gonococcal transconjugants was made on plates containing penicillin at 0.2 to 1.0 μg/ml, rifampin at 5.0 μg/ml, fusidic acid at 1.0 μg/ml, and nalidixic acid at 2.0 μg/ml. Recipients of *N. flava* were $Rif^R$ $Nal^R$, and selection was made on plates containing penicillin (1.0 μg/ml), rifampin (5 μg/ml), and nalidixic acid (2 μg/ml). *Escherichia coli* transconjugants were selected on ampicillin (30 μg/ml) plates.


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10 January 1977


## Conditioned Narcotic Withdrawal in Humans


Abstract. *Subjective and physiological manifestations of the narcotic withdrawal syndrome were produced as a conditioned response. Withdrawal reactions precipitated by the narcotic antagonist naloxone in methadone-dependent volunteers were the unconditioned response. These data support clinical anecdotes of withdrawal symptoms occurring in former addicts when they return to their drug-related environment.*


Resumption of narcotic use is a serious problem among treated addicts. Clinical evidence suggests that conditioning factors may play a role in relapse (*1*). Former addicts have reported the return of withdrawal symptoms when they visit areas of drug use (*2*). Wikler first proposed a conditioning explanation for these reports (*3*), and subsequent animal research (*4*) has provided support. Recently we reported an experimental method for producing conditioned withdrawal in human subjects (*5*). We now report objective evidence of conditioned narcotic withdrawal experimentally produced in eight male former heroin addicts.

The subjects were maintained on a constant daily dose of methadone, with daily urine test evidence confirming their lack of street drug use. Their median age was 26 years (mean, 26 years; range, 22 to 29 years), median education was 12 years (mean, 12 years; range 9 to 16 years), and median duration of addiction was 7 years (mean, 7 years; range, 4 to 10 years). Five subjects were white and three were black. There were two controls, one white and the other black. All volunteered for the study and gave their written informed consent after a detailed explanation of the procedure. The events that would take place during a session were described to participants; they were also informed that they would receive either saline or naloxone, a short-acting narcotic antagonist, which would cause mild withdrawal symptoms. Intramuscular injections of naloxone (0.1 mg) were the unconditioned stimulus. These injections caused the onset of tearing, rhinorrhea, yawning, decreased skin temperature, increased respiratory rate, and increased heart rate; these reactions lasted about 20 to 30 minutes and were conditioned to environmental cues. Methadone dosage ranged from 25 to 70 mg (mean, 43 mg; median, 40 mg). By using a constant dose of naloxone, the unconditioned withdrawal reactions were kept at approximately the same magnitude within and among participants (*6*).

Participants were tested individually in 1-hour sessions in a sound-attenuating chamber where heart rate, skin temperature, respiration, motor responses, and subjective reports were monitored and recorded. Introductory instructions were presented each day at the beginning of the session. At this time the patient had certain procedures explained to him (such as where he should place his head for pupil measurements and how he should answer questions about severity of withdrawal symptoms). After instructions, the participant heard background music for a 10-minute period of baseline measurement. Naloxone was then administered by a nurse using a standard procedure. The background music gradually became softer as a tone and odor (compound conditioning stimulus) (CS) became stronger. The tone (700-hertz, audiogenerator) was tape-recorded and was therefore the same for all subjects. It began at a low level 3 minutes after injection, became audible 7 to 8 minutes after injection, reached peak intensity (70 db) at 13 minutes, and remained at that intensity until 18 minutes, when it decreased at the same rate and became inaudible again 28 minutes after injection. The odor was administered by passing compressed air through a flask that contained gauze soaked in oil of peppermint. Both components of the CS complex increased and decreased at the same rate. This timing of the CS was selected because it corresponded to the pattern of unconditioned withdrawal as determined in pilot studies. As the withdrawal subsided, the tone and odor were replaced by music different from the first. At this time the participant received his daily dose of methadone. Physiological and psychological monitoring was conducted continuously throughout the session; the session ended 20 minutes after ingestion of methadone, which corresponded with termination of withdrawal.

