RESPONSIVITY TO AVERSIVE ODORS AND THE AGONISTIC BEHAVIOR OF MALE MICE: HORMONAL AND EXPERIENTIAL EFFECTS

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ABSTRACT

A series of experiments examined the nature of the relationship between hormones, responsivity to odors, and the agonistic behavior of male mice. The central hypothesis was that manipulations that modify the perception of and the responsivity to male mouse urine odors would also modify the subsequent agonistic behavior of the subjects.

Experiment 1 found that intact male mice would avoid an area of an open field that had been spotted with the urine of male donors, while castration of the subjects eliminated the response. Hormone replacement was found to be effective in reinstating the aversion, clearly demonstrating the androgen-dependent nature of the response.

Experiment 2 indicated that the effects of castration do not generalize to responses to alarm odors excreted by castrate mice. That is, both intact and castrate males exhibited a pronounced aversion to the odors of castrates that had been subjected to a prolonged period of stress.

In Experiment 3, intact and castrate mice were subjected to avoidance training using the urine of intact and castrate donors as stimuli. Both intacts and castrates learned to discriminate between the odors, and training modified their subsequent aversion response in the predicted fashion. This result suggested that the lack of spontaneous behavioral responsiveness in castrates, as found in Experiment 1, was not due to a sensory deficit.

Experiment 4 also utilized avoidance training so as to modify responsiveness to intact odors. Subjects were then tested for aggression directed at castrate opponents to which the odor had been applied. The results indicated that training intacts so as to increase their aversion response also increased their aggression, while training intacts to avoid the odor had little effect on aggression. Furthermore, modification of the castrates' responsiveness had no effect on aggression, regardless of the direction. This indicated that replacing the responsivity to urine odors is not equivalent to replacing the hormone.

It was suggested that a possible basis for the failure to modify the agonistic behavior of castrates was the lack of any social significance of the odor to them, an aspect that the avoidance training presumably could not replace.

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The importance of male gonadal hormones to intermale aggression in rodents has been well established. For over three decades various investigators have consistently demonstrated that castration greatly reduces fighting behavior, and treatment with testosterone replaces it (e.g. Barfield, Busch, and Wallen, 1972; Barkley and Goldman, 1977; Beeman, 1947; Luttge, 1972). However, the basis for the action of androgen, in terms of possible influences on behavioral systems important in agonistic interactions, has not been specified. The present study represents an investigation of androgen effects on the responsiveness of male mice to particular olfactory cues known to be involved in the agonistic interactions of mice. More specifically, the present study extends the results of a previous experiment which indicated that castrate male mice deviated substantially from intact males in their spontaneous behavioral responsiveness to the urine odors of intact male mice (Sawyer, Note 1).

The hypothesis that androgen influences an animal's responsiveness to particular sorts of stimulation is not new. Scott and Fredericson (1951) have suggested that testosterone lowers the threshold of sensitivity to painful stimuli. Such a notion has been supported by the finding that androgen influences pain-induced aggression in rats (Conner and Levine, 1969). Similarly, Moyer (1976) has suggested that testosterone lowers the threshold of the

brain system involved in intermale aggression so that it is activated by the "adequate stimulus complex". Furthermore, a model proposed by Leshner (1975) suggests that the "baseline hormonal state" of the organism predisposes it to react in a specific way and to a specific degree to a standard stimulus. Thus modification of the hormonal state of an organism via experience or through experimental manipulations may alter the manner in which the animal perceives the situation, and hence, the way in which it behaves. In a social interaction the stimuli of primary importance in activating or modifying the behavior of the animal, or in Moyer's terms "the adequate stimulus complex", are the stimulus qualities of the other animal. Of considerable importance in the agonistic interactions of male mice are urine odors (Ropartz, 1968).

In summary, the working hypothesis of the present paper is that the manner in which a male mouse perceives and responds to the odors of another male influences the resulting agonistic behavior. Thus, it is suggested that manipulations, whether hormonal or experiential, which are found to modify the perception of and responsivity to odors will also be found to modify agonistic behavior. The balance of the introduction will concentrate on the evidence for olfactory involvement in rodent aggression, independent lines of evidence suggestive of the present hypothesis, and the rationale for the experiments which were performed.

Olfaction and Agonistic Behavior

The evidence for olfactory involvement in rodent aggression is striking. Ropartz (1968) found bulbectomy in male mice to be completely effective in eliminating isolation-induced aggression. However, the results of such a manipulation should be considered with caution, as it is not clear whether the surgery had the effect of eliminating olfactory sensitivity or in destroying neural structures functioning in other than olfactory ways. For example, the olfactory bulbs have influences on the endocrine system (Cain, 1971), suggesting that the bulbs may influence aggression via their effects on gonadal functioning. However, Rowe and Edwards (1971) found that castrate, bilaterally bulbectomized male mice did not respond to exogenous testosterone treatment, while unilaterally bulbectomized castrates and sham-operated control castrates responded in an identical fashion, exhibiting high levels of fighting behavior. While the results of bulbectomy studies should be interpreted with care, the correlation between the effects of bulbectomy and castration on agonistic behavior should be recognized.

Other evidence for olfactory involvement in rodent aggression has accumulated. For example, anosmia induced peripherally by bathing the olfactory mucosa in a zinc sulfate solution has been shown to reduce greatly the frequency, as well as the vigor of fighting behavior in

rats (Alberts and Galef, 1973; Flannelly and Thor, 1976). Also, artificially deodorizing opponent male mice prior to an agonistic encounter significantly reduced the frequency of fighting relative to unscented opponents (Lee and Brake, 1971; Ropartz, 1968). Mackintosh and Grant (1966) were able to disrupt an established social group of male mice by applying the urine of a strange male to the coat of a group member. These studies combined with several performed by Jones, Nowell, Mugford, and others (e.g. Kessler, Harmatz, and Gerling, 1975; Jones and Nowell, 1973a, 1973b; Mugford and Nowell, 1970) have led to the conclusion that olfaction plays a primary role in the agonistic behavior of male mice and rats. Furthermore, it appears that male mice excrete odorous substances, primarily in their urine, which promote aggression in conspecifics (e.g. Mugford and Nowell, 1970).

The androgen-dependency of these aggression-promoting odors has been well established. Castrate mice and rats not only exhibit less aggression as discussed previously, they are also less frequently attacked by intact males (Barfield et al., 1972; Lee and Brake, 1972). However, swabbing castrates with urine obtained from intact males has been found to be effective in increasing the frequency and duration of attack directed at them by trained fighters (Jones and Nowell, 1973a, 1973b; Mugford and Nowell, 1970). It is interesting that urine from dominant male mice was

found to be more effective in producing attact than that from subordinate donors (Jones and Nowell, 1973a; Mugford and Nowell, 1970). Thus, it appears that intact males are responsive to odors produced by other male mice, and respond differentially on the basis of the hormonal and social status of the donor animal.

A characteristic of male mouse urine which appears to be closely related to its apparent aggression-promoting properties is its aversiveness to other males. Recently, a number of studies have been performed investigating this effect.

Urine Aversiveness and Behavioral Responsiveness [1]

Jones and Nowell (1973b) found that group-housed male responders avoid an area spotted with the urine of other male mice. This effect was found to be androgen-dependent, as castration of the donor eliminated the aversiveness of the urine, while testosterone replaced it in a dose-dependent manner (Jones and Nowell, 1974a; Sawyer, 1978). The findings of Jones and Nowell (1973a, 1974b) also suggested an effect of previous agonistic experience on the aversiveness of an animal's urine; dominant, aggressive males produced highly aversive urine, but the urine of their subordinate counterparts was no more aversive than that of castrates. However, Sawyer (1978) was unable to replicate this latter result.

Jones and Nowell (1974a) have found that previous agonistic experience or the social status of the responding animal influences the response made when it is confronted with urine odors. The urine of aggressive donors was aversive to both dominant and subordinate subjects. However, the aversion response was much more pronounced in the subordinate responders. This result suggests that an experiential factor (prior victory or defeat) that has previously been determined to have a strong effect on the future expression of fighting behavior (Scott, 1944, 1975) also modifies responsiveness to urine odors.

Sawyer (1978) found differences in the aversion response to urine odors between isolate male responders who later won or lost an agonistic encounter (i.e. differences that were somewhat predictive of the outcome). This finding suggests that differential responsivity to urine odors may play a role in determining the nature of the agonistic interaction between two mice.

With regard to hormonal effects on responsiveness to urine odors, a recent study found that castration of an isolate responder eliminated the tendency to avoid the urine spots of aggressive male donors (Sawyer, Note 1). The urine was found to be aversive to intact, isolate responders. This finding suggests that androgens may play a primary role in mediating behavioral responsivity to those odors important to agonistic interactions of male mice.

Other evidence suggests that androgens have an analogous effect on sexual interactions, in terms of the attraction of male rats to female rat odors. Examination of this evidence as part of the present discussion is warranted by the parallels between androgen effects on sexual and agonistic behavior.

Hormones and Sex Attractants

While evidence indicates that intact male mice exhibit an aversion to male mouse urine odors, a number of studies have shown sexually experienced male rats to exhibit an attraction to female body or urine odors with a definite preference for stimuli from receptive females over nonreceptive or ovariectomized females (Carr, Loeb, and Dissinger, 1965; Carr, Loeb, and Wylie, 1966; Pfaff and Pfaffman, 1969). However, castrate rats exhibited a reduction in the amount of time spent investigating the odors, and no differential responsiveness as a function of the hormone status of the donor, regardless of the castrate's previous sexual experience (Carr et al., 1965; Carr et al., 1966; Stern, 1970). This occurred despite the fact that thirsty castrate rats were able to learn to discriminate between the odors of receptive and nonreceptive females for water reward (Carr and Caul, 1962). That is, in terms of the detection and the discrimination of the odors, castrates behave as intact animals, but they deviate greatly in their spontaneous behavioral responsiveness.

The results of these studies are paralleled in electrophysiological studies performed on castrate rats. Pfaff and Pfaffman (1969) and Pfaff and Gregory (1971) using single-unit recording techniques (particularly in the preoptic area) have found that testosterone increases the spontaneous activity and magnitude of responses to female urine odors, but it does not make differential responsiveness to receptive and nonreceptive odors any more discriminable. That is, it appears neither the differential responsiveness to the two odor types that is found in certain brain cells, nor the ability to discriminate behaviorally between the odors, is androgen-dependent. However, the spontaneous differential responsiveness at the level of behavior is androgen-dependent.

A number of studies performed by Whitney, Nyby, and colleagues have provided evidence of androgen effects for another type of behavior related to sexual interactions, namely male mouse ultrasonic vocalization. It has been found that adult male mice begin emitting 70-kHz ultrasounds shortly after being placed in the presence of a female, and continue doing so throughout the initial investigation of the female (Whitney, Coble, Stockton, and Tilson, 1973). This "ultrasonic courtship" declines across intromissions, and ceases altogether following ejaculation (Whitney et al., 1973). The stimuli that give rise to such vocalizations are primarily olfactory in

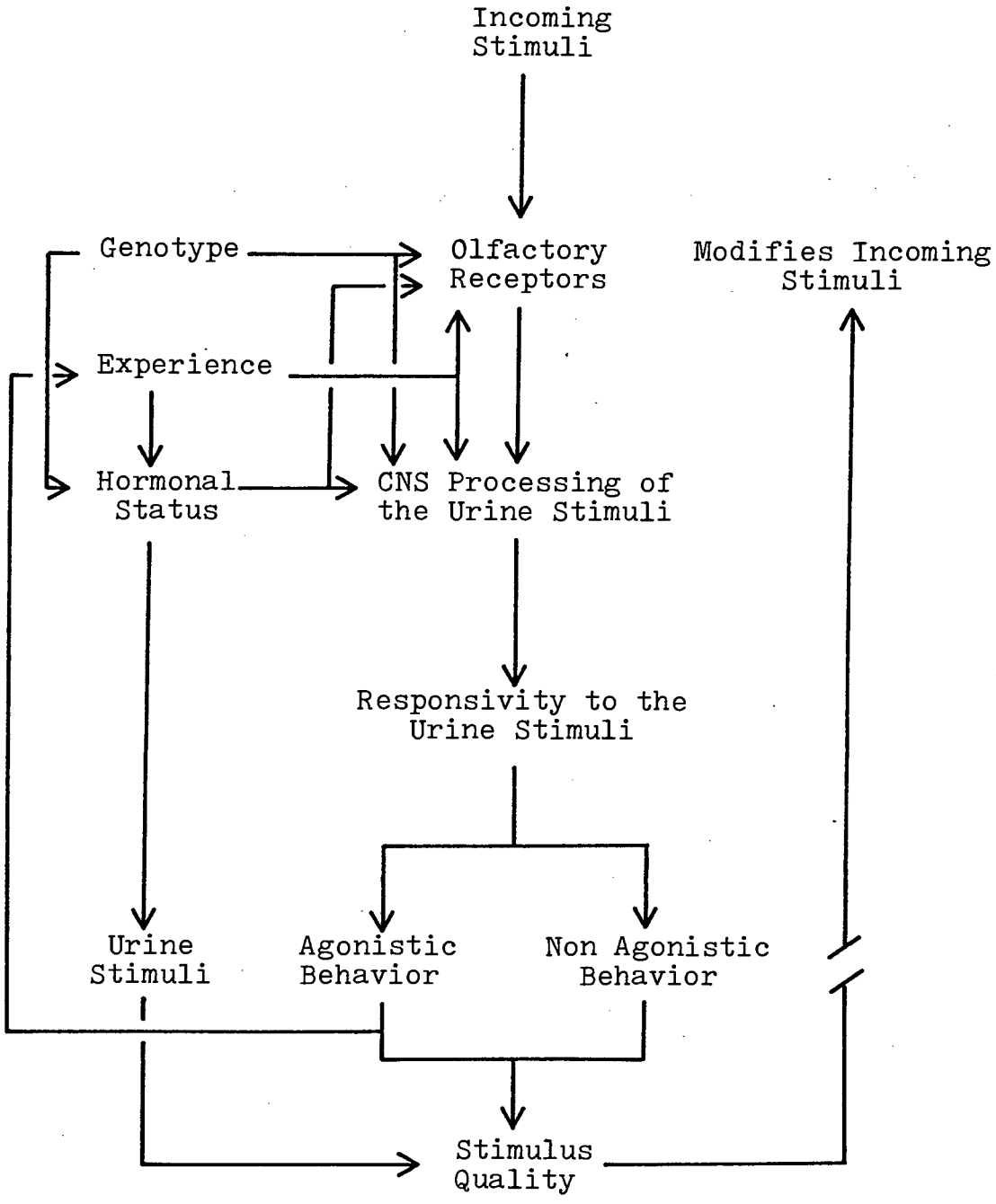
nature, as it has been found that female mouse urine odors are sufficient to elicit and maintain ultrasonic vocalization for prolonged periods, while visual presentation of a female is not (Whitney, Alpern, Dizinno, and Horowitz, 1974; Nyby, Wysocki, Whitney, and Dizinno, 1977). Furthermore, it appears that such behavior is androgen-dependent as castration of the males eliminates their ultrasonic vocalizations to female urine odors, while testosterone replaces it (Dizinno and Whitney, 1977).

