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Conditioned Suppression of Sexual Behavior in Stallions and Reversal With Diazepam

S.M. McDONNELL,*†1 R.M. KENNEY,* P.E. MECKLEY† AND M.C. GARCIA*

**University of Pennsylvania School of Veterinary Medicine, Section of Reproductive Studies New Bolton Center, 382 W. Street Road, Kennett Square, PA 19348*

Department of Animal Science and Agricultural Biochemistry, University of Delaware, Newark, DE 19711

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McDONNELL, S. M., R. M. KENNEY, P. E. MECKLEY AND M. C. GARCIA. ***Conditioned suppression of sexual behavior in stallions and reversal with diazepam.*** **PHYSIOL BEHAV** 34(6) 951-956, 1985.-Sexual behavior dysfunction unaccompanied by detectable physical or endocrine abnormality is an important cause of reproductive failure among domestic stallions. Several authors have suggested that such dysfunction may be psychogenic, related to negative experience associated with intense handling and training. An experimental model of experience-related dysfunction was developed by exposing pony stallions to erection-contingent aversive conditioning. This resulted in rapid, specific suppression of sexual arousal and response similar to spontaneously occurring dysfunction. Subsequently, treatment with a CNS-active benzodiazepine derivative (diazepam) reversed these effects.

Male sexual behavior, Stallion sexual behavior, Aversive conditioning, Diazepam, Benzodiazepine

SEXUAL behavior dysfunction has been identified as an important cause of reproductive failure among domestic stallions [9]. Specific behavioral problems include low sexual arousal, apparent shyness or preferences for certain mares or handling conditions, extreme aggressiveness, and unexplained disruption of the copulatory sequence. In many cases no physical or endocrine abnormalities can be identified. Clinical evidence strongly suggests that negative experience, such as painful injury during copulation, punishment for showing sexual arousal at inappropriate times, or rigorous handling and discipline during training or breeding may account for some behavioral dysfunction [10, 13, 15].

Despite the common belief that sexual behavior may be suppressed by negative experience, there are no conclusive experimental data to confirm this. Among the few reported experimental studies, researchers have found with male rats either no effect [2], inhibitory effects [2, 8, 12] or facilitatory effects [3,14] of aversive conditioning on copulating behavior.

With laboratory species it has been shown that negative experience, such as punishment, negative reinforcement, and conditioned fear, can suppress goal-directed eating and drinking behavior [16]. Aversively suppressed eating and drinking behaviors in rats have been used extensively as models for study of central nervous system mediation and psychopharmacological manipulation of negative experience effects. Although the mechanisms are not yet fully understood, a number of CNS-active drugs have been found to block or reverse experience-related suppression of eating and drinking behavior.

Experiment 1 was designed to characterize the effects of response-contingent aversive experience on sexual behavior of domestic pony stallions, with the specific aim of developing an experimental model of experience-related dysfunction. Experiment 2 was designed to assess the behavioral effects of the anti-anxiety benzodiazepine derivative, diazepam (7-chloro-l,3dihydro-l-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one), on aversively suppressed sexual behavior. Diazepam reliably attenuates the effects of aversive experience on eating and drinking behavior in laboratory animals, and is used widely for treatment of anxiety and depression in humans [7]. It has not been studied in regard to suppressed sexual behavior.

METHOD

Experiment I

Design. Precopulatory behavior of six pony stallions was measured during adaptation trials in a standard behavior test and ranked from high to low performance based on erection responses. Subjects were then assigned as rank-matched pairs to Groups A and B. Experiment la consisted of six baseline trials for each subject, followed by six conditioning trials in which the three Group A subjects received aversive conditioning and the three Group B subjects served as con-

'Requests for reprints should be addressed to S. M. McDonnell, Section of Reproductive Studies, University of Pennsylvania, New Bolton Center, 382 W. Street Road, Kennett Square, **PA 19348.**

temporary controls. After completion of Experiment la, Experiment lb consisted of subjecting the three Group B stallions to aversive conditioning. This was done in order to study the process of suppression in additional subjects.