Each subject received 21 sessions, five per week. The first three sessions consisted of normal saline baseline trials. Three consecutive conditioning sessions were then given, followed by a single test session. This sequence of three conditioning sessions and one test session was presented four times (total of 12 conditioning sessions); the last block of conditioning trials was followed by three test trials rather than one. This sequence and number of sessions was chosen as the maximum that outpatient volunteers could be expected to complete. Test trials were arbitrarily included every fourth session to obtain data on acquisition of conditioning. The two controls were treated identically to the eight subjects, except that they received only saline injections during all sessions.

Evidence of conditioning was obtained by comparing trials 2 and 3 of the baseline block (saline trials, before conditioning) with trials 19 and 20 (saline trials, after conditioning). The first baseline trial was omitted from the analysis because many participants were anxious and anticipated severe withdrawal reactions. The last test trial was also omitted because any conditioned response that had developed began to be extinguished by the third consecutive nonreinforced trial. The degree of conditioning was assessed by using a $t$-test for paired observations in which each subject's baseline scores were compared to his test trial scores. When data for all eight conditioning subjects were pooled, respiration rate showed a significant increase on the test trials ($t = 2.50$, d.f. $= 7$, $P < .025$) and skin temperature showed a significant decrease ($t = 1.92$, d.f. $= 7$,

$P < .05$) in the test trials after the CS. Heart rate of subjects increased from baseline to test trials ($t = 1.61$, d.f. = 7, $.10 > P > .05$). However, one subject reacted to the naloxone injections with a slowed heart rate (unconditioned response) rather than the more typical tachycardia; this subject's heart rate during test trials (conditioned response) was in the same slowed direction when compared to baseline. If absolute change in heart rate rather than heart rate increase is treated as the conditioned response, the difference between baseline and test trial heart rates becomes more significant ($t = 2.49$, d.f. = 3, $P < .025$). No significant difference between baseline and test occurred in the control group on any of the above measures.

The motor responses of each participant were assessed by reviewing videotapes of each session and counting discrete behaviors. Counts were made by a single observer who was not informed as to the type of session (baseline, conditioning, or test). An ethogram consisting of 33 discrete behaviors was constructed and the frequency of each behavior was recorded for every minute of the session. After this ethological analysis, eight behaviors were selected as being particularly indicative of the withdrawal state: touching the eyes (an indication of tearing), touching the nose (an indication of rhinorrhea), ptosis (eyelids partially closed), yawning, sniffing, frowning, position shifting, and flexing. The total frequency of all eight behaviors for each participant was calculated across blocks of 5 minutes for each session. The mean frequency of all eight behaviors during the baseline sessions was then compared to that for the test sessions. This difference was almost significant for subjects ($t = 1.81$, d.f. = 7, $.10 > P > .05$), but not for controls ($t = 0.15$, d.f. = 1, $P > .30$).

Examples of temperature, respiration, heart rate, and withdrawal behavior responses are shown in Fig. 1. These graphs depict typical responses in subjects who showed conditioning of these variables. The prestimulus values for heart rate and respiration increased across trials. We interpret this as generalization of response from the CS complex to the test chamber itself. It would have been preferable to have each subject sit in the chamber before beginning the trial until values reached preconditioning baseline levels. This was not possible because the participants were outpatients whose time was limited. Controls (saline injections only) did not show this baseline increase across trials.

Participants were also asked to rate their symptoms at three points during the session: 2 minutes before CS, 18 minutes after CS, and 10 minutes after methadone ingestion. They were questioned concerning eight symptoms: tearing, running nose, sweating, tremors, feeling cold, anxiety, depression, and anger. Of the eight subjects, four reported tearing on test trials, five reported running noses, and four reported sweating. Verbal reports were pooled for these three symptoms and averaged across the eight subjects. The average ratings for these symptoms taken after CS onset were significantly greater for test trials than for baseline trials ($t = 2.14$, d.f. = 7, $P < .05$). The same scores for the con-

Table 1. Summary of conditioning data for eight subjects.

| Variable | Unconditioned response | Conditioned response | Subjects displaying conditioning | |
|---|---|---|---|---|
| | | | Proportion | Subject number |
| Respiration | Increase | Increase | 6/8 | 1 to 6 |
| Skin temperature | Decrease | Decrease | 5/8 | 1, 2, 4, 6, 8 |
| Heart rate | Increase* | Increase* | 6/8 | 2, 3, 5, 7, 8, 6* |
| Motor responses | Increase | Increase | 5/8 | 2, 3, 5, 7 |
| Pupil diameter | Increase | Changes (nonsignificant) | | |
| Subjective reaction | Simulates narcotic withdrawal | Indistinguishable from UR at this dose (0.1 mg) | 8/8 | All |

*In subject 6 heart rate decrease was the unconditioned response (UR) and the conditioned response.