Thus, castrate male rats deviate from intact male rats in their spontaneous responsivity to olfactory stimuli associated with sexual behaviors, and castrate male mice deviate from intacts in their ultrasonic vocalization elicited by female odors. With regard to aggression, it is suggested that castrate male mice may deviate from intact male mice in their spontaneous responsivity to olfactory stimuli associated with agonistic behavior.

Model of Relationships Between Olfaction and Other Factors Affecting Aggression

In order to organize previously presented findings and to describe possible relationships between experience, hormones, responsiveness to urine odors, and agonistic behavior, a model will be proposed. The model, presented in Figure 1, is similar to one proposed by Leshner (1975), but has been designed to reflect the importance of olfaction. For comparison purposes Leshner's model is provided

Figure 1. A model of possible relationships between genotype, experience, hormonal status, olfaction, and agonistic behavior. The arrows represent proposed causal effects on, or modifications of one factor by another.

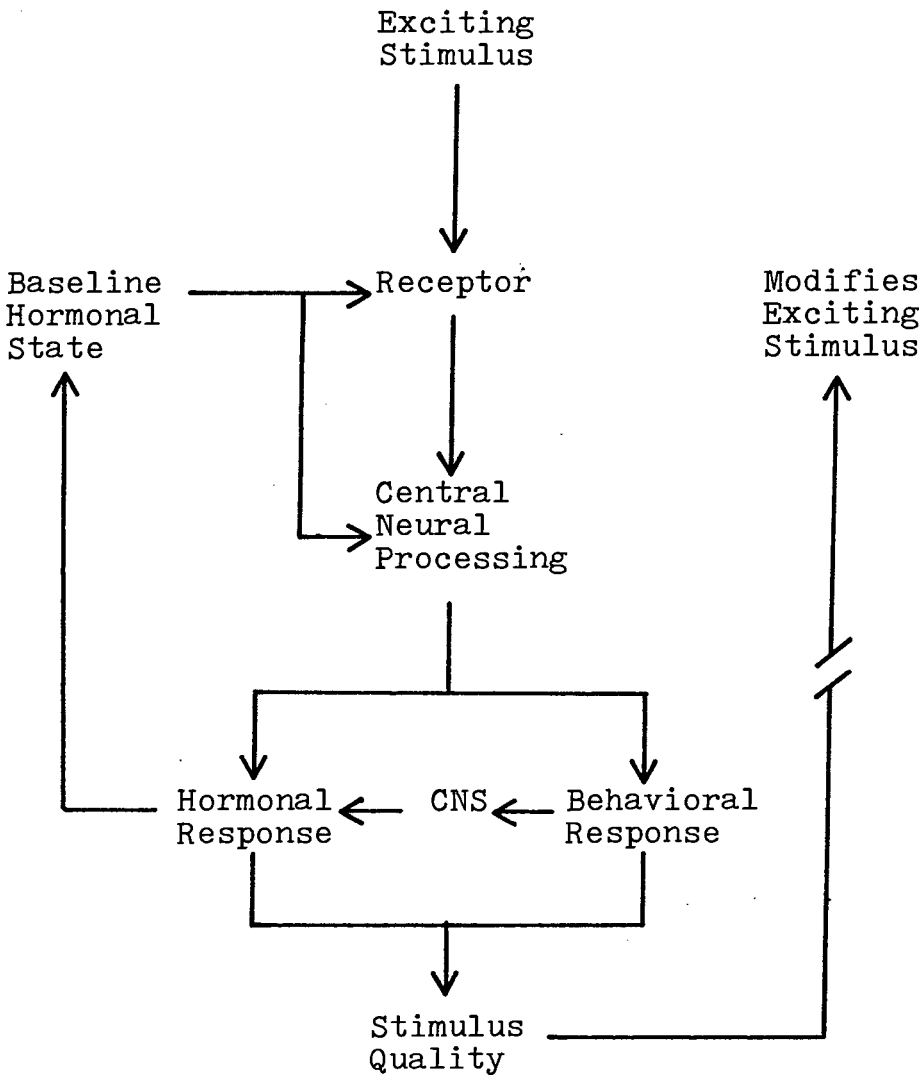

Incoming Stimuli
Genotype
Olfactory Receptors
Modifies Incoming Stimuli
Experience
Hormonal Status
CNS Processing of the Urine Stimuli
Responsivity to the Urine Stimuli
Urine Stimuli
Agonistic Behavior
Non Agonistic Behavior
Stimulus Quality

in Figure 2. Both of the models emphasize the possible role of hormonal status in influencing receptors and/or central nervous system (CNS) processing of stimuli. However, the present model (Figure 1) emphasizes the particular nature of the stimuli, namely olfactory, and suggests the importance of responsivity to such stimuli in influencing the resulting behavior. An important and interesting aspect of Leshner's model (Figure 2) is the feedback effect of behavioral responses on the baseline hormonal state. The same aspect is included in the present model (Figure 1) with behavior influencing hormonal status via experience. However, this aspect will not be emphasized in the discussion to follow. The remainder of this section will consist of a detailed examination of the model shown in Figure 1, and hypotheses which it suggests.

As can be seen in Figure 1, the starting point lies with the incoming stimuli to which the animal is subjected. The most important of these for the present purposes are characteristics of the urine stimuli of the opponent animal, but may also include the opponent's physical and behavioral characteristics. Other stimuli such as painful ones (which may originate from the opponent) may also influence agonistic behavior as has been previously shown through the study of shock-induced fighting in rats (Conner and Levine, 1969).

Figure 1 indicates that various factors, such as genotype, experience, and hormonal status, may influence the

Figure 2. A model of hormone-agonistic behavior interactions (after Leshner, 1975).


Exciting Stimulus
Baseline Hormonal State
Receptor
Modifies Exciting Stimulus
Central Neural Processing
Hormonal Response
CNS
Behavioral Response
Stimulus Quality

the reception of the urine cues, thus influencing behavior. The same factors are also expected to alter the manner in which the stimuli are processed at the level of the CNS. This CNS processing of the urine odors is conceived of as analogous to the animal's perception of the odors, and subsequently is considered to directly affect the responsivity to them. Thus, the responsivity would also be expected to be indirectly modifiable by those factors proposed to have an influence on the CNS. Previously cited studies indicate genotype (Kessler et al., 1975), experience (Jones and Nowell, 1974a), and hormones (Sawyer, Note 1) may modify the central processing of the urine odors (i.e. perception of the odors) by the recipient animal when differences in responsiveness to the odors are taken as an indication of such a modification.

The model in Figure 1 shows behavior to be affected in a general sense by CNS activity, and more specifically by the animal's perception of and responsivity to the urine odors. In other words, while genotype, experience, and hormonal status may affect agonistic behavior, it is proposed that a primary source of their effect is their influence on the perception of, and subsequently, the responsiveness to, the incoming urine stimuli.

Other aspects of the model point out relationships which, while not vital to the present discussion, are interesting. For example, the model proposes that hormonal

status exerts an effect on the quality of an animal's urine as has been previously demonstrated (Jones and Nowell, 1974a; Sawyer, 1978). Likewise, prior experience may affect urine quality as a result of modification in hormonal status (Jones and Nowell, 1974a). An animal's behavioral qualities, urine qualities, and possibly other characteristics are shown to contribute to the stimulus qualities to which another animal may respond when an encounter occurs. As previously noted, behavior should be seen to supply feedback onto the system in the form of experience.

In summary, the model proposes that the agonistic behavior of mice can be influenced by genotype, experience, and hormonal status, as well as by the nature of the incoming stimuli. However, the most important suggestion is that a primary route by which such factors exert their influence on agonistic behavior is via their influence on the perception of the odors by the recipient animal. That is, as previously hypothesized, manipulations that modify a male mouse's perception of and responsiveness to the urine odors of another male mouse should also influence the animal's agonistic behavior.

Purpose of Proposed Experiments

Four experiments were performed, and were designed to extend the findings of Sawyer (Note 1). As previously discussed, the urine of aggressive male mice was found to be aversive to intact mice but not to castrates. Specifically, responsiveness to the urine was tested by placing the subject in an open field, one-half of which had been spotted with urine. The time spent on the clean side of the field during the 300-second trial was recorded as the measure of aversion. The castrate subjects were well within the range of the random response of 150 seconds (1.e. half of the time spent on the clean side, and half on the urine treated side), while the intacts fell well outside this range. This suggested that androgens may play a role in agonistic behavior by influencing the responsiveness to the urine stimuli.

The first experiment was a more systematic demonstration of this finding. That is, a more definitive demonstration of this phenomenon would include baseline measures of responsiveness in order to verify a decrease following castration, as well as a demonstration of a rise in the aversion response following replacement of the hormone. Appropriate control groups were employed to ensure that effects were due to hormonal manipulations, rather than some extraneous factor.

A second experiment examined the generalizability of the effect of castration to another type of odor, namely the alarm odors of mice. Experiments have determined that the odors of male and female mice subjected to hypertonic saline injections (Rottman and Snowdon, 1972), or of males receiving several sessions of frequent, painful electric shocks (Carr, Martorano, and Krames, 1970) are aversive to conspecifics. Bronson (1971) pointed out that such an odor in the urine of severely stressed animals would be valuable in communicating danger to other mice in a natural population. If the effect of castration is a general reduction in either sensitivity or responsivity to olfactory or aversive stimuli one might expect the odor of stressed animals would not be aversive to castrate subjects. However, if androgen effects on responsiveness to stimuli are more specific, including primarily aggression and sex-related stimuli, and not those involved in what might be called danger, one might expect the castrates to avoid the urine odors of stressed donors. The second experiment examined the aversiveness of the urine of stressed and unstressed castrate donors to both intact and castrate subjects.

A third experiment investigated the ability of intact and castrate subjects to discriminate between the urine of intact and castrate donors. As previously noted, the urine of intact donors was aversive to intact subjects, while

that of castrate donors was not (Jones and Nowell, 1974a; Sawyer, 1978). Also, Sawyer (Note 1) found that the urine of intact donors was not aversive to castrate subjects. These findings suggested that the urine types were discriminable to intacts, but supplied no evidence concerning their discriminability to castrates. The third experiment attempted to resolve this issue by training intact and castrate subjects in an avoidance task using the urine of castrate donors as the S^D (for half the subjects) and the urine of intacts as the S^{Δ} (for half the subjects). A group "trained" with no urine stimuli present served as a control for possible cues other than the odors. The ability of castrates to learn such a discrimination would suggest that the basis for their lack of response to intact urine is not due to a sensory deficit. With regard to the previously proposed model, such a finding would suggest that the hormone effect does not lie entirely at the level of the reception of the stimuli (i.e. a receptor effect), but rather lies at the level of the CNS, or possibly motor output. Furthermore, training would be expected to result in modification in the responsivity of the subject to the urine odors as measured in the aversion test. That is, intact urine would be aversive (or attractive) to castrates, and would increase (or decrease) from the already aversive level for intact subjects. An aversion test was given following training to determine if such an effect occured.

Experiment 3 was designed to determine if subjects are able to learn a discrimination between the urine of intact and castrate donors, and if so, whether such training increased or decreased their responsiveness to the odors. As previously discussed, a central hypothesis of the present paper is that, due to the extreme importance of olfactory stimuli in rodent aggression, variation in an animal's perception of the urine odors of another mouse should influence the animal's agonistic behavior. The finding of Jones and Nowell (1974a) concerning differences in responsiveness between highly aggressive and highly submissive males supports such a notion. Likewise, the result of Sawyer's (Note 1) study concerning the lack of responsiveness of castrate males, coupled with the well-documented effect of castration on aggression (e.g. Beeman, 1947) provides some support. And finally, the finding of Sawyer (1978) that differences exist in responsivity to urine odors that are predictive of the outcome of an agonistic encounter also suggests this hypothesis. However, it is possible that the relationship seen in these studies between an animal's responsiveness to urine odors and its agonistic behavior is a spurious one. For example, the relationship between levels of aggressiveness (i.e. prior agonistic behavior) and the responsiveness found by Jones and Nowell (1974a) may be due to a causal effect of experience on both responsiveness to urine and agonistic

behavior, with no causal relationship between the latter two. On the other hand, the relationship between responsiveness to urine and agonistic behavior may be a causal one, with prior experience affecting agonistic behavior by modifying the significance of the olfactory cues. The finding of Sawyer (Note 1) concerning hormonal effects can be explained in an analogous fashion. Finally, the finding that differences exist in the aversion response which are predictive of the outcome of an agonistic encounter between two mice (Sawyer, 1978) can be explained by slight differences in prior experience and/or hormonal status affecting both responsiveness and agonistic behavior. Thus, what the above noted studies supply is a large amount of evidence suggestive of the hypothesis of a causal relationship between responsiveness to urine odors and agonistic behavior, but they are not definitive. That is, they provide only correlational evidence, and do not reveal any causality in the relationship between responsivity to odors and agonistic behavior. A fourth experiment was performed in an attempt to derive experimental/causal evidence.

