: to attack with a wing to hold the opponent.
 - Pecking: to pick up with the beak preventing opponent's access to food, or in areas far from food, after chasing (to follow quickly in order to harm).
 - Wing beating: to hit with the wings.
 - Aggressive vocalization: vocalizations close to feeder or in areas far from food, together with horizontal movements of head and body causing wing tremor in opponent or running away in the opponent.
 - Threatening: to express intention to inflict injury causing freezing or running away in the opponent.
2. Emotional behavior: time spent (s/10 min) in behaviors involving fear and anxiety responses following offensive acts of the opponent.
 - Watching: close observation of the opponent for a time.
 - Wing tremor: after an attack or aggressive vocalizations of the opponent.
 - Fear vocalizations: short vocalizations accompanied by expiration and immobility after an aggressive attack by the opponent.
 - Freezing-like behavior in a corner of the interaction cage (far from feeder).
 - Intents to leave the observation chamber (flying up to the top of the chamber).
3. Feeder control behavior (standard laboratory measure of dominance): time spent (s/10 min) in:
 - Eating attempts: up and down of the head at the feeder without eating.
 - Pushing the opponent out of the feeder.
 - Wing covering the feeder.
 - Walking around the feeder.
 - Vigilance/immobility close to the feeder to prevent eating at the feeder by the opponent.
4. Eating behavior: time spent (s/10 min) in:
 - Eating at the feeder.
 - Eating out of the feeder (food from the feeder on the surrounding floor).
5. Resting behavior: time spent (s/10 min) in:
 - Resting (sedate).

structure of offensive behavior (time spent in each component of the behavior). The other behavioral categories are reported as total time spent.

Experiment 2. Carbamazepine treatment

Non-sedative doses of carbamazepine used in mammals for testing aggressive behavior ranged from 5 to 50 mg/kg (14,15). In preliminary dose-response experiments we found that acute doses exceeding 20 mg/kg cause a dose-related locomotor ataxia and

drowsiness in pigeons. In the present experiment, one pigeon in each pair of high-aggression (N = 10 pairs) and low-aggression birds (N = 10 pairs) was treated daily with an oral dose of carbamazepine (20 mg kg⁻¹ mL saline⁻¹) for 15 days. The other member of the pair received 1 mL of vehicle. Each pair was submitted to a food competition trial on days 1, 7, and 15 of treatment as done in Experiment 1.

Statistical analysis

Comparisons between the mean behavioral scores of experimental (drug-treated) animals and their controls obtained in the trials of days 1, 7, and 15 were analyzed by repeated measure ANOVA-2 and the *post hoc* TAU multiple comparisons test, with the level of significance set at P < 0.05. Data are reported as means ± SEM throughout the text and figures.

Results

Experiment 1. Effects of sodium valproate treatment

After data analysis by ANOVA-2, with treatment vs time as factors, there was a significant variation according to treatment (F = 49.23, d.f. = 1.54, P < 0.005), time (F = 48.98, d.f. = 2.54, P < 0.005) and interaction (F = 37.76, d.f. = 2.54, P < 0.005).

Figure 1 shows no difference in the scores of total time spent in offensive aggression between the acute valproate and control groups of high-aggression females. In these pigeons, subchronic valproate caused only a trend toward lower offensive aggression scores. However, the inhibition of offensive aggression reached significance when the chronic treatment scores (15 days) were compared (TAU: P < 0.001). Analysis of the structure of offensive aggression in the chronically treated animals showed significantly lower scores of hooking (control: 25.7

