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PROGRESS REPORT

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Project Title: Integration of neurobiological and computational analyses of the neural network essentials for conditioned taste aversions

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Principal Investigator: Dr. Kathleen C. Chambers

GOALS

The general goal of the ONR project is to determine the neural basis of learning and memory, i.e., how the brain stores and retrieves memory. More specifically we are determining how the hard-wired (innate) part of the neural system interfaces with the plastic (learned) part. The special form of learning which is the focus of this project is conditioned taste aversions (CTAs), i.e., learned aversions to the taste of a food or fluid when consumption of that substance is followed by illness. In order to achieve this general goal, neurobiological and computational analyses of the neural network essentials for CTA are being integrated. The essential neurobiological network for CTA is being identified and characterized and computational models for the CTA neural circuit are being developed.

NEUROBIOLOGICAL RESEARCH

It is necessary to identify four pathways in order to gain a clear understanding of the neural basis of CTAs: the US (illness) pathway, the CS (taste) pathway, the pathway for the elicited response to the CS prior to conditioning (UR_{cs} or unconditioned ingestive response, UIR), and the pathway for the elicited response to the CS after conditioning (CR_{cs} or unconditioned aversive response, UAR). As much work has already been done on the taste and UR_{cs} pathways, we are concentrating on the illness and illness-taste integration pathways in this proposal. In addition, we also are identifying endogenous factors that modulate the acquisition and extinction of conditioned taste aversions.

Illness Pathway

We have completed 2 experiments. These were presented as a single poster at the American Psychological Society meetings in June and a draft of the manuscript submitted to Physiology and Behavior is in Appendix A. Progress also is still underway in

three other projects.

There are two known detection systems for toxins, the gastric-intestinal mucosa and the area postrema. The vagus nerve conveys information from the gastric-intestinal mucosa to the nucleus of the solitary tract (NST), pontine parabrachial nucleus (PBN) and the insular cortex (Cechetto & Saper, 1987; Norgren, 1978; Torvik, 1956). The area postrema detects chemicals in the blood and is thought to convey this information to the NST (Morest, 1967). Beyond this little is known about the illness pathways.

A wide variety of substances can induce CTAs. The detection system that is used to convey information about these substances to the brain varies with the particular chemical and the route of administration. LiCl, a widely used illness-inducing agent, acts by way of the area postrema; copper sulfate acts by way of the vagus nerve when it is administered intraperitoneally; and, apomorphine acts by systems other than the area postrema and the vagus nerve.

One of our aims is to determine the conditions under which endogenous substances and motion act as illness-inducing agents in a CTA and to determine the neural pathways by which these agents and the commonly found toxin, LiCl, produce their effect. This work is of vital importance for understanding motion sickness and the effect of stress on behavior.

Experimental Series 1: Nature of Toxin

We have completed two studies that examine the role of estradiol, an endogenous hormone found in both males and females, in CTAs (see Appendix A). When estradiol levels are elevated, animals exhibit increases in the rate of extinction of a CTA. This is true when estradiol is elevated only during acquisition and only during extinction. We suggest that this effect can be explained in terms of its nonassociative toxic effects.

Experimental Series 2: Neural Pathways

We have initiated three pilot studies which will help us identify the neural pathways for different illness-inducing agents. In the first pilot study, we have worked out the coordinates for locating the area postrema and have completed some practice lesions. We have designed a study to determine whether this neural area mediates the effects of estradiol. In the second pilot study, we have worked out the parameters for 2-DG studies. This procedure will allow us to identify active areas of the brain after administration of illness-inducing agents. We have designed a study in which we will determine the effects of the highest doses of LiCl, copper sulfate and apomorphine that have been used to induce conditioned taste aversions. Since these three drugs are mediated differently we expect to see different areas of the brain activated. These studies will be done in collaboration with Dr. Sally Hazard who

has extensive experience with the 2-DG technique. In the third pilot study, we have begun to work out the parameters for multiunit recording from the PBN and are beginning to work out the parameters for recording from the gustatory cortex during illness. The coordinates for locating the PBN have been identified and the components for recording have been assembled.

Illness-Taste Integration Pathway

We have completed 3 experiments aimed at identifying neural areas mediating illness-taste integration. Progress also is underway in one other project.