Experiment 4 was similar in design to Experiment 3. That is, an attempt was made to modify a subject's responsiveness to intact urine odors directly via training in an avoidance task. Successful training was expected to modify an animal's perception of and responsiveness to

the odor, and, as proposed in the model, alter its agonistic behavior. Again, modification was attempted in both directions (i.e. increased and decreased aversion to the odor), and was expected to result in groups being higher and lower than an untrained control with respect to aggression. Both intact and castrate subjects were used, as well as two groups of each to control for exposure to the urine odors during training, and the exposure to shock during training. Thus, Experiment 4 was designed to: (1) train an aversion or attraction to urine odors (as was done in Experiment 3), (2) test the effect of training on responsiveness through the use of an aversion test (as was done in Experiment 3), and (3) determine the effects of the modification in relative aversion to the urine odors on the subject's agonistic behavior when confronted with an animal having the odor.

EXPERIMENT I

The first experiment was an attempt to extend the results of Sawyer (Note 1) using a better, or more sophisticated design. For one group of subjects (designated the castrate experimental group, or CE) baseline measures of responsiveness to urine odors were obtained, followed by measures after castration (post-op session) and hormone replacement (replacement session). Measures for the baseline, post-op, and replacement sessions were also obtained for control groups which consisted of: (1) a group remaining intact throughout the experiment (the intact control, or IC group), (2) a group remaining intact throughout which responded to a water stimulus, rather than urine, during the post-op session (the intact water, or IW group), and (3) a castrate group which was treated with only the oil vehicle prior to the replacement test (the castrate control, or CC).

Method

Responding Subjects

The subjects were 64 male Swiss-Webster mice born and reared in the Psychology Department at Bowling Green State University. After weaning at 25 $\pm$ 1 days of age they were housed in groups of four under a partially reversed 12:12 hour light-dark cycle with lights on at 01:00 hr and off at 13:00 hr. The cages were standard 31.4 cm by 19.7 cm by 12.7 cm mouse boxes with wire mesh tops. Food and water were available ad libitum.

Urine Donors

The donors were obtained from the same stock as the responding subjects and housed under the same conditions until approximately 70 days of age. At this time they were housed individually in 24 cm by 18 cm by 18 cm rat metabolism cages. Following approximately three weeks of isolation each prospective donor was placed into a neutral cage with a group-housed opponent for 30 min. Observations were made to determine the incidence of fighting. Sixteen of the isolate males found to exhibit high rates of attack were selected to serve as the donors. Fighting sessions of 30 min duration were given at 72 hr intervals to ensure continued high levels of fighting behavior in the donors.

Urine Collection

Urine was collected while the donors were in their home cage, which consisted of a rat metabolism cage adapted for use with mice by exchanging the feces screen for a 4.72 squares/cm screen. Immediately before the onset of urine collection each donor was given a 30-min session with a group-housed opponent. Urine was obtained over a 16-18 hr period between 16:00 hr and 10:00 hr during which time food was removed to prevent contamination of the urine. The urine was stored in airtight, glass receptacles and was used within seven hr of collection. A minimum of 72 hr elapsed between each occasion of urine collection.

Aversion Testing

At approximately 70 days of age the baseline aversion measure was obtained for each of the group-housed responding subjects, with all animals remaining intact during this initial test. Urine aversion testing took place in a 45 cm by 45 cm open field with 45 cm high walls. All walls were made of unpainted aluminum except for an 18 cm high strip of Plexiglas at the base of one wall to permit observation of the subject. The floor of the apparatus was a table upon which unprinted newsprint was placed. This technique facilitated the elimination of urine odors by removing the soiled newsprint, wiping the table with a damp sponge, and placing a clean sheet under the open field. Ten spots of urine (totaling approximately .125 cc) were distributed approximately equidistant throughout one-half of the field. The subject was placed under a 10 cm by 10 cm by 10 cm Plexiglas box in the center of the field. Twenty sec later the box was removed and the subject was allowed to move freely about in the field. The time spent on the clean side of the field was measured at one-min intervals over a five-min test period. The aversion testing took place in a darkened room under red light illumination. During a trial the observer was approximately 1.5 m from the field concealed behind a fiberboard screen containing a viewing hole 10 cm in diameter. Testing took place .5-5 hr into the dark period.

Following the baseline test the subjects were divided into four groups matched in terms of their performance on the initial test. An exception to this matching procedure was that each of the treatment groups (i.e. IC, IW, CE, and CC) was represented by one animal per box of four mice.

Each animal of two of the treatment groups (i.e. two mice per box) was castrated approximately two hours after the baseline measure was taken. Castrations were performed under ether anesthesia via scrotal incision. Those animals selected to remain intact (i.e. the other two subjects per box) were sham-operated, receiving the anesthetic and incision with testes left intact.

A second measure of aversion was taken 16 days after surgery. During this phase of testing, labeled the post-op session, one of the intact groups (IW) was confronted with distilled water on one side of the aversion test apparatus, rather than urine.

Nine days after the post-op test, hormone treatment began. One group of castrates (CE) received daily subcutaneous injections of 100 μg of depo testosterone in .05 cc sesame seed oil. Intacts (IC and IW) and the remaining group of castrates (CC) received daily .05 cc injections of the oil vehicle. Treatment continued for seven days with the final aversion test (the replacement session) being conducted 2-4 hr after the seventh injection (16 days following the post-op aversion test). A similar hormone

treatment procedure has been found to result in levels of fighting comparable to intact animals of this mouse strain (Edwards, 1969).

The urine to which a subject responded was collected from a different donor during each of the test phases. Furthermore, in any particular session the measures for each of the subjects within a group represented the response to a different donor animal.

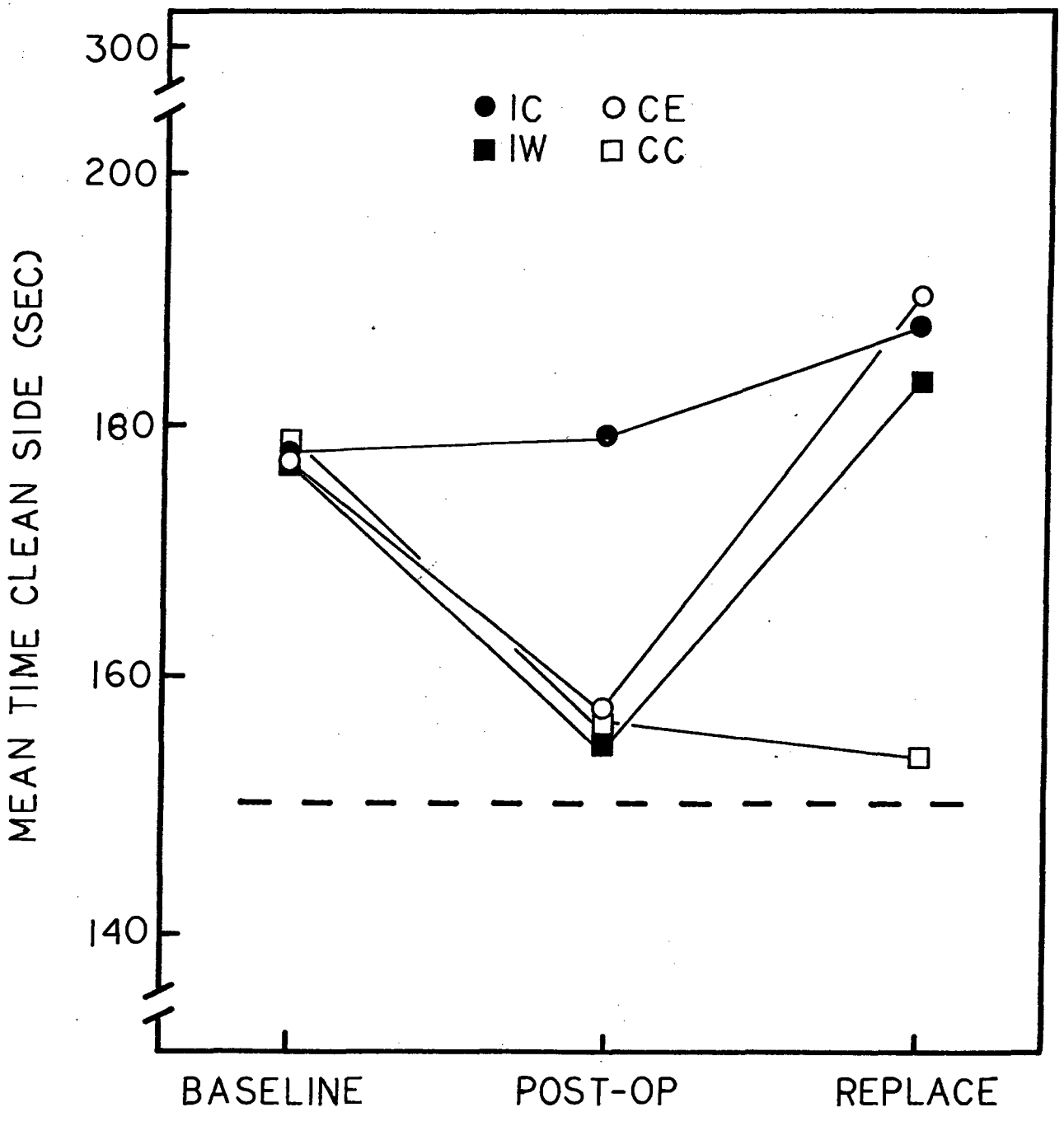
Results and Discussion

The design of Experiment 1 was a 4 x 3 x 5 (treatment group x test session x interval within session) factorial with repeated measures on the session and interval factors. The initial analysis consisted of a repeated measures analysis of variance (ANOVA). The summary table is provided in Appendix A.

The higher order (i.e. 3-way) interaction of treatment group by session by interval was found to be nonsignificant, as were the session by interval and treatment by interval interactions. However, as expected the treatment group by session interaction was found to be highly significant, $F(6, 120 = 3.19, p < .001$, indicating the divergence of treatment groups as a function of test session.

Inspection of Figure 3 suggests the interaction of treatment group and session was due to a pronounced reduction in the aversion response of both castrate groups (i.e. CE and CC) and the intact group exposed to water

Figure 3. Mean time (sec) spent on the clean side of the open field as a function of treatment group and test session (random response is approximately 150 sec). Group designations are: IC = intact control; IW = intact, water stimulus during post-op session; CE = castrate, treated with testosterone in replace session; and CC = castrate, treated with oil in replace session.


300
200
160
160
140
MEAN TIME CLEAN SIDE (SEC)
IC
CE
IW
CC
BASELINE
POST-OP
REPLACE

(i.e. IW) during the post-op session, as well as the subsequent return to higher levels in the treated castrate group (i.e. CE) and the IW group during the replacement session. Further statistical analyses revealed that the reduction in the aversion response following castration was indeed highly significant (CE baseline and CC baseline vs. CE post-op and CC post-op, matched-sample $\underline{t}(31) = 3.52$, $\underline{p} < .01$). Furthermore, comparisons of the post-op session data indicated the mean aversion response of the castrate subjects was significantly lower than that of the intact group exposed to urine (CE post-op and CC post-op vs. IC post-op, $\underline{t}(46) = 3.36$, $\underline{p} < .01$), but did not differ from that of the intact group exposed to water (CE post-op and CC post-op vs. IW post-op, $\underline{t}(46) = 0.53$, $\underline{p} > .20$). With regard to hormone treatment, it was found that testosterone administration produced a significant rise in the mean aversion response of the treated castrate group (CE post-op vs. CE replace, matched-sample $\underline{t}(15) = 3.23$, $\underline{p} < .01$). Furthermore, comparisons of the replacement test session data indicated the mean aversion response of the treated castrate subjects was significantly higher that that of the untreated castrates (CE replace vs. CC replace, $\underline{t}(30) = 3.52$, $\underline{p} < .01$), while not differing substantially from either the IC group, $\underline{t}(30) = 0.53$, $\underline{p} > .20$, or the IW group, $\underline{t}(30) = 1.74$, $\underline{p} > .07$.

Thus, the results of Experiment 1 provide a replication and extension of the previously reported results of Sawyer (Note 1). That is, the results indicate that the aversion response is indeed androgen-dependent, as the tendency to avoid the urine odors was eliminated by castration and replaced by exogenous testosterone treatment.

EXPERIMENT II

As briefly discussed previously, Experiment 2 was performed to test the generalizability of the effects of castration to those odors associated with stress. More precisely, Experiment 2 attempted to determine whether castrate male mice deviate from intact male mice with regard to their responsiveness to an entirely different aversive odor, namely alarm odors.

Method

Responding Subjects

The subjects were 24 male mice obtained from the same stock and housed under the same conditions (e.g. groups of four) as in Experiment 1.

Urine Donors and Stress Induction

The urine donors were 16 male mice obtained from the same stock as the responding subjects. Each donor was castrated by scrotal incision when approximately 50 days of age. At approximately 70 days of age the donors were housed in pairs in a urine collection cage.