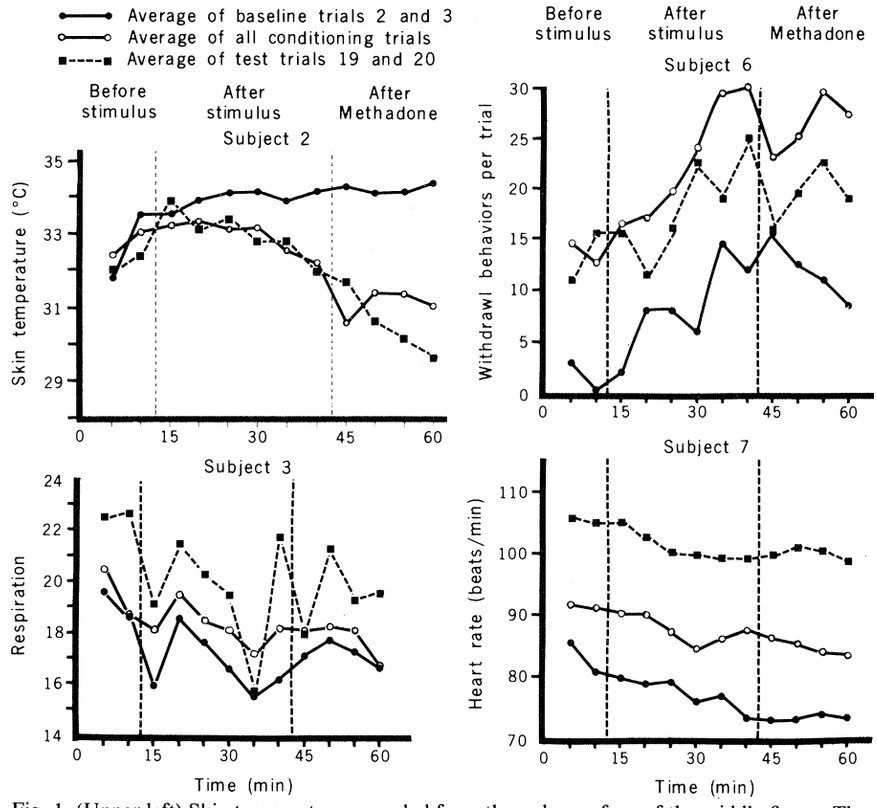


Fig. 1. (Upper left) Skin temperature recorded from the volar surface of the middle finger. The CR is a decrease in temperature. Mean standard deviations (S.D.'s) were 0.6°C for baseline, 0.8°C for conditioning, and 1.3°C for test trials. (Upper right) Frequency of withdrawal behaviors counted from a videotape by a viewer who did not know whether the tape represented a baseline, conditioning, or test trial. Mean S.D.'s were 1.1 for baseline, 2.8 for conditioning, and 2.0 for test trials. (Lower left) Conditioned increase in respiration rate. In addition to the rate change, both UR and CR also consisted of a loss of respiratory regularity. Mean S.D.'s (in inspirations per minute) were 1.2 for baseline, 0.9 for conditioning, and 2.0 for test trials. (Lower right) Heart rate response. Heart rate in this subject increases before stimulus onset in anticipation of precipitated withdrawal. This prestimulus increase was seen also with respiration and is thought to represent a response to entering the conditioning chamber after repeated conditioning trials. Mean S.D.'s (in beats per minute) were 3.9 for baseline, 3.0 for conditioning, and 2.6 for test trials.

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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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.

19 April 1976; revised 19 August 1976

## 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-