trols were not significant ($t = 3.09$, d.f. $= 1$, $P < .10$) and in fact went in the opposite direction.

Pupil size was also monitored during these sessions by use of a video pupilometer system in ordinary room light. One minute of continuous pupillary measurement was obtained before and on two occasions after stimulus onset. When pupil diameter during baseline trials was compared to that during test trials 18 minutes after injection, four of eight subjects showed slight dilation. However, these differences were not significant in the pooled data.

The proportion of subjects who showed a conditioned response during the test sessions is indicated in Table 1. A conditioned response was defined as a consistent difference between the baseline and final test trials which was in the same direction as the unconditioned response (that is, the average response to naloxone). Qualitatively the conditioned withdrawal response (CR) was similar to the unconditioned withdrawal response (UR). In pilot work with larger naloxone doses (*5*) the UR was of much greater magnitude than the CR. The small naloxone dose in this experiment (0.1 mg) produced a mild UR and the CR was of comparable magniture; subjects were unable to reliably distinguish CR from UR.

Acquisition curves (not shown) were plotted by comparing baseline trials (trials 2 and 3) with test trials (trials 7, 11, 15, 19, and 20). Maximum responses for respiration, temperature, ethogram, and subjective responses were present by the second test trial (trial 11) and for heart rate by the third test trial (trial 15).

These data suggest that both objective and subjective elements of the narcotic withdrawal syndrome in humans can be conditioned experimentally. This lends credence to clinical reports of such phenomena occurring naturally when treated addicts return to their former environment. The strength of this phenomenon under clinical conditions is uncertain. It may account for only a small part of the tendency to relapse to drug use. Nevertheless, a program to locate and extinguish these responses in individual patients would be feasible and might improve treatment outcome.

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6. It was not ethically possible to place all subjects on the same dose of methadone. In pilot work with other subjects, we attempted to vary dose of naloxone in order to obtain a constant magnitude of UR. This method was less satisfactory than the constant naloxone dose reported here. The magnitude of the unconditioned response did not vary significantly as a function of methadone dose.
7. Dr. Barbara Chaddock (deceased) participated in the early planning of this study. Supported by grants IRO 1 DA 00586 and DA 01218 from the National Institute of Drug Abuse.

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Species Identification in the North American Cowbird: Appropriate Responses to Abnormal Song

Abstract. *Female cowbirds raised in auditory isolation from males responded to the songs of male cowbirds with copulatory postures. The songs of males reared in isolation were more effective in eliciting the posture than the songs of normally reared males. The females did not respond to the songs of other species. These results indicate one mechanism of species identification for this parasitic species.*

The brown-headed cowbird, *Molothrus ater*, is reported to parasitize more than 200 species (*1*). Thus, unlike most vertebrates, young cowbirds are not exposed to stimulation by conspecific parents during their earliest interactions with adults or peers. Although much remains to be learned about the developmental impact of such stimulation, there is growing evidence to demonstrate its facilitative role in avian species identification (*2*). How, then, does the cowbird, naturally deprived of such experience, come to identify other cowbirds? This question has puzzled students of development and evolution for many years, and, until now, only speculative solutions existed (*3*). We report a series of experiments detailing a mechanism of species identification that allows us to understand how cowbirds have evolved to overcome the fact that they were not reared by conspecifics.

Two findings led to the discovery of this mechanism. First, we had raised a female cowbird in the laboratory in complete auditory and visual isolation from other cowbirds. At 8 months of age, she was exposed to a recording of male cowbird courtship song. She adopted a posture we term the "copulatory response": her wings were lowered and spread apart, her neck and body were arched, and the feathers around the cloacal region were separated (Fig. 1). A series of playbacks confirmed the specificity of the response: she showed no response to the songs of a red-winged blackbird (*Agelaius phoeniceus*) or to the cowbird flight call.

The second finding related to the development of the male song. Male cowbird song, which can be transcribed as "burble burble tsee," occurs during courtship and is accompanied by a bowing display. We collected data from a small group of males that suggested that male peer experience was sufficient to produce normal adult courtship song. However, males reared in isolation from both male peers and adults, developed songs that differed from the normal version, particularly in the extent to which some of the notes are modulated (Fig. 2).