Twelve days after being placed in the metabolism cage eight of the donors (i.e. four of the pairs) were subjected to a stress-inducing procedure similar to that employed by Carr et al. (1970). Twice a day each of these donors was separated from its cagemate for a 15 min session during which 20 one ma scrambled electric shocks of variable duration (mean duration = 5 sec) were presented in an

unpredictable fashion. The apparatus consisted of a 15 cm by 15 cm by 15 cm Plexiglas box with a grid floor of .158 cm diameter rods through which the shock was presented. The shock source was a BRS/Foringer, Model #SG-901. The four donor pairs that were not selected to receive the stress-inducing procedure were left undisturbed. Following a minimum of 12 such shock sessions (i.e. six days with two per day) urine was collected from both the stressed and unstressed donor pairs for use in aversion testing. The urine was collected and stored as described in Experiment 1.

Aversion Testing

Two subjects from each group of four males (a total of 12 subjects) were castrated at 60 days of age, while the remaining subjects of each box were subjected to sham-surgery. At 80 days of age the aversion test was given. The method was similar to that employed in Experiment 1, except that 10 drops of urine from a stressed castrate donor pair were placed on one side of the open field, and 10 drops of urine from an unstressed castrate donor pair were placed on the opposite side. The use of castrate donors insured that any aversiveness of the odors was due to the stress-inducing procedure, as castrates do not produce aversive urine (e.g. Sawyer, 1978). The dependent measures consisted of the time spent on the side containing the urine of unstressed donors during each of the one-min intervals of the five-min test session.

Results and Discussion

The design of Experiment 2 was a 2 x 5 (hormone status x interval within session) factorial with repeated measures on the interval factor. The initial analysis consisted of a repeated measures ANOVA. The summary table is provided in Appendix B.

The ANOVA revealed no effect of hormone status, $F(1, 22) = 0.08$, $p > .20$, no effect of interval, $F(4, 88) = 2.21$, $p > .07$, and no interaction between hormone status and interval within session, $F(4, 88) = 0.32$, $p > .20$. In other words, intacts and castrates did not differ in terms of their responsiveness to the odors of stressed donors. However, the odor of stressed castrate donors was found to be aversive, and comparisons with the random response value of 150 sec revealed that both castrates (mean = 169.61, $t(11) = 4.06$, $p < .001$) and intacts (mean = 171.58, $t(11) = 4.43$, $p < .001$) spent more time on the side with the urine of unstressed castrate donors.

Thus, it appears that the effects of castration do not generalize to another naturally occurring type of aversive odor. This suggests the effect of castration is not simply an overall lowering of the sensitivity or responsivity to either olfactory or aversive stimuli. Rather, these results, combined with previous results concerning sex odors (e.g. Carr et al., 1965, 1966), suggest that androgen effects on responsiveness to stimuli

may be somewhat more specific, including primarily aggression-related and sex-related stimuli, and not those involved in what might be called danger. However, these conclusions must be qualified as they are only applicable to the odors of stressed castrates, and not the odors of stressed intacts.

EXPERIMENT III

Experiment 3 was designed to determine whether the gonadal state of male mice influences their ability to discriminate between the urine odors of intact and castrate male mice. As suggested in the model, the failure of castrates to exhibit an aversion response to the urine odors of intact males may be due to a hormonal effect on receptor sensivity (i.e. sensory deficit). On the other hand, the lack of responsiveness on the part of castrates may be due to hormonal effects on the CNS in terms of the animal's perception of the urine odors. If the former suggestion (sensory deficit) accounts for the effect, then castrates would not be expected to be able to learn a discrimination between the urine types. However, if the effect is due to central action of the hormone, then castrates may be able to learn to discriminate between the urine types by pairing one type with a stimulus that already has behavioral effects on castrates.

If the stimulus paired with urine were aversive, such as electric shock, one would expect that the responsiveness to the odor, as measured in the aversion test, could be increased or decreased depending upon shock contingencies. That is, training subjects to avoid the urine of intact donors and approach that of castrate donors in order to avoid shock should produce a pronounced aversion to odors of intact subjects in such subjects. Likewise,

training subjects to avoid the urine of castrates and approach that of intacts should produce a lack of aversion, or even an attraction to the urine odors of intact donors.

In Experiment 3 a group of intact and a group of castrate subjects were trained to avoid the side of a modified T-maze in which intact odor was present, and approach the side which had the odor of castrates. Another group of intacts and one of castrates were trained with the opposite requirements. Also, a group of each hormone status was "trained" with no odors present to ensure any improvement in performance was due to the presence of the olfactory cues.

The importance of using the odors of both intact and castrate donors, rather than just that of intact mice, should be stressed. While it is highly possible, as was suggested by Sawyer (Note 1), that castrates are able to detect the odor of the urine of intact donors, it is conceivable that they are unable to detect that characteristic of the urine which distinguishes it from that of castrates. That particular characteristic was suggested by Jones and Nowell (1973c) to be the secretion of the coagulating gland, which is an androgen-dependent gland that secretes directly into the urine. Thus, the use of urine from both intacts and castrates would suggest that any learning exhibited is due to the detection and discrimination of that aspect of the urine that presumably gives it the

aversive quality. However, it is also possible that the urine of castrates and intacts differ in some other manner.

Also, it was necessary to train a subject with the urine of more than one intact and castrate donor to ensure that they were not discriminating between those features of an individual's urine that makes it distinctive from the urine of any other mouse (e.g. Bowers and Alexander, 1976; Hahn and Simmel, 1968). This was accomplished both by pooling urine from several intacts or several castrates to get the stimuli, and by requiring the subjects to respond on some trials to urine samples pooled from different sets of intact and castrate donors. Pooling of the urine also allowed the collection of an adequate quantity with which to train subjects which individual donors did not reliably provide, and has previously been determined not to affect the aversiveness of the urine (Jones and Nowell, 1974c; also pilot observations), or its aggression-promoting properties (Jones and Nowell, 1975).

Method

Responding Subjects

The subjects were 60 male mice obtained from the same stock and housed under the same conditions as the subjects of Experiments 1 and 2.

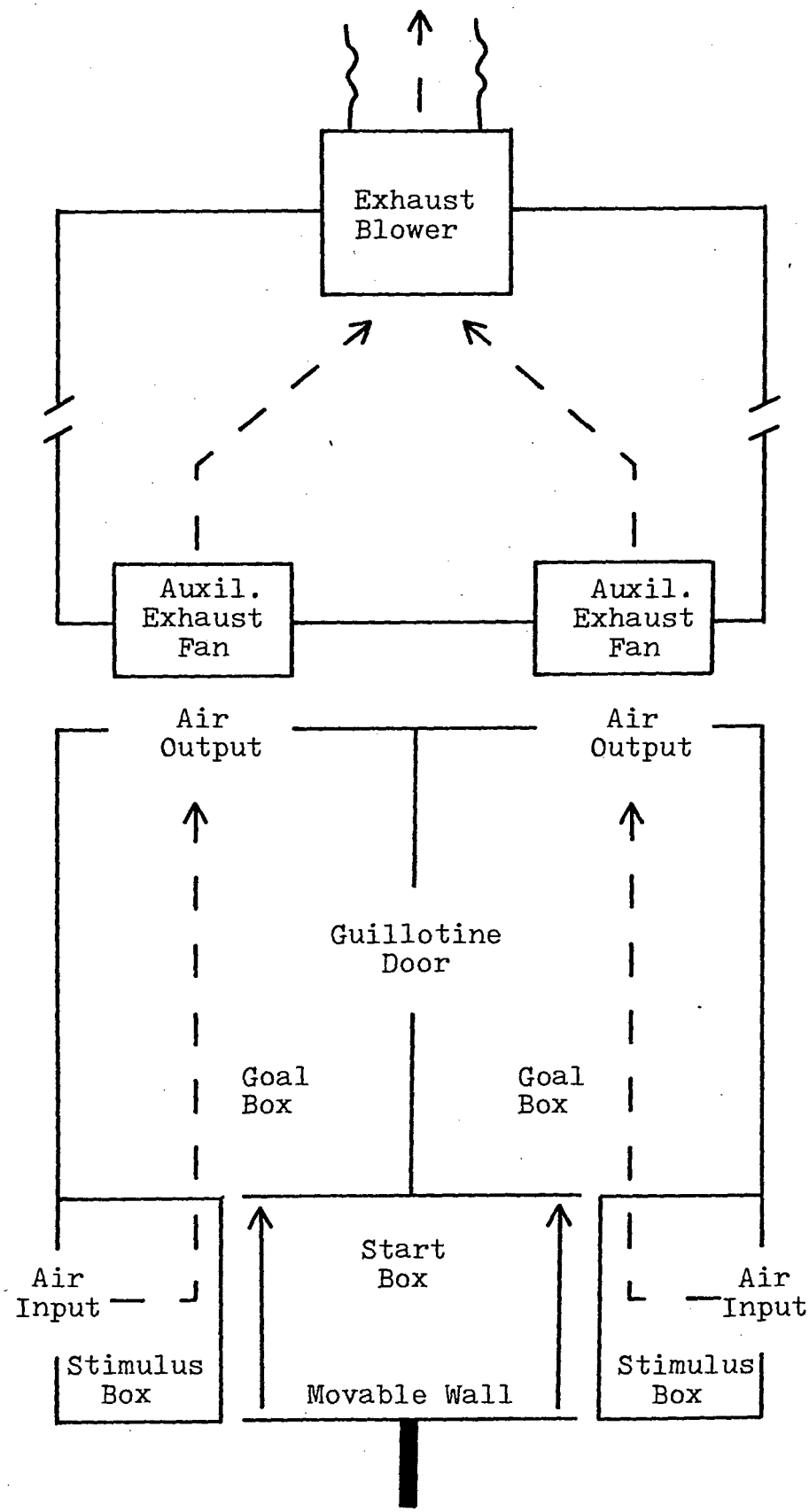
Donor Animals

The donors were 24 male mice obtained from the same stock as the responders. At approximately 70 days of age, 12 of the donors were castrated; the remaining 12 were sham-operated. Following surgery the donors were housed individually in metabolism cages for a minimum of three weeks prior to urine collection.

Apparatus

Avoidance training took place in an apparatus designed for use with olfactory stimuli. As pictured in Figure 4 it consisted of a start box (11 cm by 14 cm by 15 cm) which opened out to two goal boxes (each 18 cm by 14 cm by 15 cm). Each of the three chambers was covered by a hinged top. The wall between the goal boxes contained an 8 cm diameter hole permitting movement of the subject from one side to the other, and yet could be closed off through the use of a guillotine door. Likewise, pushing the movable back wall of the start box closed this section off from the goal boxes, as well as forcing a subject placed in the start box to move from it. Located on either side of the start box were removable stimulus boxes (11 cm by 7 cm by 15 cm) which contained holes on the side towards the goal boxes to permit passage of the odors. On the outside wall of each stimulus box was a hole four cm in diameter to allow air input. Holes 8 cm in diameter at the end of each goal box provided output of both air and odors into an adjacent enclosure

Figure 4. Olfactory avoidance training apparatus used in Experiment 3. The flow of air is depicted by the dashed lines.


Exhaust Blower
Auxil. Exhaust Fan
Auxil. Exhaust Fan
Air Output
Air Output
Guillotine Door
Goal Box
Goal Box
Start Box
Air Input
Air Input
Stimulus Box
Stimulus Box
Movable Wall

(30 cm by 30 cm by 30 cm). The air contained in this enclosure was drawn out via an exhaust blower and exited the room through flexible tubing attached to the air output vent located in the ceiling of the room. Thus, while the exhaust blower was on, air passed through the stimulus boxes, into the goal boxes, out of the test chamber, and finally out of the room. Auxiliary exhaust fans located at the end of each goal box provided further replacement of air to the apparatus between trails. The apparatus was constructed entirely of Plexiglas and rested on a grid of .158 diameter rods through which shock was delivered. The shock source was the same as that used in Experiment 2.

Procedure

At approximately 70 days of age two subjects from each box of four were chosen at random and castrated. The remaining subjects were sham-operated. At this time subjects were arbitrarily assigned to one of the treatment groups. Twelve of the intact subjects were subsequently trained to avoid intact urine (designated intact avoid, or IAV), while 12 were trained to approach intact urine (intact approach, or IAP). Likewise, 12 castrates were trained to avoid (CAV) and 12 to approach (CAP) intact urine. The remaining six intacts and six castrates were "trained" with no stimuli present (designated no stimuli, or NS). Approximately 16 days after surgery, avoidance training began.

Avoidance training consisted of 80 trials distributed

across four sessions of 20 trials. Forty-eight hr elapsed between each session. Within a session, a responder was given 10 trials with an intertrial interval (ITI) of 60 sec, followed one hr later by another 10 trials with the same ITI. Shortly before each session, the subjects were removed from their home cage and housed individually in clean boxes until the session was completed. They were then transferred back to the home cage at the same time as their cagemates. Pilot observations indicated no evidence of fighting when subjects were reunited, provided they were placed back in their original soiled cage.

The intact urine stimuli used during training were obtained from arbitrarily designated sets of four intact, isolated donors, with each contributing approximately .20 cc to the pool. Castrate stimuli were obtained from analogous sets of castrate donors. During the initial three training sessions subjects were trained with urine from different intact and castrate donor sets each session. During the fourth session, the intact and castrate stimuli came from the same sets as in the first session. The urine stimuli were presented by applying 20 drops of one urine type (approximately .25 cc) to an absorbant cotton ball which was placed in one of the stimulus boxes. The other urine type was presented in a similar fashion in the other stimulus box. Following each set of 10 trials each stimulus was replenished with approximately .06 cc of urine. Also,

after each set of 10 trials the apparatus was thoroughly cleaned with a mild disinfectant solution.