Subjects. Six mature, sexually experienced pony stallions were used for this study. These stallions had been acquired at local auction and used as semen research subjects during the three years preceding the present study. These animals were housed together in adjacent individual tie-stalls in an unshuttered barn, and were maintained on hay, grain, and water.

Behavior trials. All trials were conducted in a rectangular **(6.7~ 12.8** m) enclosure formed by five-rail tubular steel farm gates (1.4 m high) within a three-sided pole barn located approximately 40 m from the stallion barn. Two ovariectomized, estrogen primed (ECP, Upjohn, 2 mg/week, IM) stimulus mares were tethered behind the rail along one side of the test pen. This allowed precopulatory access to the mares and mounting of the rail, but prevented intromission. Domestic stallions readily display a full range of precopulatory behavior under these conditions, and similar arrangements are commonly employed on breeding farms to identify mares in estrus [6].

The aversive stimulus was a 3-4 second pulse of unipolar AC current (l-7 kHz) with a maximum of 2500 v at 25 mA, delivered from a radio-controlled electronic dog training collar (Sensitronix Electra-Trainer, Model 103) fitted to a belly girth placed anterior to the prepuce (Fig. la). This stimulus elicited behavior indicative of transient local discomfort similar to the response of a horse following contact with an electric fence. Shock of this type has been used to discourage aerophagia in the horse [1]. A dummy unit of the same weight and shape with identical electrodes was used for all non-shock trials.

Subjects were introduced to the test procedures during daily four-minute trials for one week before the baseline measurement period. Baseline and aversive conditioning trials were conducted three days weekly (Monday, Wednesday, and Friday) during October and November. On each test day, the order of stallions was random. Subjects were taken under halter from the stall and fitted with the belly girth, then taken to the test pen and released for a period of four minutes. Shock was delivered as punishment on an immediate continuous schedule, contingent on erection, and continued at five-second intervals for the duration of erection as negative reinforcement of penis withdrawal (Fig. 1).

A self-contained microcomputer event recorder (Observational Systems, Inc., Model OS-3) was used to record on a time base 11 specific investigatory, olfactory, aggressive and sexual responses commonly exhibited during this type of exposure to a mare. (Excellent descriptions of these responses can be found in Waring [19]). The following endpoints were recorded or derived for analysis: 1. attention latency-the time from the beginning of a trial to the first approach (within 1 meter) to a mare; 2. sniffs to mare-frequency of sniffs to any region of a mare; 3. sniffs to the floor-frequency of sniffs to urine, feces, or bedding on the floor of the test pen; 4. flehmen responses-frequency of flehmen responses; 5. vocalizations-frequency of whinny, snort, or nicker vocalizations; 6. rolls-frequency of rolls; 7. penis drop latency-time from the start of the trial to the fast penis letdown from the prepuce; 8. penis drop time-total duration of penis drops; 9. erection frequency-number of erections; 10. erection latency-time from the start of the trial to the first erection; 11. erection time-total duration of erections; 12. mount frequency-the number of mounts to the tease rail; 13. mount latency-the time from the start of the trial to first mount to the tease rail; 14. mount time-the total duration mounting the tease rail; 15. kicks-frequency of kicks or strikes to a mare; 16. bites-frequency of bites to a mare.