The following experiments were designed to elaborate upon these observations by testing other naive females. The songs of both normal and isolate males were included to help clarify the role of peer experience in song development.

The subjects were six female cowbirds raised in auditory and visual isolation from adult cowbirds. Their prenatal auditory experience was controlled in that eggs were obtained from a captive colony of cowbirds and incubated in isolation. Their postnatal social contact was limited to a 1- to 5-day period during which the individual eggs hatched into barn swallow (*Hirundo rustica*) nests, and the young birds were fed by their hosts. After being returned to the laboratory, they were hand-reared as a group without contact with adult cowbirds. They were placed in soundproof chambers between the ages of 35 and 60 days (*4*). The birds were housed in pairs to allow the opportunities for social interaction and auditory experience thought to be essential for the birds to come into breeding condition. Chambers 1 and 2 each housed two female cowbirds; chamber 3, a female cowbird and a female red-winged blackbird; chamber 4, a female cowbird and a male cowbird; chamber 5, a male cowbird and a male cardinal (*Richmendena cardinalis*). The light phase of their photoperiods was gradu-

Fig. 1. Female cowbird displaying the copulatory response.

ally extended during the spring months.

The playback period began in May 1975, when the birds were 9 months old, and was conducted in two phases. In the first phase, the females were exposed to recordings of normal cowbird song obtained from a wild-caught male and the control songs of male red-winged blackbirds, meadowlarks (*Sturnella magna*), and Baltimore orioles (*Icterus galbula*). There were four playbacks spread throughout each day; the order of presentation varied each day but was the same for each bird. In the second phase, the procedure was identical except that the playback series included the abnormal song of an isolate male. The response measure was the presence or absence of the copulatory posture (*5*).

During the first phase, four of the six female cowbirds responded more often to normal song than to any of the control songs (Table 1). One of these, female DB, showed a dual preference for cowbird and meadowlark song. The fifth cowbird, female W, did not come into breeding condition and hence did not respond. The sixth, female CB, was housed with the male cowbird and also showed no response. She was observed, however, to copulate frequently. The variability across the females was probably due to variations in breeding condition, which was indicated by the numbers of eggs laid.

During the second phase, four females demonstrated a clear preference for the abnormal song (Table 1). For each of the four birds, the abnormal song produced the highest rates of response, the normal song the second highest and the control songs of the other species the lowest rates.

In order to pursue this finding in greater detail, the birds were maintained in isolation for another year and retested in the spring of 1976. Several procedural changes were also made. Female CB, which had been reared with the male cowbird, was transferred to a chamber housing a female canary 3 weeks before the 1976 testing. Also, two samples of parts of the abnormal song were used: (i) the "burble-burble" phrase alone, (ii) the "tsee" alone, and (iii) a new sample of abnormal song from another isolate male.

The abnormal song retained its superior effectiveness, and abnormal, normal, and partial-abnormal songs were all more effective than the controls (Table 1). The differences among the conditions were reliable (Friedman two-way analysis of variance, $\chi_r^2 = 23.3$, $P < .001$). Both samples of abnormal song were equally effective, and one of the partial songs was more effective than the full normal song (*6*). Female CB, despite her sexual experience and her lack of responding in the earlier experiment, responded as strongly as the sexually naive females (*7*).

Another piece of evidence attests to the potency of the abnormal song as a sexual releaser. Two wild female cowbirds were captured and placed in the laboratory in the winter of 1976. While in these surroundings, they heard, but could not see, caged male cowbirds and male and female free-living cowbirds. Although a full series of playbacks was not carried out owing to the birds' nervousness in captivity, the first time the abnormal song was played to them, one of the females immediately adopted the copulatory posture. In subsequent exposures, she continued to respond to the abnormal song but never to the normal. Thus, response to abnormal song was not restricted to females raised in isolation. Furthermore, the response of this female and that of female CB suggest that auditory and sexual experience with males does not diminish the effectiveness of the abnormal song.