Each trial began by placing the subject in the start box. If after 15 sec a choice (defined as the entire body excluding the tail entering a goal box) was not made, one-half of the start box was closed off by moving the back wall. This forced the subject directly to the choice point, but did not necessarily force a choice. If another 15 sec elapsed, the subject was then forced into a goal box by completely closing off the start box. Entry into the correct box resulted in removal from the apparatus (approximately 5 sec later), while entry into the incorrect side resulted in a two-sec .4 ma scrambled shock. The guillotine door separating the goal boxes was then raised and the subject was required to escape the shock by moving to the correct side, followed approximately 5 sec later by removal from the apparatus. Unforced entry into a goal box resulted in the closing off of the start box followed by either removal or shock, as in forced entry. Essentially, the technique was a forced-choice, correction procedure.

Following a trial the stimulus boxes were removed and the auxiliary fans were turned on for 45 sec. This allowed the removal of the residual odors of the previous trial. Also, absorbent paper beneath the apparatus was drawn out, simultaneously replacing it with a clean piece. Approximately 15 sec before the next trial the stimuli were re-

placed in their appropriate position and the auxiliary exhaust fans turned off. The location of the stimuli was determined by a modified Gellerman series in which strategies, such as alternation and/or win-stay, lose-shift were unsuccessful in producing performance above 50% correct. Subjects in the NS group were "trained" using an identical procedure except for the use of distilled water in place of the urine stimuli.

Following training, the subjects of the IAV, IAP, CAV, and CAP groups were tested for their aversion response to the urine of the intact donor set which supplied the stimulus odor during the last training session. The aversion test was conducted 48 hr after the last training session, and employed a procedure similar to that described in Experiment 1. The only modification was to apply the urine of the castrate donor set which supplied the castrate stimulus odor for the last training session to the side of the open field opposite the intact urine. That is, in the aversion test the subject had a choice between intact and castrate odor, rather than intact odor and no odor.

The dependent measure for the training phase of Experiment 3 was the number of choices toward the castrate odor during each block of ten trials, with eight such blocks. The dependent measure during the aversion testing was the amount of time spent on the side of the open field to which castrate urine had been applied during each one-min interval of the five-min test period.

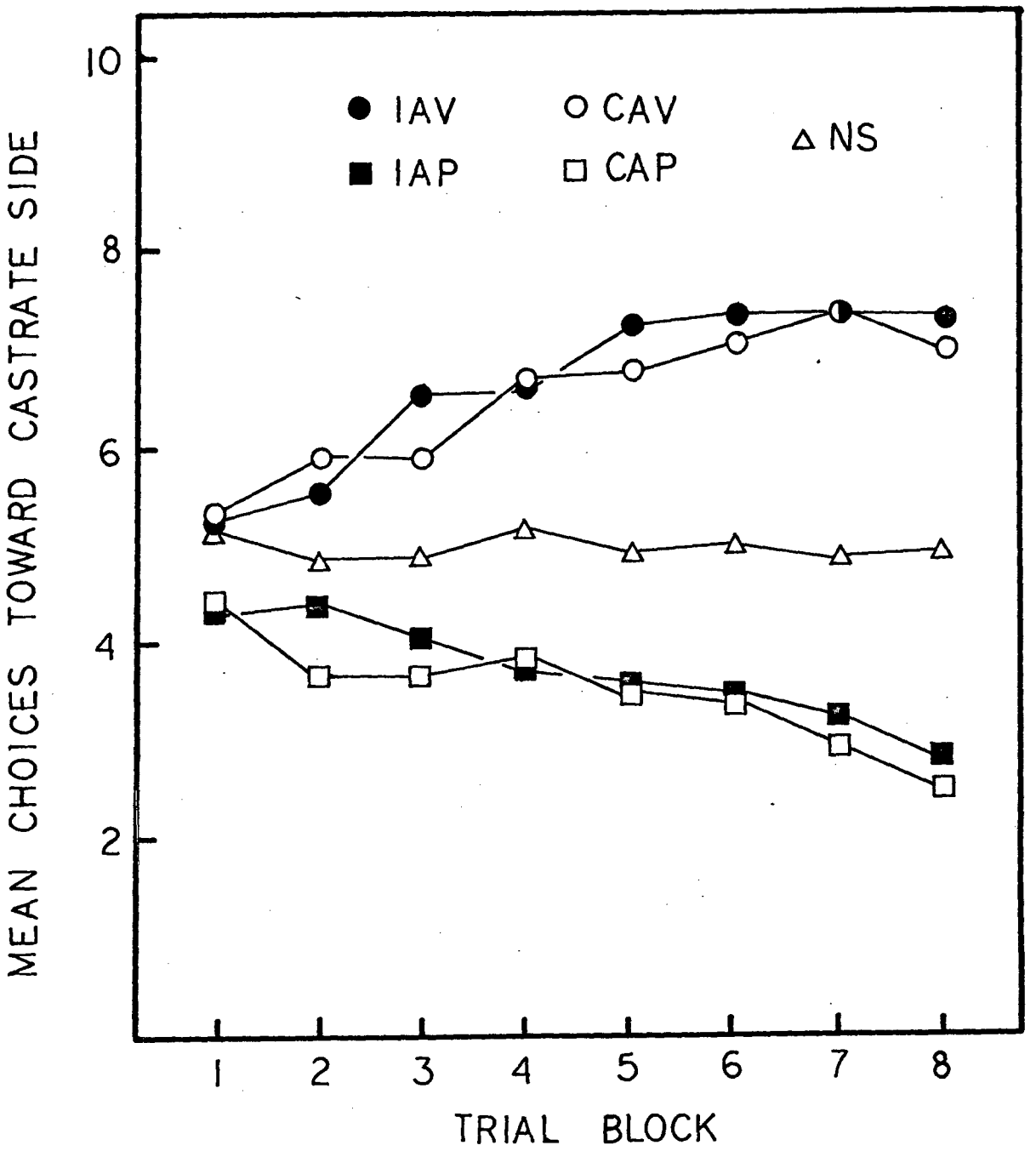
Results and Discussion

Training

During the training phase the design of Experiment 3 was a 5 x 8 (treatment group x trial block) factorial with subjects repeated across the eight blocks of trials. The initial analysis consisted of a repeated measures ANOVA. The ANOVA summary table is provided in Appendix C.

The results of the ANOVA for the training data of Experiment 3 demonstrated a highly significant treatment group by trial block interaction, $F(28, 385) = 5.38$, $p < .001$. This indicated that the trend in the mean number of choices toward the castrate odor across the trial blocks was not the same for each of the treatment groups. As Figure 5 shows, the NS group exhibited a relatively flat function at about five of ten toward the castrate side, while the AV groups (i.e. IAV and CAV) exhibited a consistent increase in responses toward the castrate odor across blocks, and the AP groups (i.e. IAP and CAP) exhibited a consistent decrease across blocks. That is, training subjects to avoid the odor of intacts increased the frequency with which they approached the castrate odor, while training the subjects to approach intact odor decreased the frequency. Analysis of the simple effects of treatment group at the various trial block levels using the Satterthwaite correction for repeated measures (Winer, 1971), indicated no difference on the first trial block,

Figure 5. Mean number of choices toward castrate odor as a function of treatment group and trial block, in Experiment 3 (chance response is approximately five per block). Group designations are: IAV = intact trained to avoid intact odor, IAP = intact approach, CAV = castrate avoid, CAP = castrate approach, NS = no stimuli present during training.


MEAN CHOICES TOWARD CASTRATE SIDE
10
8
6
4
2
IAV
CAV
NS
IAP
CAP
1
2
3
4
5
6
7
8
TRIAL BLOCK

$F(4, 326) = 2.21$, $p > .10$, while there was a substantial effect of treatment group at all other trial blocks (all F's > 7.25, all p's < .001). Post-hoc analyses using Scheffe's S-method (Kirk, 1968) indicated that while the IAV and CAV did not differ from one another at any trial block, their mean differed from that of the NS group at trial blocks three through eight (critical mean difference = 1.37, $\alpha = .05$). Likewise, while the IAP and CAP were not found to differ from one another on any trial block, the mean of these two groups differed from that of the NS group, and hence the AV groups, on trial blocks four through eight. Thus, as was expected, both AV groups learned to approach the castrate odor and to avoid the intact odor, while both AP groups avoided the castrate odor and approached the intact odor, with no evidence of any hormonal effect.

An ANOVA performed on the number of correct trials in each trial block for each of the subjects in the four trained groups indicated only a significant main effect of trial block, $F(7, 308) = 19.56$, $p < .001$. Trend analysis indicated a significant linear trend, $F(1, 44) = 123.32$, $p < .001$, with no higher-order trends reaching significance. That is, all groups learned the discrimination, and did so approximately equally well. It is interesting that the IAP group performed as well as the IAV group during the training phase. That is, it was no more difficult to train intact

males to approach the odor of an intact, an odor which in itself is somewhat aversive, than it was to train intact males to avoid the odor. This result is probably due to the extreme aversiveness of the shock stimulus relative to the mild aversiveness of the odor of intact mouse urine.

Thus, the results of the training phase of Experiment 3 indicate that castrate male mice were able to learn a discrimination between the odors of intact and castrate male mice, just as castrate rats were able to learn a discrimination between the odors of estrous and nonestrous female rats (Carr and Caul,1962). This suggests that the failure of castrate mice to exhibit an aversion to the odors of intact male mice, as demonstrated in Experiment 1, is not due to a sensory deficit, or an inability to detect and discriminate the odor from others.

Aversion

During the aversion testing phase the design of Experiment 3 was a 2 x 2 x 5 (hormone status x shock contingency x interval) factorial with subjects repeated across the five trial intervals. The initial analysis consisted of a repeated measures ANOVA. The AVOVA summary table is provided in Appendix D.

The ANOVA revealed a significant shock contingency by interval interaction, $F(4, 176) = 7.56$, $p < .001$. This indicated that the trend in the aversion test scores across the five-min test period for the AV groups differed

from the trend exhibited by the AP groups. Figure 6 shows the mean time spent on the side with castrate urine as a function of interval for each of the four groups. This figure suggests that the interaction was due to a slight rise across intervals for the AV groups compared with the gradual decrease across intervals for the AP groups. It is interesting that the aversion response exhibited to the odors, whether to intact odor or to castrate odor, did not show any degree of extinction during the aversion test.

While the shock contingency by interval interaction was significant, discussion of it should not detract from the result of primary importance, which was the pronounced main effect of shock contingency, $F(1, 44) = 137.04$, $p < .001$. That is, the previous training was found to generalize to the aversion test situation with the AV groups showing a pronounced aversion to the intact urine, and the AP groups an attraction to the same odor. The mean aversion score (sum of the five interval scores) for each group is provided in Table 1. Comparisons of each group with the random response of 150 sec indicated that the AV groups exhibited a significant aversion to intact urine (IAV, $t(11) = 8.37$, $p < .001$; and CAV, $t(11) = 4.27$, $p < .001$), and the AP groups exhibited a significant attraction to the intact urine odor (IAP, $t(11) = -5.05$, $p < .001$; and CAP, $t(11) = -7.77$, $p < .001$).

Figure 6. Mean time (sec) spent on the castrate side of the open field as a function of hormone status, shock contingency, and test interval, in Experiment 3 (random response is approximately 30 sec).

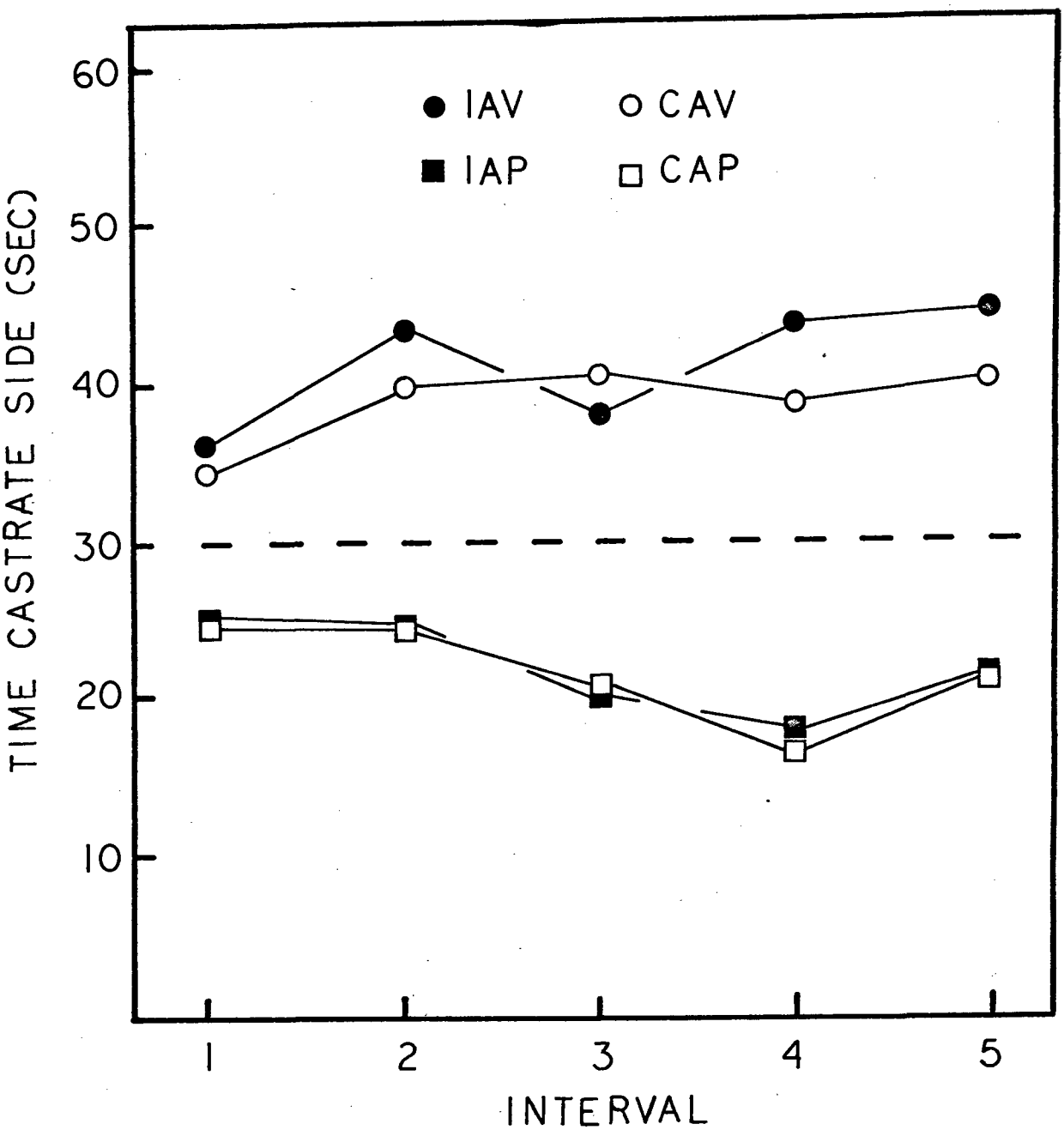

IAV
CAV
IAP
CAP
TIME CASTRATE SIDE (SEC)
60
50
40
30
20
10
1
2
3
4
5
INTERVAL

TABLE 1

Mean Time (sec ± standard error) Spent on the Castrate Side of the Open Field as a Function of Shock Contingency and Hormone Status [a]

	Hormone Status	
Shock Contingency	Intact	Castrate
AV	204.32 ± 6.49 *	191.28 ± 9.66 *
AP	108.93 ± 8.33 *	107.16 ± 5.51 *

[a] p values refer to comparisons with random response value of 150 sec and were determined by t-tests.

