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6. P. Seeman and C. Ulpian, *Eur. J. Pharmacol.* **94**, 145 (1983); ______, J. Wells, *Soc. Neurosci. Abstr.* **8**, 718 (1982); P. Seeman, C. Ulpian, K. A. Wreggett, J. Wells, *J. Neurochem.* **43**, 221 (1984). The human postmortem brains were stored at −70°C for as long as approximately 4 years. The dissected tissues were flown to Toronto on dry ice from Vienna, Cambridge, and Los Angeles. All tissue slices were kept at −70°C until used (within 4 months of arrival in Toronto). Separate experiments on the same human brain tissue indicated little loss (less than 10 percent) in D_2 receptor density over 4 years (unpublished). The brain tissue was weighed frozen, and 9 ml of buffer was added for each gram of original tissue. The buffer contained 50 m*M* tris-HCl (*p*H 7.4 at 20°C), 120 m*M* NaCl, 5 m*M* KCl, 1.5 m*M* $CaCl_2$, 4 m*M* $MgCl_2 \cdot 6H_2O$, 1 m*M* EDTA, 0.1 percent ascorbic acid, and 12 μ*M* nialamide. The suspension was homogenized in a glass homogenizer with rotating piston (eight strokes). The homogenate (0.8 ml) was diluted with 35 ml of buffer, centrifuged (39,000*g* for 30 minutes at 4°C), and the pellet resuspended in 35 ml and recentrifuged; the final pellet was resuspended in 30 ml of buffer, yielding a concentration of 2.7 mg of original tissue per milliliter. The suspension was homogenized with a Polytron (Brinkmann PT-10, 20 seconds, setting 7). The density of D_2 dopamine receptors was measured by saturation analysis, through the use of 12 concentrations of [^{3}H]spiperone (10 to 1000 p*M*; 28 to 34 Ci/mmol, New England Nuclear). Each incubation tube received 0.5 ml of buffer, 0.5 ml of [^{3}H]spiperone, and 0.5 ml of homogenized tissue suspension. It was important to use a final concentration of less than 1 mg of (original) tissue per final milliliter of incubate, because the apparent values for the K_d and the receptor density varied directly with the final concentration of tissue above 1 mg/ml (latter reference). Our method generally resulted in a K_d of about 84 p*M* for control human striatum; this value can be compared with values of between 120 and 700 p*M* (*1–5*) reported with high concentrations of tissue. Thus, the final concentration of tissue was about 0.9 mg of original tissue per milliliter (equivalent to less than 70 μg of protein per milliliter of final incubate). The mixtures were incubated at room temperature (20°C) for 120 minutes and then filtered (12 tubes simultaneously) by a Titerteck cell harvester (Skatron, Lier, Norway), with a glass fiber filter mat (two stapled together; Flow Lab 78-195-05) and a vacuum of 400 to 500 mmHg. The filter mat was rinsed for 15 seconds with 10 ml of 50 m*M* tris-HCl (*p*H 7.4 at 20°C). The filters were monitored for tritium by scintillation spectrometry at 45 to 50 percent efficiency. Specific binding was defined as that binding of [^{3}H]spiperone inhibited by the presence of 1 μ*M* (+)-butaclamol (Ayerst, Montreal). The values for the maximum number of binding sites (B_{max}) and K_d were derived by Scatchard analysis, wherein the 12 data points for each analysis fitted a straight line with a correlation coefficient always greater than 0.98. Replicate determinations of B_{max} on different days (for the same human tissue) agreed to within 5 percent. The diagnosis of schizophrenia was made by the postmortem examination of the case record by at least two psychiatrists. The criteria used were either identical or similar to those of DSM-III [*Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association, Washington, D.C., ed. 2, 1980)].
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Siblicidal Aggression and Resource Monopolization in Birds

Abstract. *In Texas, great egret* Casmerodius albus *chicks attack younger nestmates, often fatally (siblicide). By contrast, the young of neighboring great blue herons* Ardea herodias *seldom strike or kill siblings. These interspecific differences seem related to prey size: only fish provided by egret parents are small enough for chicks to monopolize (a process facilitated by aggression). Experimentally cross-fostered heron chicks raised on small prey by egret parents became siblicidal, but the reverse procedure of cross-fostering egret chicks did not reduce aggression or siblicide.*