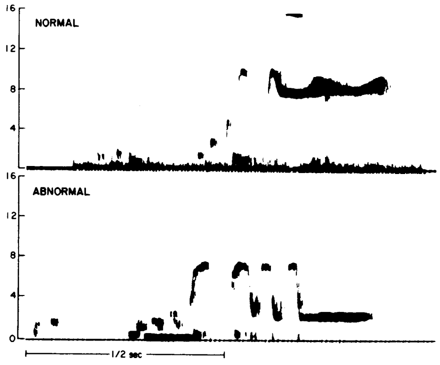

Fig. 2. These recordings were made on a sonograph (Kay) with a wide-band filter and set for flat response and linear display. The units on the ordinate are kilohertz. The most striking difference is the rapid modulation between 2 and 8 khz present in the abnormal but not the normal song. This type of modulation was not present in any of a sample of 522 song spectrographs obtained from a captive colony of normally reared male cowbirds (*10*).

In summary, these data indicate that neither male nor female cowbirds require experience with adult cowbirds in order for species identification to occur. Naive females respond adaptively to male song; naive males produce an acoustically atypical, but highly effective, song. Isolate song, then, appears to have qualities of a "supernormal" releaser. Why normal song appears less effective is as yet unspecified, but it may reflect the fact that males sing in contexts other than courting. The songs of males, therefore, may contain more complex messages indicating not only the species, sex, or breeding condition of the singer, but agonistic or territorial information as

Table 1. Percent of copulatory responses to each category of song in the springs of 1975 and 1976; *N*, total number of playbacks in each category (not the number per bird).

Category	*N*	Response rate (%)						
		Female						$\bar{X}$
		DG	Y	WW	DB	W	CB*	
				1975				
Phase 1 normal	157	25	29	50	95	0	0	50
Phase 2 normal	157	0	24	0	62	0	0	22
Phase 2 abnormal	116	88	100	62	95	0	0	88
Control	220	0	2	0	32	0	0	8
				1976				
Abnormal								
One	105	78	81		54	74	88	75
Two	81	79	71		75	84	79	78
Partial								
"Burble"	105	36	69		50	64	64	57
"Tsee"	130	0	5		47	0	42	19
Normal	121	31	38		28	32	48	35
Control	72	0	0		13	0	0	3

*Because CB and W never responded to song, their data were not included in any 1975 analysis.

well. A closer assessment of the functional properties of the songs of normally reared males may help to clarify these speculations. In addition, these data suggest the need to examine in more detail the developmental role of self-stimulation. It may be that the self-generated sounds of the isolate males channeled their song development toward an emphasis on the acoustic properties that contain the sexual message in cowbird song. Males reared with other males, however, whether age mates or adults, may receive acoustically more varied stimulation, may learn to modify their song components as a result of auditory and behavioral feedback from their companions (thereby leading to a dilution of the purely sexual message), or both. In any case, these data implicate self-stimulation as possibly a very important form of species-typical experience for cowbirds as has been hypothesized (*8*). Finally, although it is tempting to label the cowbird's response to isolate song as idiosyncratic or unrepresentative of other songbirds, this cannot be done, as little comparative information exists regarding the responses of other species to isolate songs. Such information would be useful in species that, like the cowbird, have courtship songs. Songs of a primarily territorial nature may be more difficult to assess.

Although these results provide one answer to the question of the mechanism by which cowbirds identify one another, they do not explain how young or juvenile cowbirds first come to recognize one another. Little is known about cowbird social behavior, yet data from our laboratory indicate that cowbirds have highly structured dominance hierarchies and complex intraspecific behaviors that may also facilitate identification or maintain social integration among acquainted cowbirds (*9*). The mechanism we have described might represent an independent system designed to ensure identification during the most important context, the breeding season, and to enhance reproductive opportunities by inducing sexually appropriate behavior in the female. Thus, although cowbirds may have multiple means to identify one another, the presence of a mechanism geared directly for reproduction may represent a critical adaptation for a parasitic species.