* $p < .001$

Thus, Experiment 3 was successful in answering two important questions concerning the relationship between hormonal status and responsiveness to urine odors. First, castrate mice are capable of learning a discrimination between intact and castrate odors, which suggests that their lack of spontaneous behavioral responsiveness to intact ddors is not due to a sensory deficit. Secondly, the spontaneous responsiveness, as measured by the aversion test, is easily modifiable via experimental manipulations. That is, the effect of training an aversion to an odor generalizes to at least on other situation (the aversion test situation), and persists for at least 48 hours.

EXPERIMENT IV

Previous findings have determined that varying the nature of the urine odors of an opponent animal modifies the agonistic behavior of the subject (e.g. Mugford and Nowell, 1970). Experiment 4 was designed to examine how modifying the responsivity of subjects to a standard urine odor would influence their agonistic behavior when confronted with an animal having that odor. That is, as the previously proposed model suggests, altering either the nature of the incoming stimuli, or the perception of and responsivity to these stimuli by the recipient animal, should influence subsequent agonistic behavior.

Modification of a subject's responsivity to urine odors was accomplished by employing a training procedure similar to that used in Experiment 3. That is, subjects were trained to approach or avoid intact urine odors. However, in Experiment 4 the side of the apparatus opposite to that containing intact urine had no odor (distilled water). The reason for using intact versus no odor, rather than intact versus castrate odor, lies in the confounding effect training with both odors could have had on later aggression testing. In order to standardize the opponent animals used during aggression testing, castrate males swabbed with the urine of intact donors were employed. However, the castrate opponents would, along with the experimentally applied odor, have the odor of a castrate.

Thus, subjects would have been faced with two odors, which could have presented a confusing situation if they had previously been trained with both of the odors.

The subjects were trained to discriminate between intact odor and no odor in order to avoid shock. Following training, the subjects were tested both for the aversion or attraction to the intact odor so as to determine the effects of training on responsiveness, and for aggression toward a castrate opponent swabbed with the urine of intacts so as to determine the effects of modifying responsiveness on the subsequent agonistic behavior. Four groups of intact and four groups of castrate subjects were employed. Two groups of each hormone status were trained to discriminate between intact and no odor. Two groups, one of each hormone status, were treated as the NS group of Experiment 3. And, finally one group of intact and one group of castrate subjects were exposed to the odor stimuli and the training situation, but no shock was ever presented (designated the stimulus groups, or ST). All groups received the aversion and the aggression tests.

Method

Responding Subjects

The subjects were 112 male mice obtained from the same stock and housed in groups of four under the same conditions as in previous experiments.

Donor Animals

The donors were 24 male mice obtained from the same stock as the responders. At approximately 70 days of age each donor was housed individually in a metabolism cage for a minimum of three weeks prior to the onset of urine collection.

Opponent Animals

The opponent animals used during aggression testing were 112 males obtained from the same stock as the responders, and housed in groups of four under the same conditions. At approximately 35 days of age each opponent was castrated. The opponents were used when 60-75 days of age.

Apparatus

The avoidance training apparatus was the same as that employed in Experiment 3. Also, the aversion test apparatus was the same as that described previously. Aggression tests took place in a clean cage similar to that in which the subjects were housed.

Procedure

At approximately 70 days of age 56 of the subjects (two per box of four mice) were castrated, while the remaining 56 were sham-operated. At this time the intact and castrate subjects were assigned to one of the following groups:

IAV, 14 intacts trained to avoid the odor,
IAP, 14 intacts trained to approach the odor,
INS, 14 intacts "trained" with no odor present,
IST, 14 intacts "trained" with the odor present, but no shock,
CAV, 14 castrates trained as IAV,
CAP, 14 castrates trained as IAP,
CNS, 14 castrates "trained" as INS,
CST, 14 castrates "trained" as IST.

The groups designated IST and CST were allowed to make a choice in the training apparatus, and were then removed from the goal box as if the response were correct. No shock was ever presented to these two groups.

Approximately 20 days after castration avoidance training began. Training was conducted as described for Experiment 3, except that only urine stimuli from intact donors were used. The other stimulus box of the training apparatus contained an equivalent amount of distilled water. The urine used during training was collected as described for the previous experiments, and was pooled prior to its use as described for Experiment 3. Also, the subjects were required to respond to the urine from a different set of donors during each of the initial three training sessions, while the last session was conducted with urine from the same donor set as was used during session one. Each subject received a total of 80 trials distributed in the same manner as described for Experiment 3.

Aversion and aggression testing began 48 hr after the last training session. Half of the subjects in each group

were initially subjected to the aversion test, followed 80-90 min later by aggression testing. The remaining subjects received the behavioral tests in the opposite order with the same interval between tests.

Aversion testing proceeded as described for Experiment 1. The donors supplying the urine during the last training session for a particular subject, also supplied the urine for aversion testing.

During the aggression phase, each subject was tested for aggression directed toward a castrate opponent swabbed with approximately .06 cc of urine. A different opponent was used during each aggression test in order to eliminate possible effects of prior use on the measures of aggression obtained from a responding subject. The urine was obtained from the same pool as that used (or for subjects receiving aggression tests first, that subsequently used) during aversion testing.

The procedure for aggression testing consisted of initially placing a subject in a clean mouse box with clean bedding on the floor. A Plexiglas cover was placed over the box, and a barrier inserted through a slit in the top to divide the box into two sections of equal size. The opponent was then swabbed with urine and placed in the box on the side opposite that occupied by the subject. Approximately 15 sec later the barrier was removed and testing began. Frequency and total time the subject

engaged in the following behaviors were recorded for a 10-min period: investigative nosing, vigorous nosing, chasing, tail-rattling, biting attack, and defensive (submissive) postures. Immediately after the session the subject was rated on the following seven-point scale, which is similar to that previously used by Ebert and Hyde (1976) and Hyde and Sawyer (1977): 0 - occasional or frequent submission, with little or no nosing; 1 - occasional nosing, little contact; 2 - frequent nosing, moderate contact; 3 - frequent vigorous nosing; 4 - chasing and tail-rattling; 5 - biting attack, including wrestling; and 6 - frequent fierce attack, including biting and wrestling. All aggression testing was conducted approximately 1-5 hr into the dark period under red light illumination.

Results and Discussion

Training

The design for the training phase of Experiment 4 was a 2 x 4 x 8 (hormone status x training condition x trial block) factorial with subjects repeated across trial blocks. The analysis consisted of a repeated measures ANOVA. The ANOVA summary table is provided in Appendix E.

The results provided by the ANOVA were relatively straightforward. As expected, a pronounced training condition by trial block interaction was found, $F(21, 728) = 6.87$, $p < .001$, which renders the main effects of training

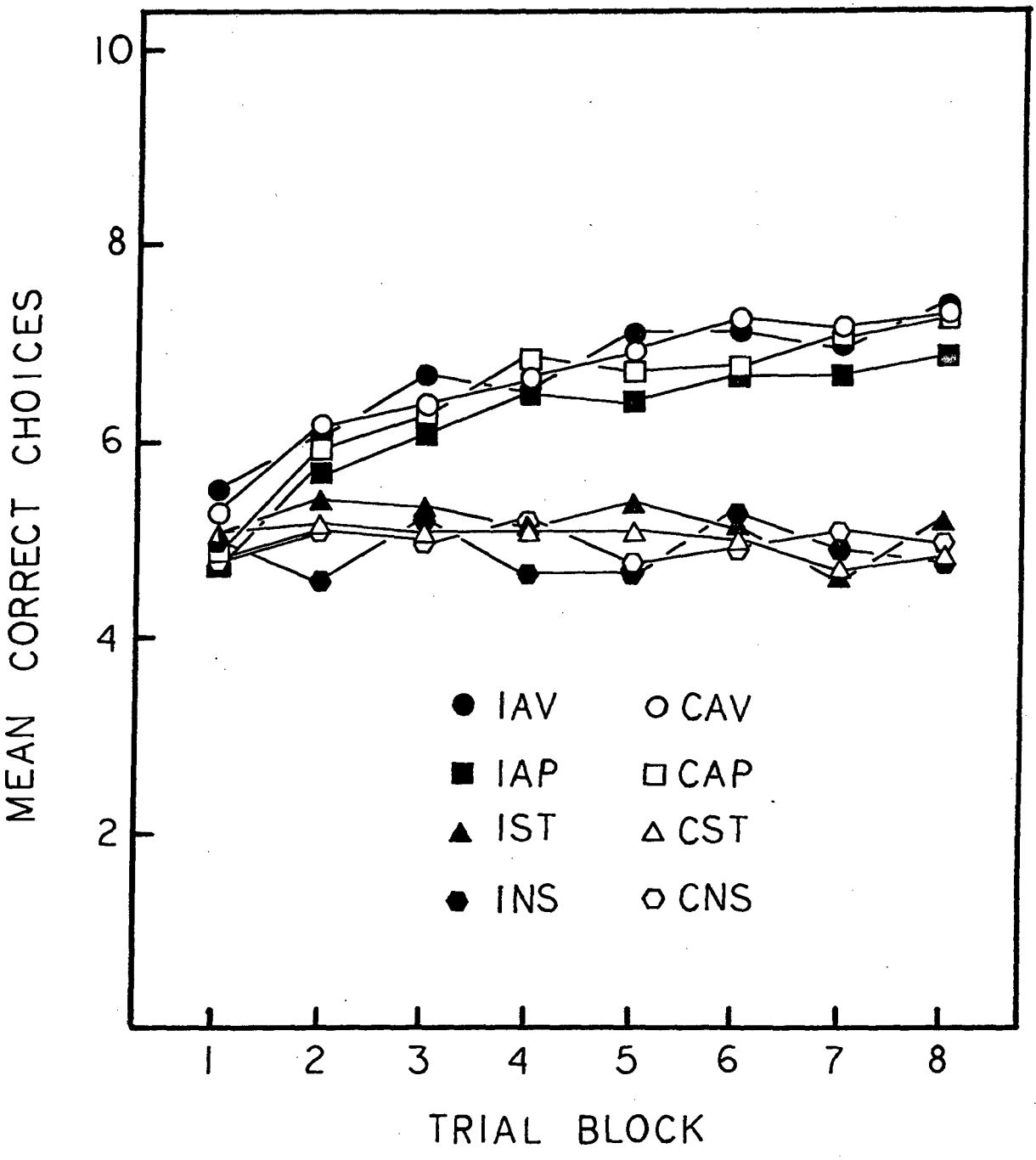
condition, $F(3, 104) = 86.44$, $p < .001$, and trial block, $F(7, 728) = 15.10$, $p < .001$, somewhat obscure. It is obvious from inspection of Figure 7, which shows correct choices as a function of group and trial block, that the interaction was due to the fact that the AV and AP groups (whether intact or castrate) had learned the avoidance task, while the ST and NS groups (whether intact or castrate) had not. That is, the AV and AP groups exhibited essentially a linear increase in correct choices across blocks, while the ST and NS groups exhibited relatively flat functions at approximately 50% correct.

It should be noted that, as in Experiment 3, the main effect of hormone status did not approach significance, nor was hormone status found to interact with any other variable. Thus, these results support those of Experiment 3, in suggesting that castrate male mice are capable of detecting the urine odors of isolate males, and can learn to respond to such odors.

Aversion Testing

The design for the aversion testing phase of Experiment 4 was a 2 x 4 x 2 x 5 (hormone status, training condition x test order, i.e. before or after aggression testing x interval with session) factorial with subjects repeated across intervals. The initial analysis consisted of a repeated measures ANOVA. The ANOVA summary table is provided in Appendix F.