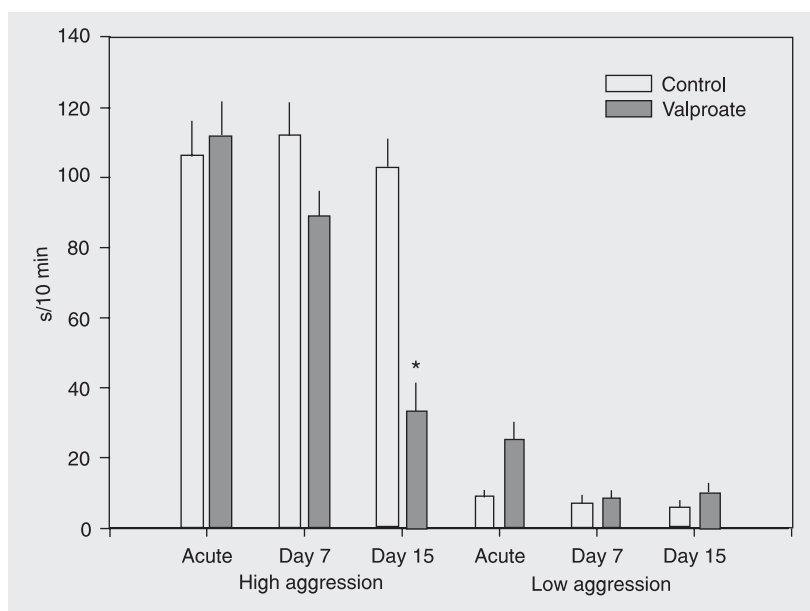


Figure 1. Acute, day 7 and day 15 effect of valproate treatment (50 mg/kg) on the total time spent by high- (N = 10 pairs) and low-aggression pigeons (N = 10 pairs) in offensive aggression. Data are reported as means ± SEM. *P < 0.001 vs control (TAU test).

Table 2. Time spent by control and valproate-treated (15 days) high- and low-aggression pigeons in various behaviors.

Behavior	High aggression (N = 10 pairs)		Low aggression (N = 10 pairs)	
	Control	Valproate	Control	Valproate
Emotion	16.3 ± 1.9	20.0 ± 2.3	23.4 ± 4.0	21.6 ± 3.8
Resting	35.6 ± 5.7	64.2 ± 15.7	171.5 ± 30.9	145.8 ± 29.6
Feeder control	18.2 ± 4.2	30.6 ± 8.7	16.7 ± 5.7	25.2 ± 12.8
Eating	424.0 ± 42.3	407.8 ± 38.6	307.3 ± 30.5	316.1 ± 32.3

Data are reported as mean ± SEM s/10 min.

± 2.0 vs valproate: 3.2 ± 0.7 s/10 min; $P < 0.05$) and aggressive vocalizations (control: 11.1 ± 0.8 vs valproate 2.0 ± 0.3 ; $P < 0.05$) and a trend toward lower scores of pecking (control: 18.2 ± 3.5 vs valproate: 10.8 ± 4.0), wing beating (control: 4.0 ± 1.2 vs valproate: 1.2 ± 0.6), threatening (control: 18.5 ± 3.6 vs valproate: 8.2 ± 2.3), and pursuing (control: 13.0 ± 5.4 vs valproate: 5.8 ± 1.1).

The time spent by high-aggression pigeons in emotional behavior, feeder control behavior, eating behavior, and immobility time was not changed by chronic valproate treatment (Table 2).

The scores of total time spent in offensive aggression by low-aggression control pigeons were very low. The acute, subchronic and chronic valproate treatment did not affect offensive aggression in these animals (Figure 1). The same was true for running away behavior, feeder control behavior, eating behavior, and immobility time (Table 2).

Experiment 2. Effects of carbamazepine treatment

After data analysis by ANOVA-2, with treatment vs time as factors, there was a significant variation according to treatment ($F = 561.71$, d.f. = 1.54, $P < 0.001$), time ($F = 48.98$, d.f. = 2.54, $P < 0.001$) and interaction ($F = 79.89$, d.f. = 2.54, $P < 0.001$).