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4. Each chamber consisted of two concentric boxes constructed of plywood and sheetrock. Wood and acoustic tile baffles between the boxes were designed to be most effective between 2 and 16 khz. Suppression was greater than 39 db at 1000 hertz, and it increased with higher frequencies to greater than 50 db between 8 and 16 khz. The interior box was a 1.1-m cube, fabric-lined to reduce sound reflection, lighted by two 40-watt Vita Lite tubes and continuously ventilated. White noise was broadcast in the room housing the chambers.
5. The songs were recorded with a dynamic microphone (Uher 517) and tape recorders (Uher 4000). The recordings were played through a driver (JBL 2420) and horn (JBL 2340). Playback levels were determined with a sound pressure meter (General Radio 1933). The same sound pressure levels (SPL's) were used for playbacks of the normal and the abnormal songs. At 0.35 m from the speaker along the axis, the SPL was 86 ± 1.5 db slow reading and 104 ± 1.5 db impulse. Control songs of the other species were adjusted to the slow reading. The A-weighted SPL inside the chamber was 50 db slow reading.
6. Differences between categories of song were tested with t-tests. Reliable differences were obtained for the following comparisons: abnormal one or two versus normal song (abnormal one: $t = 11.04$, $P < .001$; abnormal two: $t = 9.95$; $P < .001$); abnormal versus control songs (abnormal one: $t = 8.85, P < .001$; abnormal two: $t = 19.43$, $P < .001$); normal versus control song ($t = 6.09, P < .001$); the "burble" phrase versus the "tsee" phrase ($t = 3.17$, $P < .05$); and either partial song versus the normal "burble" ($t = 4.22$, $P < .001$). There was no reliable difference between the two samples of abnormal song or between the "tsee" phrase and normal song.
7. Female CB's lack of response the previous year probably resulted from her being housed with a male cowbird, who repeated his song to her many times each day, thus raising her threshold for song responsiveness. Furthermore, her companion's song was undoubtedly of superior acoustic quality, a factor that might also have diminished her interest in the recorded songs.
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* Request for reprints should be sent to M.J.W.

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Phenylethanolamine: A New Putative Neurotransmitter in *Aplysia*

Abstract. *Phenylethanolamine is present in the* Aplysia *nervous system in concentrations similar to that of octopamine. There are receptors that are very specific for phenylethanolamine, which on different neurons mediate sodium, chlorine, or potassium conductance increase responses. These observations indicate that phenylethanolamine may act as a neurotransmitter in* Aplysia.

Phenylethanolamine is a biogenic amine that differs from norepinephrine in lacking two ring hydroxyl groups. In mammals, it is synthesized from phenylalanine through the action of aromatic amino acid decarboxylase (to make phenylethylamine) and dopamine β-hydroxylase (*1*). Phenylethanolamine is present in mammalian fetal brain (*1*) but dramatically decreases in concentration with prenatal development (*2*). The presence of phenylethanolamine has usually been considered to be an accident of catecholamine synthetic pathways without a physiological function, although sympathetic nerve terminals can take up phenylethanolamine and store it to a limited extent (*1*).

The nervous system of the marine mollusk *Aplysia californica* lacks norepinephrine but contains considerable amounts of dopamine (*3*) and octopamine (*4*). Some *Aplysia* neurons have specific receptors for dopamine (*5*) and octopamine (*6*). We have detected the presence of a considerable amount of phenylethanolamine in the *Aplysia* nervous system and established the existence of specific receptors for this amine.

Phenylethanolamine was assayed as described by Saavedra and Axelrod (*1*) from buccal, pleural, pedal, cerebral, and abdominal ganglia and from various nerves (Table 1). It is present in all ganglia, its concentration being highest in the buccal (2.32 ng per milligram of protein) and lowest in the pedal (0.73 ng per milligram of protein). The identity of endogenous phenylethanolamine was confirmed by thin-layer chromatography (*1*). The amount of phenylethanolamine in these tissues does not significantly differ from that of octopamine (*4*). Phenylethanolamine is present in the ganglionic neuropil, the region of normal synaptic contact. Its concentrations in this area are lower than those in the whole ganglion in the case of the buccal, pedal, and pleural ganglia. Thus, the possibility remains that phenylethanolamine is also present in neuronal cell bodies. However, to our knowledge no phenylethanolamine has yet been found in isolated, identified neurons. Phenylethanolamine was also found in the nerves, in particularly high concentration in the pleural-abdominal connective nerves.

Electrophysiological studies were performed as previously described (*6*). Phenylethanolamine was dissolved in dis-