Siblicide, fatal aggression among siblings (*1, 2*), is a taxonomically widespread phenomenon that can occur prenatally (*3*) or postnatally (*4*). Because it involves the killing of close kin, inclusive fitness theory predicts that siblicide would evolve only when the principal's own survival is seriously jeopardized. Although such risk could be expected when critical resources are currently or prospectively inadequate for all broodmates to survive (*5*), little is known about the way in which such resources affect sibling aggression. I have proposed that the degree to which small food (*6*) can be monopolized profitably is an important ecological determinant of sibling aggression and, therefore, of siblicide (*7*). I now partially confirm this prey size hypothesis by a cross-fostering experiment involving two species of colonial Ardeidae (Aves, Ciconiiformes).

I studied great egrets (*Casmerodius albus*) and great blue herons (*Ardea herodias*) on three islands in Lavaca Bay, Texas (30°39′N, 96°34′W) during the summers of 1979, 1980, 1981, and 1982. Both species are piscivorous and monogamous, with males and females sharing all aspects of parental care. Incubation typically begins after the first egg is laid, so the 1- to 2-day interval between the laying of successive eggs produces comparable hatching intervals. Both species frequently experience brood reduction, but the demise of the youngest chick is effected differently between species. Whereas siblicide is the apparent cause of death in many, perhaps most, egret brood reductions, it was significantly rarer in heron nests (*8*).

During the first month, aggression in egret broods is on the average 18 times higher than aggression in heron broods, most of which do not fight at all (Table 1). Egret nestmates quickly form stable, age-dependent dominance hierarchies that confer distinct feeding advantages to

Table 1. Fighting rates of broods (three and four chicks) of great egrets and great blue herons in Texas. All data are from the first 25 days after the completion of hatching and are expressed as means ± 1 standard deviation.

Brood size	Natural broods		Foster broods*	
	Broods (No.)	Fighting rates†	Broods (No.)	Fighting rates†
Great egrets				
Three chicks	9	1.46 ± 1.32	9	1.09 ± 1.11
Four chicks	2	1.42 ± 0.16	1	0.69
Pooled	11	1.45 ± 1.22	10	1.05 ± 1.05
Great blue herons				
Three chicks	4	0.08 ± 0.17	7	1.51 ± 0.75
Four chicks	9	0.08 ± 0.14	2	0.38 ± 0.29
Pooled	13	0.08 ± 0.14	9	1.26 ± 0.83

*Egret chicks raised by heron adults and heron chicks raised by egret adults (see text). †Fighting rates were standardized across brood sizes by $F_t/N_d/N_r$, where F_t is the total fights, N_d is the number of possible dyads, and N_r is the number of days recorded. The number of possible dyads varies with brood size: three-chick broods have three possible dyads, and four-chick broods have six. Fighting rates between species show significant differences [$P < 0.001$, analysis of variance (ANOVA) with Scheffé test]. Rates between brood sizes within each species were not significantly different (*17*).

Table 2. Effects of cross-fostering on the relative frequency of siblicidal mortality, presented as a comparison of the 1981–1982 foster broods with the 1979–1981 observed broods of three or four chicks (natural parents). The fates presented are for the youngest sibling in each brood.

Parents	Great egret chicks* (No.)			Great blue heron chicks† (No.)		
	Alive by day 25	Siblicidal deaths‡	Other deaths	Alive by day 25	Siblicidal deaths	Other deaths
Natural	5	8	4	8	1	10
Foster	4	6	0	1	6	2

*No significant differences in frequency of siblicide between treatments for great egret chicks ($P = 0.163$, Fisher's exact test). †$P = 0.006$, Fisher's exact test. ‡Observed beatings to the point of visible wounding [see (*7*)].

the senior siblings; this fighting effectively intimidates junior siblings (*7*). After approximately 1 week of indirect feeding (fish regurgitated by parents onto the nest floor), older siblings begin direct feeding, by which they can monopolize the whole regurgitated bolus by gripping the parent's bill and catching the discrete mass of fish directly. By contrast, the typical single fish regurgitated by heron parents is so large that it is impossible to catch and, therefore, cannot be monopolized (*7, 9*).