Figure 2 shows that acute treatment with carbamazepine had no effect on the behaviors scored for the high-aggression pigeons. The 7-day and the 15-day treatments, however, were effective in decreasing significantly the scores of offensive aggression (TAU: $P < 0.01$ and 0.001 , respectively) in these animals, without affecting the other behaviors scored (Table 3). Analysis of the structure of offensive aggression in the 15-day treatments revealed significantly lower scores of hooking (control: 39.7 ± 3.2 vs carbamazepine: 11.7 ± 1.9 ; $P < 0.03$) and aggressive vocalizations (control: 25.1 ± 1.9 vs carbamazepine: 8.3 ± 1.4 ; $P < 0.05$) and

only a trend toward lower pursuing (control: 15.3 ± 3.2 vs carbamazepine: 8.1 ± 2.4), pecking (control: 5.0 ± 0.8 vs carbamazepine: 3.2 ± 0.6) and wing beating (control: 7.1 ± 1.9 vs carbamazepine: 2.5 ± 0.6) scores. In the group of low-aggression subjects the acute, subchronic and chronic carbamazepine treatments did not affect any behavior scored (Figure 2 and Table 3).

Discussion

The present results show that chronic sodium valproate ($50 \text{ mg kg}^{-1} \text{ day}^{-1}$) selec-

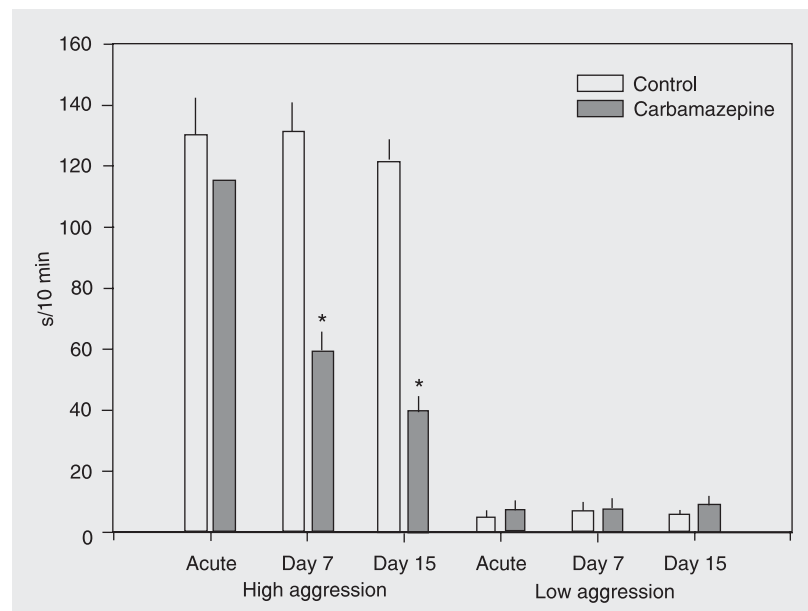


Figure 2. Acute, day 7, and day 15 effect of carbamazepine treatment (20 mg/kg) on the total time spent by high- ($N = 10$ pairs) and low-aggression pigeons ($N = 10$ pairs) in offensive aggression. Data are reported as means \pm SEM. * $P < 0.01$ vs control (TAU test).

Table 3. Time spent by control and carbamazepine-treated (15 days) high- and low-aggression pigeons in various behaviors.

Behavior	High aggression ($N = 10$ pairs)		Low aggression ($N = 10$ pairs)	
	Control	Carbamazepine	Control	Carbamazepine
Emotion	24.3 ± 4.8	37.2 ± 8.3	20.5 ± 8.4	45.8 ± 12.3
Resting	20.6 ± 4.5	28.4 ± 5.2	66.6 ± 2.8	65.3 ± 32.8
Feeder control	38.7 ± 9.4	40.0 ± 9.8	23.7 ± 6.5	20.2 ± 5.5
Eating	354.6 ± 49.8	417.3 ± 41.7	407.2 ± 36.3	376.7 ± 34.8

Data are reported as mean \pm SEM s/10 min.