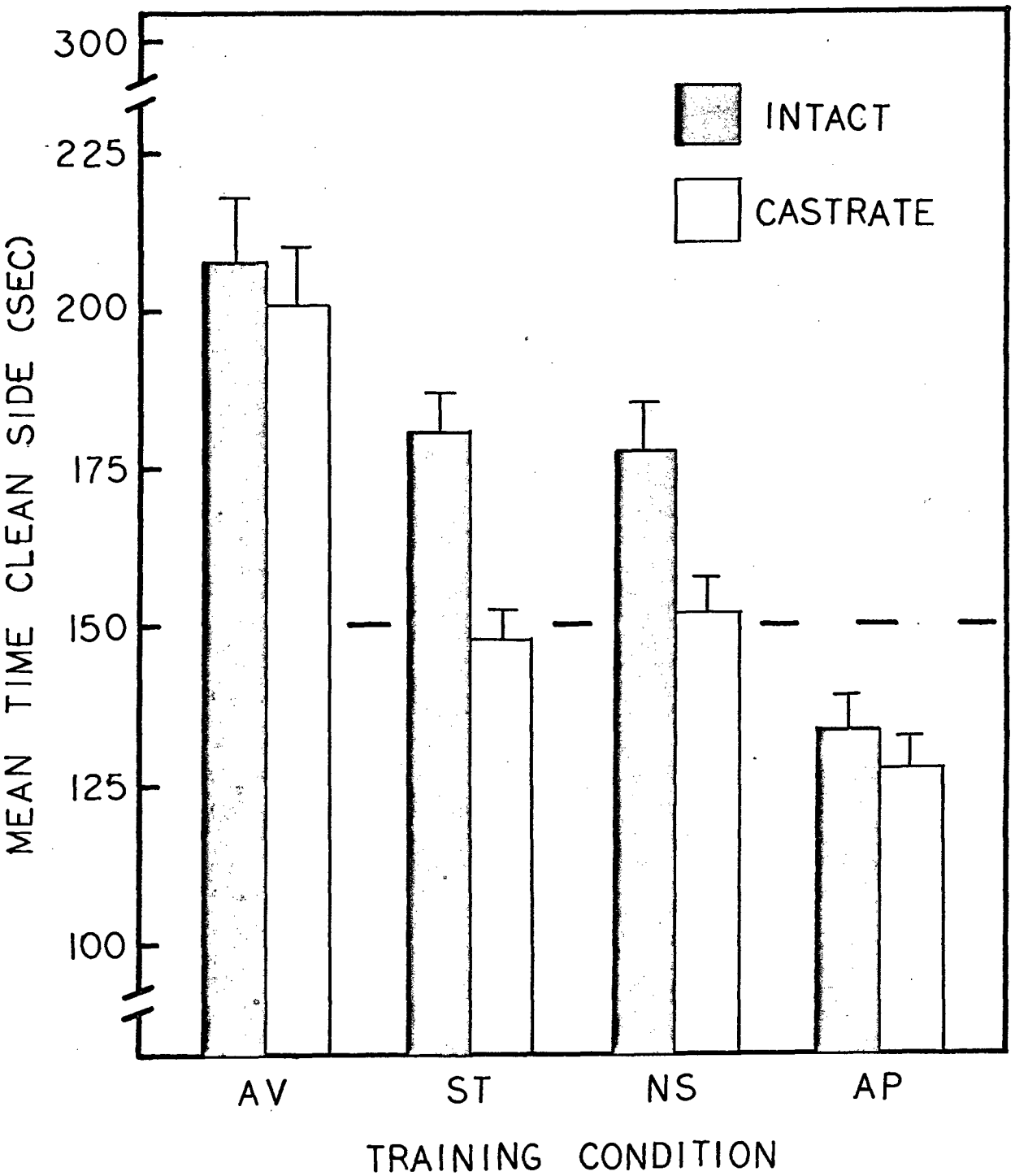
Figure 7. Mean number of correct choices as a function of hormone status, training condition, and trial block, in Experiment 4 (chance response is approximately five correct per block). Group designations are, IAV = intact trained to avoid odor, IAP = intact approach, CAV = castrate avoid, CAP = castrate approach, IST = intact stimulus present but no shock, CST = castrate stimulus, INS = intact no stimulus, CNS = castrate no stimulus.


MEAN CORRECT CHOICES
10
8
6
4
2
1
2
3
4
5
6
7
8
TRIAL BLOCK
IAV
CAV
IAP
CAP
IST
CST
INS
CNS

The ANOVA revealed a significant effect of interval within session, $F(4, 384) = 9.71$, $p < .001$, which was due to a relatively consistent, though small, increase across intervals in the time spent on the clean side of the open field (mean for interval 1 = 31.07 sec, interval 2 = 31.24, interval 3 = 34.19, interval 4 = 34.04, interval 5 = 35.51). The greatest portion of this increase was contributed by the AV groups, and to a lesser extent the IST and INS groups. This is reflected in the training condition by interval interaction effect which approached, but failed to reach, conventional levels of significance, $F(12, 384) = 1.71$, $p > .07$.

A significant hormone status by training condition interaction, which is plotted in Figure 8, was also found, $F(3, 96) = 2.74$, $p < .05$. Analysis of the simple effects of hormone status at different levels of the training condition factor indicated that the interaction was due to the differences between the intact and castrate subjects of the ST groups, $F(1, 96) = 14.68$, $p < .001$, and NS groups, $F(1, 96) = 9.37$, $p < .005$, while no differences were found between the AV groups, $F(1,96) = 0.59$, $p > .20$, or the AP groups, $F(1, 96) = .046$, $p > .20$. That is, as the previous experiments have demonstrated, castrate males that have been trained to avoid or approach the odor of intact donors do not differ from trained intact responders during subsequent aversion testing (see results

Figure 8. Mean time (sec ± standard error) spent on the clean side of the open field as a function of hormone status and training condition, in Experiment 4 (random response is approximately 150 sec). Training condition designations are: AV = trained to avoid, AP = trained to approach, ST = "trained" with stimulus present, but no shock, and NS = "trained" with no stimulus present.


300
225
200
175
150
125
100
MEAN TIME CLEAN SIDE (SEC)
INTACT
CASTRATE
AV
ST
NS
AP
TRAINING CONDITION

of Experiment 3). while castrates without such training do-differ from intact subjects in their spontaneous behavioral responsiveness to the odors of intacts (see results of Experiment 1). Thus, this portion of Experiment 4 represents a replication of the combined results of Experiments 1 and 3.

Post hoc comparisons were performed on the aversion test data to assess the effects of training and hormone status further. The intact control groups (i.e. IST and INS) were not found to differ significantly from one another, $F(1, 96) = 0.14$, $p > .20$, nor were the CST and CNS groups, $F(1, 96) = 0.16$, $p > .20$. Comparison of IAV with the average of the intact controls indicated that training intact subjects to avoid the odor was effective in increasing the time spent on the clean side of the open field during the aversion test, $F(1, 96) = 15.06$, $p < .01$. The castrates trained to avoid the odor (i.e. CAV) were found to exhibit a substantially greater aversion response than the castrate controls (i.e. CST and CNS), which indicated the effectiveness of training castrates to avoid the odor, $F(1, 96) = 48.59$, $p < .01$. Also, the IAP group was found to exhibit a significantly lower aversion response (i.e. less time on clean side) than did intact controls, $F(1, 96) = 38.78$, $p < .01$, as did the CAP group when compared with the castrate controls, $F(1, 96) = 9.18$, $p < .01$. Thus, as expected, training had the effect of

raising the aversion score for the AV groups and lowering it for the AP groups relative to the appropriate controls. Finally, it should be noted that receiving the aggression test prior to the aversion test (i.e. the main effect of test order) did not appear to influence the aversion response of the subjects, $F(1, 96) = 1.04$, $p > .20$.

Aggression Testing

The analyses of the aggression test data were done separately for the aggression rating and the component behaviors. The design for the rating measure, to be discussed first, was a 2 x 4 x 2 (hormone status x training condition x test order) factorial. An ANOVA constituted the initial analysis. The summary table is provided in Appendix G. The mean aggression rating, as well as the number attacking, for each group as a function of test order is provided in Table 2.

The ANOVA revealed a highly significant effect of hormone status on the rating measure, $F(1, 96) = 26.60$, $p < .001$, with intact subjects (overall mean rating = 3.20) substantially more aggressive than castrates (overall mean rating = 1.71). This demonstrates the well-documented effect of castration on aggression (e.g. Beeman, 1947). This result is further substantiated by the finding that of the 56 intact subjects, 21 of them (37.5%) attacked the opponent at least once (i.e. received a rating of 5 or 6), while only 5 of 56 castrate subjects (8.9%) attacked

TABLE 2

Mean Aggression Rating (± standard error) and the Number of Subjects (n = 7) that Attacked as a Function of Hormone Status, Training Condition, and Test Order [a]

		Test Order			
Hormone Status	Training Condition	Aversion First	Attacked	Aggression First	Attacked
Intact	AV	4.71 ± 0.77	5/7	3.57 ± 0.84	3/7
Intact	AP	3.29 ± 0.80	3/7	1.86 ± 0.59	1/7
Intact	ST	3.00 ± 0.74	2/7	2.86 ± 0.59	2/7
Intact	NS	3.71 ± 0.93	4/7	2.57 ± 0.51	1/7
Castrate	AV	2.57 ± 0.46	1/7	1.14 ± 0.15	0/7
Castrate	AP	1.71 ± 0.20	0/7	1.71 ± 0.15	1/7
Castrate	ST	1.86 ± 0.59	1/7	1.29 ± 0.20	0/7
Castrate	NS	1.71 ± 0.61	1/7	1.71 ± 0.61	1/7

[a] Ratings range from a possible low of 0.00 to a possible high of 6.00.

the opponent. A chi-square test indicated this to be significant, $\chi^2(1) = 8.65$, $p < .01$.

An interesting and unexpected effect of test order was also found. That is, the main effect of test order was found to be significant, $F(1, 96) = 6.49$, $p < .05$; interestingly enough subjects that were subjected to the aversion test prior to aggression testing received higher ratings (overall mean rating = 2.82) than did those that received the aggression test first (overall mean rating = 2.09). While the difference was small it was quite consistent across groups , as the subjects receiving the aversion test first received higher ratings than those tested for aggression first in all but two groups (CAP and CNS), and in those groups there was no difference (see Table 2). While it was also found that subjects receiving the aversion test first were somewhat more likely to attack than those receiving the aggression test first (17 of 56 or 30.4% vs. 9 of 56 or 16.1%), the chi-square test was not significnat, $\chi^2(1) = 1.89$, $p < .10$.

It is interesting that a five-min exposure to the urine of a male can influence subsequent agonistic behavior toward an opponent with the odor, particularly after an 80-90 min interval. An answer as to why it occurred cannot at this time be provided, but will require further research. However, it can be suggested that the prior exposure may have served as a "warmup period", to heighten the arousal

state of the subject. Scott and Fredericson (1951) have observed that mice will be more vigorous if, "they have a sort of warmup period and are thoroughly excited before the actual fight starts" (p. 291). However, one would expect any heightened state of arousal would gradually diminish over the 80-90 min period. This would suggest that the effect of test order might be even larger if the interval between exposure to urine and aggression testing were to be reduced, and smaller if the interval were increased. Another possible explanation, which is not at all inconsistent or contradictory with the arousal notion, is that the exposure to urine could have had a priming function. That is, it may have caused endocrine responses in the subjects in much the same manner as male odors influence, for example, the estrous cycle of female mice (Bronson, 1971). The result may have been a heightened state of sensitivity to the particular odor, or to any stimulus event. Obviously, any priming action on the castrate subjects could not have involved testosterone release, and as the effect of prior exposure was quite substantial for the CAV group, the release of other types of hormones could be examined.

The main effect of primary interest, namely training condition, was not found to be statistically significant, $F(3, 96) = 1.76$, $p > .10$. While the overall test was not significant, a planned comparison between the combined AV

and AP groups revealed the AV (overall mean rating = 3.00) subjects to exhibit more aggressive behavior than did the AP (overall mean rating = 2.14) subjects, $F(1, 96) = 4.45$, $p < .05$. Thus, as expected, subjects trained to avoid the urine odor exhibited more aggression than those trained to approach the odor when tested with an opponent having the odor. However, further planned comparisons revealed that while the IAV (mean rating = 4.14) group had a higher mean rating than the IAP (mean rating = 2.57) group, $F(1, 96) = 7.48$, $p < .01$, the same was not true for CAV (mean rating = 1.86) compared with the CAP (mean rating = 1.71) group, $F(1, 96) = 0.14$, $p > .20$. That is, intact subjects trained to avoid the odor did receive higher ratings than intacts trained to approach, while castrate subjects trained to avoid did not receive higher ratings than castrates trained to approach. These results are substantiated by the finding that twice as many IAV subjects (8 of 14 or 57.1%) attacked the opponent than did IAP (4 of 14 or 28.6%), while an equal number of CAV and CAP subjects attacked (1 of 14 or 7.1% of each group).

Thus, the results of Experiment 4 do not provide complete support for the previously stated hypothesis that manipulations, whether hormonal or experiential, which are found to modify the responsivity to the urine odors will also be found to modify agonistic behavior. That is, experimentally manipulating responsivity of intact subjects

does, while experimentally manipulating responsivity of castrates does not, modify agonistic behavior. This implies that while the influence of the male hormone on responsivity to stimulus odors may be involved in aggression, such an influence is not the sole route, or possibly even the primary route by which the hormone exerts its effects on agonistic behavior. In other words, replacing responsivity does not appear to have the same effect as replacing the hormone.

While the results for the aggression rating were of primary importance, a 2 x 4 x 2 (hormone status x training condition x test order) Multivariate Analysis of Variance (MANOVA) using Finn's Multivariance (1974) computer program was performed on the component behaviors. These behaviors consisted of the frequency and total time the subject engaged in investigative nosing, vigorous nosing, chasing, tail-rattling, biting attack, as well as the latency to the first attack (equalled 600 sec if subject did not attack), yeilding a total of eleven dependent measures. Frequency and time for defensive (submissive) postures were dropped from the analysis as only four of the 112 subjects exhibited a total of five such postures. The means of each of these dependent measures, as a function of hormone status and training condition, are provided in Table 3.