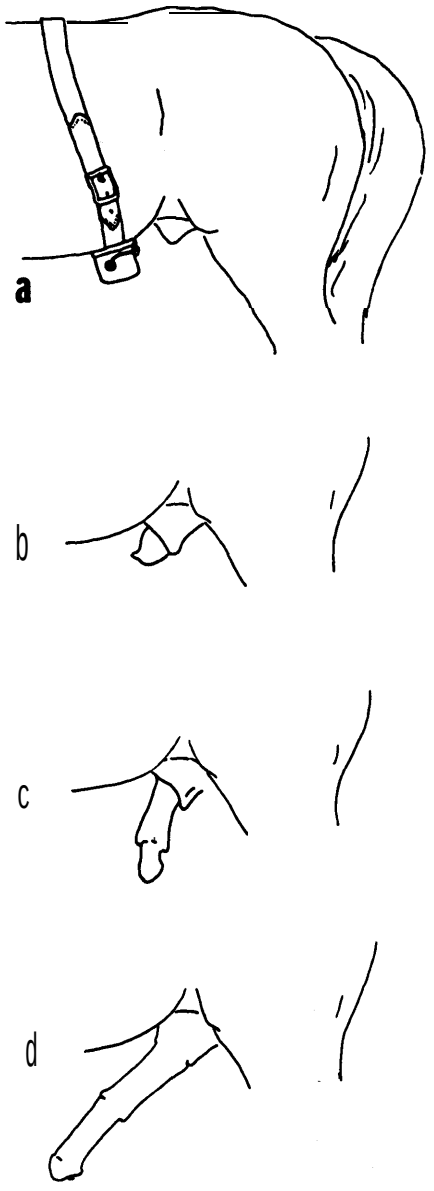

FIG. 1. Belly girth position and stages of erection in the horse: (a) penis fully withdrawn; (b) early penis drop; (c) middrop; (d) full drop with erection.

Analyses. Each behavioral variable was analysed by repeated measures analysis of variance [5]. Factors included trials (12 levels), and group (2 levels). When factor or interaction effects were significant, appropriate means were compared using Fisher's LSD [18].

Experiment 2

Six pony stallions that had been sexually suppressed as described in Experiment 1 were randomly assigned as

TABLE 1

FREQUENCY, LATENCY, AND DURATION OF PRECOPULATORY RESPONSES IN PONY STALLIONS

	mean*	range*
Attention Latency (sec)	5	0-30
Sniffs to Mare	12	4-29
Sniffs to Floor	3	0-14
Flehmen Responses	3	0-6
Vocalizations	11	0-23
Rolls	0.5	0–4
Penis Drop Latency (sec)	57	O-227
Penis Drop Time (sec)	157	13-240
Erection Frequency	1.5	0-5
Erection Latency (sec)	103	0-240
Erection Time (sec)	75	O-219
Mount Frequency	0.6	o-4
Mount Latency (sec)	193	6-240
Mount Time (sec)	5	0-41
Bites	4	0-21
Kicks	1	0–4

*Based on 36 baseline trials (six stallions, six trials each).

rank-matched pairs based on pre-suppression sexual performance to diazepam (n=3) and saline control (n=3) groups for six no-shock extinction trials and four post-treatment extinction trials. The behavior trial procedures and endpoints were the same as in Experiment 1. The belly girth with a dummy shock unit was used throughout. Diazepam treatment consisted of slow (about 4 ml/min) intrajugular injection of 0.05 mg/kg diazepam (Valium, Hoffmann-LaRoche, Inc.) five to ten minutes before the start of each trial. In preliminary trials (McDonnell, unpublished observations) this dose produced mild ataxia and muscle fasciculations for one to five minutes following injection. Control subjects received equivalent volume injections of saline. Trials were conducted three days weekly (Monday, Wednesday, and Friday) during January. Data from treatment period trials were analysed by repeated measures analysis of variance for the effects of treatment (2 levels) and trials (6 levels). Similar analyses were used to evaluate the effect of group (2 levels) and trial (4 levels) during the post-treatment period.