I propose that prey size plays a major indirect role in the evolution of siblicidal aggression (as compared to less violent alternative forms of brood reduction), simply by influencing the economic defendability (*10*) of a key limiting resource (*7*). In this egret population, sibling aggression appears to be automatic [even broods experimentally provided with freely accessible food continue fighting (*7*)] and remains consistently profitable to the older or dominant chicks. Because Texas heron prey are never defendable, even when the chicks are several weeks old, the potential profit for fighting remains lower in those broods. According to this hypothesis, siblicidal aggression may be facultative, with prey size (specifically, its monopolizability) serving as a key proximate cause (*7*).

To test this hypothesis, a fostering experiment was conducted in which ten heron broods were raised by egret parents and ten egret broods were raised by heron parents (*11*). If siblicidal aggression is facultative, then normally pacifist heron chicks raised on small fish by egret parents should show the combination of direct feeding and fighting that characterize natural egret broods. Conversely, egret chicks fed fish too large for them to monopolize should show reduced fighting if their siblicidal aggression is similarly facultative.

Complete broods were exchanged between species as eggs or recently hatched chicks (maximum age, 3 days posthatch) (*12*). These nests were kept under continuous daylight observation from a blind less than 30 m away for at least 25 days. Parents appeared to adopt the new broods readily. To compensate for the possibility that egret parents might not supply sufficient quantities of food to the larger heron chicks, half of the foster heron broods were given daily food supplements (*12*).

As predicted, heron chicks raised by egret parents fought at significantly higher frequencies during the first month than when raised by their natural parents (Table 1) (Mann-Whitney *U* test: $U = 114$, $P < 0.001$), showing an average increase of more than 15-fold. The foster broods that received extra food did not show reduced fighting relative to the unprovisioned broods (*13*). Heron chicks in egret nests also made an abrupt transition to direct feeding (*14*) and performed siblicidal brood reduction at a significantly higher rate (Table 2).

However, the results for egrets in foster heron nests are difficult to interpret. Though fighting rates showed a slight decline, they were not significantly lower than in natural egret broods (Table 1). The egret chicks persistently scissored the foster parent's bill, although the large prey could not be intercepted directly (*14*). The resulting frequency of siblicidal deaths was not reduced relative to the observed natural broods (Table 2).

These experimental results indicate that prey size and monopolizability may elicit preemptive sibling aggression in heron nestlings. Because the associated changes in feeding behavior accompanied the increase in sibling fighting, and because great blue herons take a wide variety of prey types and sizes across that species' broad range, such a system of facultative siblicidal brood reduction may be an adaptive behavioral complex whose flexibility is matched to the ecological diversity encountered.

The lesser behavioral flexibility found in the fostered egret chicks is puzzling and may be due to several factors: (i) less variance in prey sizes (that is, if prey are always small enough for chicks to monopolize) across the species' cosmopolitan range, such that sibling aggression is essentially obligate in all populations; (ii) a population-by-population pattern of obligate as opposed to facultative sibling aggression that matches the size ranges of local prey types; or (iii) additional factors not controlled by this cross-fostering procedure.

Although not previously advanced as an influence on sibling aggression (or brood reduction generally), the apparent relation between prey size and siblicidal aggression reported may be widespread in other predatory birds. Among avian taxa known to practice siblicide, most (if not all) feed offspring by presenting them with units of food small enough to monopolize that could be subject to control through various degrees of sibling intimidation. Some of these species (for example, certain boobies, cranes, kittiwakes, and three more species of egrets) capture small prey (*2, 4, 7, 15*), while others (for example, some eagles, hawks, owls, pelicans, and skuas) capture larger prey that the parents tear (or digest) into small pieces before presenting to the offspring (*2, 4, 16*). The possible influence of food size on sibling aggression in these species remains to be examined.