Just as for the aggression rating, the MANOVA revealed a highly significant effect of hormone status, $\underline{F}(11, 86) =$

TABLE 3

Mean Values (frequencies and times [a]) for each Component Behavior Measured in Experiment 4

Group		Component Behaviors					
		Nose	Vig. Nose	Chase	Tail Rattle	Attack	Attack Latency
IAV	Freq	25.63	8.36	1.21	5.79	7.00	
	Time	79.67	23.29	1.52	6.32	15.06	381.40
IAP	Freq	17.04	2.88	0.16	1.13	0.82	
	Time	62.99	7.12	0.19	2.80	2.51	558.80
IST	Freq	20.50	3.93	0.14	2.64	2.29	
	Time	61.19	9.65	0.15	3.09	2.27	479.50
INS	Freq	17.64	5.93	0.21	2.71	2.79	
	Time	49.96	12.37	0.13	2.51	3.43	459.20
CAV	Freq	19.71	1.57	0.14	0.36	0.21	
	Time	53.14	2.59	0.07	0.30	0.19	559.60
CAP	Freq	16.29	0.57	0.00	0.00	0.29	
	Time	56.29	2.04	0.00	0.00	0.33	583.40
CST	Freq	13.64	0.64	0.00	0.21	0.50	
	Time	48.75	1.51	0.00	0.51	0.84	592.90
CNS	Freq	10.29	1.00	0.07	0.29	0.86	
	Time	24.44	2.80	0.04	0.33	1.32	552.90

[a] Time in seconds.

5.48, $p < .001$. Subsequent univariate ANOVAs revealed that hormone status influenced each of the eleven component behaviors (all p's $< .03$), with intacts scoring higher on all except for a lower attack latency. The MANOVA also revealed a significant effect of training condition, $F(33, 254) = 2.09$, $p < .01$. Univariate ANOVAs indicated that training condition influenced investigative nosing time and frequency, as well as chasing time and frequency (all p's $< .05$), while its effect on attack time approached significance ($p < .10$). Multivariate comparisons indicated the AV (i.e. IAV and CAV) groups were significantly different from the AP (i.e. IAP and CAP) groups, $F(11, 86) = 2.03$, $p < .05$, and the control groups, $F(11, 86) = 3.87$, $p < .01$, on nosing frequency and time, chasing frequency and time, and attack time (all p's $< .05$). However, the AP groups did not differ significantly from the controls, $F(11, 86) = 0.92$, $p > .20$. Finally, the results of the MANOVA revealed that the effect of test order approached, but did not reach conventional levels of significance, $F(11, 86) = 1.66$, $p < .10$. Also, none of the interaction effects approached statistical significance (all p's $> .12$).

DISCUSSION

The experiments reported in the previous pages were designed to examine a number of questions concerning the relationship between androgens, responsivity to odor stimuli, and agonistic behavior in male mice. Each of these questions, briefly stated previously, will now be discussed with regard to the results.

Experiment 1 provided replication and elaboration of the previously cited study of Sawyer (Note 1). It was clearly demonstrated that castration eliminated the spontaneous behavioral responsiveness of male mice to the urine odors of intact, isolate mice. Furthermore, exogenous testosterone treatment was found to replace responsivity, clearly indicating that the aversion response to male odors is androgen-depaendent.

Experiment 2 provided an extension of these results to another type of "naturally-occuring" aversive odor, namely alarm odors. It was found that responsivity to such odors is not androgen-dependent as both intact and castrate subjects avoided them. This suggested that male hormone effects on responsivity to odors may be more specific, including aggression-related and sex-related (Carr et al., 1965, 1966) odors, and not those involved in danger.

The results of Experiment 3 suggested that a castrate subject's lack of responsivity to male stimulus odors is not due to a sensory deficit, as castrate males were

able to learn a discrimination between the odor of intact male donors and that of castrate donors. Furthermore, learning of the discrimination had a pronounced effect on subsequent spontaneous behavioral responsivity as measured in the aversion test. That is, it was possible to modify the responsivity of castrate subjects, as well as intacts, to the odors of other mice. However, it is not certain that during avoidance training and later aversion testing the castrate subjects were responding to the particular aspect of intact urine that makes it aversive. That is, the urine of intact and castrate male mice may differ in ways other than in the particular aversive substance, which appears to be the secretions of the coagulating gland (Jones and Nowell, 1973c).

Experiment 4 examined the central question stated in the introduction, which was whether or not manipulations that modify responsiveness to urine odors also act to modify agonistic behavior. The results indicated that modification upward in the aversion response exhibited by intact subjects to a standard urine stimulus resulted in a substantially higher aggression rating, and doubled the incidence of attack directed at an opponent having the odor relative to intact males subjected to downward modification in their aversion response. This finding supports the notion that threat may be an important component of aggression (Sawyer, 1978; Scott and Fredericson, 1951);

it also supports the central hypothesis. However, with regard to castrate subjects, modifications in the responsivity to urine odors was found not to influence subsequent agonistic behavior. That is, subjecting castrate males to training that resulted in "intact-like" responsivity to urine odors, did not result in their being "intact-like" with regard to aggression. As previously stated, modifying or replacing responsivity to odors does not appear to have the same effect on castrate subjects as replacing the hormone. This result does not support the previously stated hypothesis, and suggests the model presented in Figure 1 is not completely adequate.

There are some reasons that may be suggested as to why modifying the responsivity of castrate subjects did not influence subsequent agonistic behavior. For example, while it was determined that castration does eliminate spontaneous behavioral responsiveness to the aversive odors of intact male mice, it may have this effect by eliminating the social significance of such odors to the castrates. That is, intact animals may perceive the odor to have some specific social significance, such as threat of attack by another, while castrates do not. Training castrates to avoid the odor, while it does replace the aversion response, would not necessarily replace the social significance of the odor. Thus, when castrates were tested for aggression with the opponent having the odor, there may have been

no change in social behavior because the odor may still have not had any particular social significance to the subject. The argument presented here is based on two notions: (1) that modifying the response to a socially insignificant or neutral stimulus will not modify subsequent social behavior directed at an animal having that odor, while (2) modifying the response to a socially significant stimulus will modify subsequent social behavior. This would suggest that training intacts to avoid some unaversive and socially neutral stimulus, as the urine odors of intact males apparently are to castrate males, would not modify their social behavior toward an animal to which that odor had been applied.

Thus, perhaps modifying the responsivity of castrates did not influence agonistic behavior because the stimulus odors had no social significance to the castrates. The problem with this suggestion is that it is not immediately apparent as to how it could be subjected to scientific test. That is, it might be extremely difficult to modify the social significance of odors to the castrate subjects without subjecting them to social interactions. Such interactions could provide the subjects with the opportunity to learn appropriate social responses, and as was demonstrated in both Experiment 3 and Experiment 4 the learning of responses to odors can readily occur in the absence of androgens. An approach that could prove

productive would be to manipulate the type of odors that come to be socially significant during an animal's development, and to test the effect of hormonal and experimental manipulations on the responsiveness to them. Such an attempt has demonstrated that the odors to which male mice emit ultrasonic vocalization can be manipulated during early development (Nyby, Whitney, Schmitz, and Dizinno, in press; Nyby, personal communication).

Another possible explanation for the failure of modifications in responsivity to influence the agonistic behavior of castrates, involves the possibility of a hormone effect on motor systems involved in the output of particular agonistic responses. That is, perhaps androgen influences not only the neural systems involved in the perception of and responsivity to odors, but may also influence neural systems involved in the output of the appropriate agonistic response, or the muscle systems involved in making the response. This notion is not very tenable due to the fact that male mice with a great deal of prior experience may continue to fight with other males for some time following castration (e.g. Beeman, 1947). Also, observations during Experiment 4 indicated that the castrate opponents would occasionally fight back when attacked, suggesting such agonistic responses are possible in the absence of circulating androgens. Finally, it has been found that small amounts of

testosterone applied directly to discrete brain locations (e.g. septum and preoptic area) is capable of replacing agonistic behavior in castrated male mice (Owen, Peter, and Bronson, 1974).

Thus, neither the hypothesis of primary concern, nor the model presented in Figure 1, were entirely supported or refuted by the four experiments performed, which suggests that future research and modification of the model is in order. Also, other unresolved questions, such as the basis for the test order effect on aggression which was found in Experiment 4, need to be investigated further in the future.

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FOOTNOTE

[1] Before proceeding, a point concerning the use of terms such as donor, responder, and subject should be made in order to eliminate possible confusion. Unless otherwise specified, donor refers to an animal that supplies the urine stimuli to which another animal responds. The term responder refers to an animal tested for its response to the urine of a donor. The term subject will be used primarily to refer to responders, and only when it is clear from the context that it is being used in this way. Thus, it should be clear that in the coming section and the balance of the paper, urine aversiveness refers to the quality of a donor's urine as measured by a responder's aversion to it, while responsiveness, responsivity, or reactivity refers to the tendency on the part of an animal (a responder) to react to the urine odors of another mouse, and will be operationally defined by the aversion response. Lastly, the term opponent will be used to refer to the stimulus animal used when testing a subject for aggression.

APPENDICES

APPENDIX A

ANOVA Summary Table for Experiment 1

Source of Variance	SS	df	MS	F	
Between Subjects					
Treatment Group	1697.39	3	565.80	2.75	
Error	12355.23	60	205.92		
Within Subjects					
Session	2196.17	2	1098.08	8.91	*
Ses. x Treat.	2361.11	6	393.52	3.19	*
Error	14782.93	120	123.19		
Interval	612.45	4	153.11	1.73	
Int. x Treat.	918.58	12	76.55	0.87	
Error	21227.26	240	88.45		
Ses. x Int.	370.65	8	46.33	0.63	
Ses. x Int. x Treat.	1927.61	24	80.32	1.08	
Error	35579.41	480	74.12		
Total	94028.79	959			

* $p < .001$

APPENDIX B

ANOVA Summary Table for Experiment 2

Source of Variance	SS	df	MS	F
Between Subjects				
Hormone Status	4.64	1	4.64	0.08
Error	1246.67	22	56.67	
Within Subjects				
Interval	340.41	4	85.10	2.21
Interval x Horm. St.	48.75	4	12.19	0.32
Error	3392.83	88	38.56	
Total	5033.30	119		

APPENDIX C

ANOVA Summary Table for Experiment 3 (Training Phase)

Source of Variance	SS	df	MS	F	
Between Subjects					
Treatment Group	855.97	4	213.99	115.67	*
Error	101.93	55	1.85		
Within Subjects					
Trial Block	10.06	7	1.44	1.44	
Block x Treat.	150.53	28	5.38	5.38	*
Error	384.14	385	1.00		
Total	1502.65	479			

* $p < .001$

APPENDIX D

ANOVA Summary Table for Experiment 3 (Aversion Test)

Source of Variance	SS	df	MS	F	
Between Subjects					
Hormone status	131.56	1	131.56	0.93	
Shock Contingency	19333.65	1	19333.65	137.04	**
Horm. x Sk.	76.26	1	76.26	0.54	
Error	6207.61	44	141.08		
Within Subjects					
Interval	514.96	4	128.74	3.27	*
Int. x Horm.	250.38	4	62.60	1.59	
Int. x Sk.	1189.00	4	297.25	7.56	**
Int. x Horm. x Sk.	99.11	4	24.78	0.62	
Error	6922.26	176	39.33		
Total	34724.78	239			

* $p < .05$

** $p < .001$

APPENDIX E

ANOVA Summary Table for Experiment 4 (Training Phase)

Source of Variance	SS	df	MS	F	
Between Subjects					
Hormone Status	0.45	1	0.45	0.22	
Training Condition	531.16	3	177.05	86.44	*
Horm. x Tr.	7.08	3	2.36	1.15	
Error	213.03	104	2.05		
Within Subjects					
Trial Block	84.60	7	12.09	15.10	*
Block x Horm.	3.80	7	0.54	0.68	
Block x Tr.	115.56	21	5.50	6.86	*
Block x Horm. x Tr.	6.45	21	0.31	0.38	
Error	582.82	728	0.80		
Total	1544.95	895			

* $p < .001$

APPENDIX F

ANOVA Summary Table for Experiment 4 (Aversion Test)

Source of Variance	SS	df	MS	F	
Between Subjects					
Hormone Status	1599.63	1	1599.63	16.67	**
Training Condition	14875.43	3	4958.48	51.67	**
Test Order	99.44	1	99.44	1.04	
Horm. x Tr.	789.13	3	263.04	2.74	*
Horm. x Or.	6.00	1	6.00	0.06	
Tr. x Or.	250.84	3	83.61	0.87	
Horm. x Tr. x Or.	63.07	3	21.02	0.22	
Error	9212.11	96	95.96		
Within Subjects					
Interval	1722.27	4	430.57	9.71	**
Int. x Horm.	134.68	4	33.67	0.76	
Int. x Tr.	909.54	12	75.80	1.71	
Int. x Or.	274.72	4	68.68	1.55	
Int. x Horm. x Tr.	228.09	12	19.01	0.43	
Int. x Horm. x Or.	70.44	4	17.61	0.40	
Int. x Tr. x Or.	499.52	12	41.63	0.94	
Int. x Horm. x Tr. x Or.	544.33	12	45.36	1.02	
Error	17031.36	384	44.35		
Total	48310.58	559			

* $p < .05$

** $p < .001$

APPENDIX G

ANOVA Summary Table for Experiment 4 (Aggression Rating)

Source of Variance	SS	df	MS	F	
Hormone Status	61.51	1	61.51	26.60	**
Training Condition	12.24	3	4.08	1.76	
Test Order	15.01	1	15.01	6.49	*
Horm. x Tr.	7.38	3	2.46	1.06	
Horm. x Or.	1.51	1	1.51	0.65	
Tr. x Or.	3.31	3	1.10	0.48	
Horm. x Tr. x Or.	4.81	3	1.60	0.69	
Error	222.00	96	2.31		
Total	327.77	111			

* $p < .05$

** $p < .001$