RESULTS

Experiment I

During adaptation and baseline trials subjects displayed a full range of investigatory and precopulatory responses. Upon release into the test pen, stallions readily approached the stimulus mares in vigorous precopulatory interaction. After this initial bout, sexual interaction with the mares usually continued intermittently between periods of general investigatory behavior (sniffing the substrate throughout the test pen, rolling and marking fecal debris with urine and feces). Some stallions, however, spent most of the trial interacting ‘with one or both mares. Three stallions regularly mounted the rail, two. of which displayed pelvic thrusting, and one of which ejaculated while thrusting the penis between the rails with the glans penis against the flank of the mare. The overall mean and range of all baseline trials (6 stallions, 6 trials each) for each response are shown in Table 1.

Repeated measures analysis of variance of the twelve trials indicated significant treatment by trial interaction effects for attention latency, F(11,33)=2.75, ***p<0.05,*** erection frequency, F(11,33)=2.03, ***p<0.06,*** erection latency, F(11,33)=2.23, ***p<0.05,*** mount latency, F(11,33)=2.21, ***p<0.05,*** and mount time, F(11,33)=2.60, ***p<0.05.*** These effects are shown in Fig. 2. During baseline trials there were no significant differences between Group A and B, except for mount latency. Also, there were no significant differences between Group B baseline and conditioning trials on any of these five measures. Aversive conditioning, however, rapidly led to increased attention, erection and mount latencies as well as decreased erection frequency and total mount time. For these measures, Group A was, on the average, different (p<0.05) from Group B conditioning and Group A and B baseline. For all other endpoints there were no significant treatment, trial, or interaction effects. A similar pattern of effects was found among B stallions during subsequent conditioning (Experiment lb), also shown in Fig. 2.

With the onset of aversive conditioning, stallions exhibited a number of-responses suggesting fear, confusion, and approach-avoidance conflict in the test situation and during preparation for trials. These included aimless chewing of non-food objects, freezing, circling, quivering, headshaking, and heightened response to extraneous auditory and visual stimuli. This behavior subsided rapidly as subjects began to avoid shock.

Although total attention time was not quantified, it was apparent that aversive conditioning affected both the duration and pattern of attention to stimulus mares. With each stallion aversive conditioning led to a marked decrease in time spent near the mares. For example, in Experiment la, one stallion did not approach the mares at all after the third conditioning trial. During the fourth and fifth conditioning trials, another Group A stallion briefly approached each mare, and then remained in the comer opposite the mares for the remainder of the trial. During the last conditioning trial this stallion stood in the opposite comer and did not approach the mares at all.

The rate of suppression varied among the six stallions, with a mean of 9.75 shocks and 3 trials required to reach the first full trial without shock. There was a high positive correlation between baseline performance rank and both the number of trials (Spearman rho=0.86, ***t=3.37, p<0.05)*** and the number of shocks (rho=0.89, ***t=3.90,p<0.05)*** to the first full trial without shock.

Experiment 2

During the treatment phase there was a significant effect of treatment on all penis drop and erection measures. Diazepam-treated subjects displayed lower penis drop latency, F(l,4)= 15.58, ***p<0.05,*** and erection latency, F(1,4)= 16.97, p<0.01Diazepam-treated subjects also exhibited increased penis drop time, F(l,4)=17.60, p<0.01, erection frequency, F(l,4)=24.20, p<0.01, and erection time, F(l,4)=9.00,p<0.05. The frequency of rolls tended to be greater, F(l,4)=7.08, ***p<0.*** 10, among diazepam-treated subjects. For all other measures, there were no. significant differences (p>0. 10) between diazepam and control groups. Erection frequency, erection latency, and erection time results for the last two trials before treatment, the six treatment trials, and the four post-treatment trials are represented in Fig. 3. During the post-treatment period there were no significant group, trial, or interaction effects for any measure.