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1. "Siblicide" is a gender-free alternative to "fratricide" and is therefore a preferable term in cases where the victim's sex is unknown [see S. J. Gould, *Nat. Hist.* **91**, 12 (1982)]. In the context of avian brood reduction, siblicidal deaths often result from the combined effects of physical beatings and socially enforced starvation [see (*7*)].
2. The most detailed published accounts of siblicide in other avian species are those of V. Gargett [*Ostrich* **49**, 57 (1978)] for a single brood of black eagles (*Aquila verreauxi*) and of B. M. Braun and G. L. Hunt, Jr. [*Auk* **100**, 469 (1983)], for kittiwakes (*Rissa tradactyla*).
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6. Or other forms of essential and nonshareable parental investment [S. A. Altmann, S. S. Wagner, S. Lenington, *Behav. Ecol. Sociobiol.* **2**, 397 (1977)].
7. D. W. Mock, *Am. Nat.*, in press.
8. Censuses of 145 egret and 41 heron nests conducted every fourth day during 1980 and 1981 showed significantly more siblicide in egret broods (22.1 percent) than in heron broods (2.4 percent) [see (*7*)].
9. The estimated mass of the median fish regurgitated by heron parents was 95 times greater than that regurgitated by egrets. Egret boluses typically contained 5 to 15 fish (5 cm, 2 g each) stuck loosely together by mucous [see (*7*)].
10. J. L. Brown, *Wilson Bull.* **76**, 160 (1964).
11. In one foster brood, the third heron egg failed to hatch, making the final experimental sample sizes nine and ten broods.
12. Provisioning consisted of depositing measured piles of small fish (approximately 33 percent of the estimated daily total brought by the parents) onto the nest floor during the afternoon change

of observers. The nestlings usually consumed this food quickly.

13. The unprovisioned foster broods performed an average of 1.07 fights per dyad per day, while the provisioned broods actually fought slightly more (mean = 1.56) (Mann-Whitney U test: U = 14, not significant).
14. Egret chicks fed by egret parents begin direct feeds on day 7 and use that method in 90 percent of their boluses by day 24. Similarly, fostered heron chicks began using it regularly on about day 9 and reached 90 percent on day 24. By contrast, neither heron young fed by heron parents nor fostered egret chicks reached a frequency of 20 percent.
15. See J. B. Nelson, *The Sulidae* (Oxford Univ. Press, Oxford, 1978); R. S. Miller, *Wilson Bull.* **85**, 436 (1973); B. Meanley, *ibid.* **67**, 84 (1955); D. F. Werschkul, *Auk* **96**, 116 (1979); D. Mock and B. J. Ploger, in preparation; M. Fujioka, unpublished data. Data on reddish egret from R. T. Paul (personal communication).
16. C. Ingram, *Auk* **76**, 218 (1959); B.-U. Meyburg, *Ibis* **116**, 224 (1974); I. Newton, *Living Bird* **16**, 51 (1977); C. H. Stinson, *Evolution* **33**, 1219 (1979).
17. Analysis of variance was used for these data as described [B. J. Feir and L. E. Toothaker, *Educ. Psychol. Meas.* **34**, 789 (1974)].
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Learned Histamine Release

Abstract. *Most of the effort directed at understanding the problems of allergy has focused on the interacting components of the immune system. The possibility that histamine may be released as a learned response has now been tested. In a classical conditioning procedure in which an immunologic challenge was paired with the presentation of an odor, guinea pigs showed a plasma histamine increase when presented with the odor alone. This suggests that the immune response can be enhanced through activity of the central nervous system.*