tively decreased the time spent in offensive aggression without affecting the other behaviors scored for the food-restricted high-aggression pigeons exposed to food competition interactions. In the low-aggression pigeons, chronic valproate did not affect any of the scored behaviors. This was also the case for subchronic and chronic carbamazepine treatment. In addition, both valproate and carbamazepine shared similar effects on the structure of offensive behavior in the high-aggression pigeons. Though only hooking and aggressive vocalizations were significantly inhibited, all other components of this behavior were reduced by these drugs. Therefore, the two anticonvulsive drugs have similar behavioral profiles, although carbamazepine administration was followed more rapidly by antiaggressive effects in the high-aggression pigeons (7-day treatment) than valproate (15-day treatment). We speculate that the lack of a decrease in aggressive responses in low-aggression pigeons after chronic valproate and subchronic and chronic carbamazepine treatment might be attributed to the low-baseline levels of aggressiveness of these animals.

The mechanism of the anticonvulsive action of valproate is centered on interactions with voltage-sensitive Na^+ channels and enhancement of brain GABA accumulation due to stimulation of GABA decarboxylase and inhibition of GABA transaminase (see Ref. 16). The mechanism by which valproate can exert antiaggressive actions is poorly known. In rats, a linear correlation was observed between GABA levels in the olfactory bulbs and inhibition of mouse killing behavior following local injection of valproic acid and gamma-vinyl GABA (17). This suggests an inhibitory role of GABA from the olfactory bulbs in the modulation of muricidal behavior. As to carbamazepine, it can decrease resting sodium fluxes as well as sodium currents that flow during action potentials (see Ref. 16). In addition, carbamazepine was shown to increase the firing

rate of noradrenaline neurons in the locus ceruleus (18) and to inhibit 5-hydroxytryptophan (5-HTP)-evoked head twitches (19) in the rat. It has also been reported that the antiaggressive effect of carbamazepine in mice is somehow related to its agonistic action on adenosine receptors (20) and to GABAergic mechanisms (15).

Little is known about the mechanisms responsible for the antiaggressive effects of valproate and carbamazepine. We have reported that subchronic treatment with diazepam inhibits offensive aggression and emotional responses in high-aggression pigeons. In contrast, this treatment causes a significant increase in the scores of offensive aggression in low-aggression pigeons (9). Acute treatment with the opiate receptor antagonist, naloxone, is also able to block food competition aggression in high-aggression pigeons, whereas it stimulates offensive aggression in low-aggression pigeons (10). This evidence shows that the offensive behavior responses of pigeons to brain GABA-A-benzodiazepine receptor agonists as well as to opiate receptor antagonists may be exactly opposite in naturally high- and low-aggression pigeons. It has been reported that some drugs (e.g., methamphetamine) increase the rate of behavior that customarily occurs at a low rate and decreases the rate of behavior that customarily occurs at a high rate in pigeons (see Ref. 21). This might be the case also for the effect of diazepam and naloxone on aggression. This was not the case, however, for valproate and carbamazepine, suggesting a mechanism of action independent of GABA-A-benzodiazepine receptors and opiate receptors for the antiaggressive properties of these drugs. However, the results do not preclude a potential contribution of other diazepam-insensitive benzodiazepine sites on GABA-A receptors. In pigeons, the density of the latter is approximately 10-20% of total benzodiazepine receptor binding in the olfactory bulb, hippocampus, thalamic nuclei, and cerebellar granule cells (22).

As shown here for valproate and carbamazepine, both subchronic and chronic treatments with the 5-hydroxytryptamine precursor, 5-HTP, have been reported to block food competition aggression in high-aggression pigeons without changing aggressive responses in low-aggression pigeons (8). This might suggest that the effects of these drugs could implicate a stimulation of brain 5-HT systems. However, it was reported that carbamazepine inhibits head twitches evoked by 5-HTP in the rat (19). This evidence is against the involvement of a serotonergic stimulation in the antiaggressive action of

carbamazepine on high-aggression pigeons. As to valproate, to our knowledge, serotonergic actions have not been reported for this drug. The mechanism of the antiaggressive action of carbamazepine and sodium valproate on high-aggression pigeons still remains to be investigated.

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