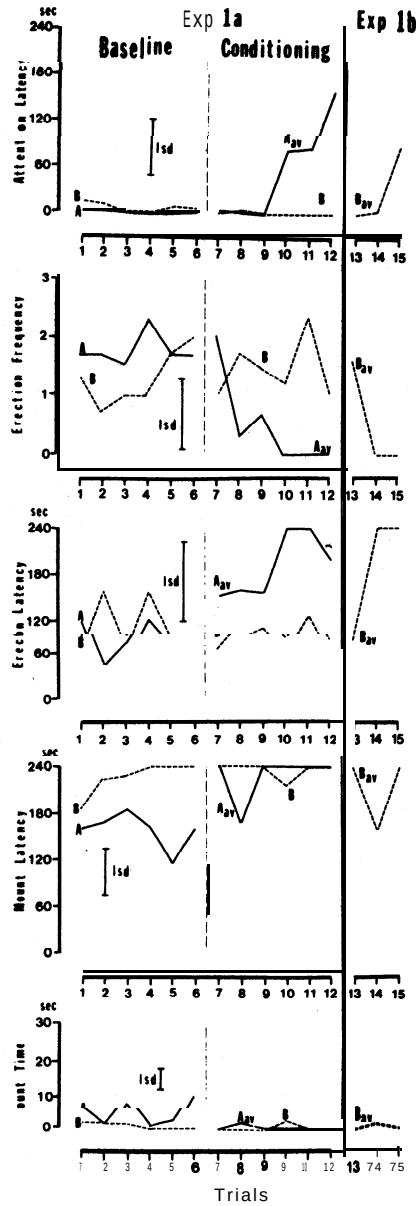

FIG. 2. Mean attention latency, erection frequency, erection latency, mount latency, and total mount time of Group A and B stallions during 4-minute trials of Experiment 1. Experiment la consisted of six baseline trials (l-6), and six conditioning trials (7-12) in which Group A received aversive conditioning (av) while Group B served as controls. In Experiment lb Group B subjects received three aversive conditioning trials (13-15).

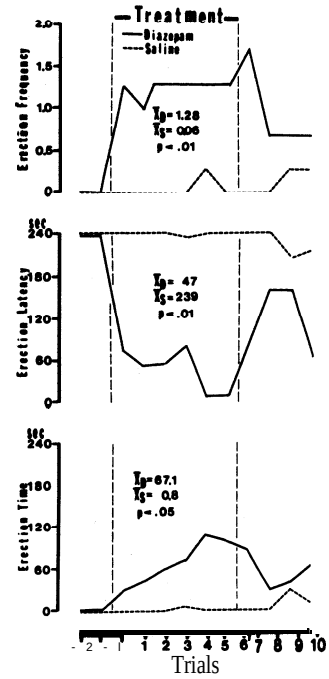

FIG. 3. Mean erection frequency, erection latency, and total erection time of diazepam treated and saline control stallions during last two aversive conditioning trials (- 2, - l), six treated extinction trials (l-6), and four post-treatment extinction trials (7-10). Means represent overall group mean during treatment period (3 animals, 6 trials).

DISCUSSION

The results of Experiment 1 indicate that stallion sexual behavior can be rapidly modified by negative experience. Erection-contingent aversive conditioning produced significant decrements in erection responses and attention to stimulus mares. As there were no significant effects of aversive conditioning on the other behavioral responses measured, suppression seemed specific to sexual response, and not the result of a general behavioral suppression in the test situation.

Certain aspects of the behavior test situation appeared to become conditioned stimuli for shock. For example, during baseline trials, stallions showed no response to the belly girth. However, with the onset of aversive conditioning subjects often kicked at the girth. After two conditioning trials one stallion developed a pattern of rolling behavior that seemed aimed at removing the girth. During two trials the rolling caused the girth to become loose enough that the electrodes no longer made contact with the skin. In each case erection occurred almost immediately and remained until the girth was repositioned by the handler. Repositioning led to immediate withdrawal of the penis, even before shock punishment of the erection could be delivered. This pattern ceased when the girth was more securely attached.