Although there is evidence that learning may modify immunosuppression (*1–3*), its effect in activating the immune system has not been demonstrated. There have, however, been many anecdotal reports of associative learning in allergic reactions. Suggestions of this phenomenon existed in the 19th century; for example, an asthmatic patient who had an allergic reaction to roses experienced an attack when exposed to an artificial rose (*4*). However, the lack of data on the mechanisms of such a response has hindered the acceptance of associative learning as a factor in immune responses. Histamine is a mediating factor in the immune system (*5, 6*) and has been found in most tissues (*7*). It is released in a variety of pathological conditions thought to be stress related, such as duodenal ulcers (*8*), asthma (*9*), cluster headaches (*10*), and premenstrual stress (*11*). This suggested to us that histamine may be released as a learned response to the pairing of a neutral stimulus with an immunologic challenge. We showed that guinea pigs (*Cavia cobaya*) had increased plasma histamine levels in response to a neutral stimulus (an odor) in a classical conditioning procedure in which an immunologic challenge was paired with presentation of the odor.

Table 1. Experimental sequence. During training, CS+ was given with the antigen, and CS− was given without the antigen. During test trials no antigen was present.

Trial No.	Stimulus
Training trials	
1	CS+
2	CS−
3	CS−
4	CS+
5	CS+
6	CS−
7	CS−
8	CS+
9	CS−
10	CS+
Test trials	
1	CS+
2	CS−
3	CS+

Four weeks before training was begun, eight adult male guinea pigs (Hartley strain) were immunologically sensitized to bovine serum albumin (BSA) by injecting into a footpad 2.5 mg of BSA in a 0.1-ml mixture of equal volumes of Freund's adjuvant and normal saline. A 5 percent solution of BSA subsequently served as the unconditioned stimulus for histamine release. All animals were handled and gentled three times weekly while they were weighed and their cages were cleaned. This also served to reduce stress to the animal caused by being handled during the testing procedures.

A classical discrimination conditioning design was used to train the animals, with each animal acting as its own control. In this design, one odor (CS+) was always paired with the unconditioned stimulus (BSA), and the other (CS−) was paired with saline. One percent solutions of dimethylsulfide (sulfur smelling) and triethylamine (fishy smelling) were used as the conditioned stimuli. For half the animals, CS+ was dimethylsulfide and CS− was triethylamine and for the other half, CS+ was triethylamine and CS− was dimethylsulfide. Each animal was given ten presentations, five trials with the CS+ and five trials with the CS−. Training trials were given 1 week apart to allow the animal to recover from any allergic reaction. The order of the presentation of the CS+ and the CS− was randomized (Table 1).

At the start of each training trial the animal was placed in a glass-walled container and a cotton-tipped swab soaked in either the CS+ and BSA or in the CS− and saline was placed on the animal's nose for 3 seconds. Ten minutes after exposure to the stimulus, the animal was anesthetized with ether, and 2 to 4 ml of blood were drawn from the retro-orbital sinus (*12*). The blood was immediately centrifuged and the plasma was tested for histamine by a radioenzymatic assay (*13*). Test trials began 2 weeks after the training. In the first trial the CS+ odor was presented without BSA. Two weeks later the CS− odor was presented. After another 2 weeks the CS+ was presented again. All other procedures were identical to those during training. The experimenter, in all phases of the study, was unaware of the order and pairing of the stimuli.

Plasma histamine levels during the test trials (extinction) were analyzed with a repeated measures analysis of variance, which achieved an overall F value of 45.57 ($P < 0.0005$). One-tailed planned comparisons between specific test conditions revealed significant differences between the CS− and the first CS+ ($P < 0.001$). All eight animals had greater histamine release in response to the CS+ (mean ± S.E.M. = 147.5 ± 28.7 ng/ml) than to the CS− (49 ± 7.7 ng/ml) (Table 2). A smaller difference was found between the CS− and the second CS+ (54.2 ± 17.0 ng/ml) ($P < 0.055$). Only one animal had a lower histamine level in response to the second CS+ than to the CS−. No significant difference was found between the CS− trial and the

Table 2. Results of test trials with the conditioned stimuli in the absence of antigen. Results are given as means ± S.E.M.

Trial	Plasma histamine (ng/ml)
Baseline	18.3 ± 4.9
Test trials	
CS+	147.5 ± 28.7
CS−	4.9 ± 7.7
CS+	54.2 ± 17.0