"Safety signal" learning, the apparent recognition of stimuli that predict a no-shock period [17], was evident among these subjects. For example, during the adaptation and baseline periods it was common for stallions to display full erection while being led to and from the test enclosure. With the onset of conditioning, these pre- and post-trial erections ceased. However, after two or three conditioning trials, these erections returned. Two stallions, in particular, repeatedly exhibited erection from the stall until the moment of release in the test enclosure when the penis was immediately withdrawn for the duration of the trial. Erection then occurred as soon as the handler approached the stallion to return him to his stall. Stimuli associated with handling, such as the lead shank or the experimenter, apparently became conditioned stimuli predicting a no-shock period.

The experimentally suppressed sexual behavior of these stallions appeared similar to spontaneously occurring

anomalous behavior seen in our stallion fertility clinic and reported by others [9, 10, 13, 15]. For example, a common problem involves the stallion with normal behavior for one or more breeding seasons that is suddenly interrupted by a negative experience such as a kick from a mare or severe discipline in the breeding situation. Despite no apparent injury that might cause pain or interfere with copulation, abnormal behavior, such as little interest in mares, tentativeness or fear of the breeding situation, preferences or aversions for certain mares or handlers, erection or ejaculation failure, or sudden disruption of the copulatory sequence, persists. Similar problems are also common among young racing and show stallions that are commonly punished for showing erection at "inappropriate" times. Because these stallions sometimes respond favorably to changes in handling and breeding conditions, the problems have been labelled "psychogenic." Dysfunction of this type may involve learning phenomena similar to those seen in our experimental subjects.

Our observations in the stallion are in direct contrast to those recently reported in male rats [14]. Both avoidance conditioning and immediate mount-contingent punishment stimulated, rather than suppressed, copulatory behavior of sexually experienced male rats, reflected in continued copulation and shorter mount, intromission, and ejaculation latencies. One difference in procedure that might account for the discrepant effects was that the rats were allowed copulatory access to the stimulus females. We have subsequently conducted trials in which stallions were allowed copulatory access to a stimulus mare. Under these conditions, shock punishment, as well as a Pavlovian conditioned aversive auditory stimulus, readily disrupted copulation and inhibited further sexual interaction (McDonnell, unpublished observations). It appears, then, that there may be a species difference in the effects of aversive stimulation on male sexual behavior. There is considerable evidence that human male sexual arousal and response may be adversely affected by negative experience, fear, and anxiety ([4,11], reviews). The stallion may be a suitable animal model for study of experience-related human sexual dysfunction.

The results of Experiment 2 indicate that diazepam attenuates the suppressive effects of response-contingent aversive conditioning on sexual behavior of stallions. As in studies of suppressed eating and drinking behavior in rats, diazepam did not significantly alter non-suppressed behaviors. Current hypotheses [7] hold that these benzodiazepine effects result from synaptic level enhancement of GABAergic inhibition of several limbic pathways involved in anxiety-related behavioral suppression.

During the four post-treatment trials diazepam-treated stallions exhibited greater sexual arousal and response than saline control stallions, but these differences were not significant. This may have been due to two factors. Diazepam-treated subjects appeared to regress toward suppression somewhat when treatment was discontinued, and at the same time control subjects appeared to be experiencing spontaneous extinction of suppression. Further work is needed to determine both how permanent the effects of diazepam treatment will be and how extinction of suppression proceeds in non-treated animals.

This study was limited to the effects of diazepam on experimentally suppressed sexual response. A related question currently under study in our laboratory is whether, as with eating and drinking behavior in rats, diazepam could also block the acquisition of suppression of sexual behavior.

In conclusion, these findings provide experimental evidence that stallion sexual behavior is suppressed by response-contingent aversive conditioning. Further, several aspects of the experimentally suppressed behavior were similar to heretofore unexplained spontaneously occurring anomalous behavior. The results of Experiment 2 suggest that experience-related suppression of male sexual behavior may involve CNS mechanisms similar to those mediating conditioned suppression of eating and drinking behavior. These fmdings also suggest further investigation of anti-anxiety drugs for use in clinical management of experience-related sexual behavior dysfunction.

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