Experimental Series 1: Role of the Amygdala

Several brain structures have been implicated in CTAs, but until recently, lesions of the amygdala (AMY), in particular the basolateral AMY, have produced the most consistent findings; they disrupt acquisition and retention of prelesion CTAs (Aggleton et al., 1981; Nachman & Ashe, 1974; Simbayi et al., 1986). But after finding that cutting the connections between the AMY and the temporal cortex produced the same deficits as lesions of the basolateral AMY, Fitzgerald and Burton (1983) suggested that it is the destruction of the fibers of passage that produces the deficits after lesions of the basolateral AMY and not the destruction of the nucleus itself. Recently, Dunn and Everitt (1988) found that neurotoxic (ibotenic)-induced lesions which spare the fibers of passage had no effect on aversion learning whereas electrolytic (ELEC) lesions which destroy both cells and fibers attenuated the aversion. Neither ibotenic or ELEC lesions had a significant effect on extinction. They concluded that it is the axons passing between the brain stem/hypothalamus and GN that are responsible for the deficits in acquisition after ELEC lesions of the AMY. In the following study, we set out to examine the effect of ELEC and neurotoxic (NMDA, N-methyl-D,L-aspartic acid)-induced lesions on acquisition and extinction of a CTA.

Two experiments have been completed. In both experiments, male rats were divided into 4 groups: nonsurgery control (CONT), sham control (SHAM), ELEC lesion (ELEC) and neurotoxic lesion (NMDA). In the first experiment, the lesions were done before acquisition and in the second experiment lesions were done after acquisition. Thus the effects of amygdala lesions on the ability to learn an aversion and the ability to retain a previously learned aversion were determined.

For the first experiment, analyses were made of the amount of sucrose solution consumed the first test day after acquisition and the number of days it took an animal to begin drinking within 1 ml of its acquisition day consumption. The four groups of males did not differ in the amount of sucrose consumed on the day of acquisition, but the ELEC animals drank more sucrose the first test day after acquisition than the NMDA and control males ($F(3,26)=9.4$, $p<0.001$, Waller Duncan T, $p<0.05$). The extinction

rates of the two groups of controls did not differ and those of the two groups of amygdala-lesioned males did not differ but the control groups extinguished more slowly than the lesioned groups ($F(3,26)=2.9$, $p<0.05$, Waller Duncan T, $p<0.05$).

Preliminary review of the data from the second experiment suggests that, as was true in the first experiment, the ELEC males drank more sucrose on the first test day after acquisition than the NMDA and control males. Also, the extinction rates of the two groups of lesioned males appears to be faster than those of the two groups of control males.

These data suggest that the amygdala plays a role in extinction but not acquisition of a CTA.

Experimental Series 2: Role of the Gustatory Cortex

Animals with lesions of the gustatory cortex (GN) exhibit slower acquisition of CTAs (Braun et al., 1972) and no retention of a prelesion CTA (Braun et al., 1981; Kiefer et al., 1984; Yamamoto et al., 1980). The effect of GN lesions on extinction is unclear. We have begun studies to determine the effect of GN lesions on acquisition and extinction of a CTA. One experiment has been completed and another is in progress. In the first experiment the lesion was made before acquisition of a CTA and in the second experiment lesions are being made after acquisition. In both experiments male rats were randomly divided into 2 groups: sham control and lesion (by aspiration). The results from the first experiment indicate that there is no effect of GN lesions on acquisition or extinction of a CTA.

Modulating Factors

We have completed 6 experiments aimed at identifying factors that modulate CTAs. Two of these experiments have been combined in a paper submitted to Behavioral Neuroscience (see Appendix B). One of the experiments has been submitted as an abstract for presentation at the Western Psychological Society meeting and two other experiments have been combined and submitted as an abstract and summary for presentation at the American Psychological Society meeting (see Appendices C and D). Also, 4 of the experiments are being incorporated into two papers.

Experimental Series 1: Hormonal Effects on Conditioned Taste Aversions

Effects of perinatal testosterone on conditioned taste aversion extinction. It has been postulated that gonadal hormones influence behavior by acting during the perinatal period (organizational effects), the postpubertal period (activational effects) or during both periods (organizational-activational effects). During the perinatal period, androgens secreted by the testes direct the development of behavioral characteristics in a masculine direction. The development of behavioral characteristics in a feminine direction proceed when the levels

of gonadal hormones are reduced. After puberty, the appropriate gonadal hormones must be present in order for feminine or masculine behavioral expressions to be present. Organizational-activational effects of the gonadal hormones occur both during the perinatal period and during the postpubertal period.

We have found that the rate of extinction of a CTA in rats is dependent on concurrent levels of testosterone. The extinction rates of males are significantly slower than those of females. Gonadectomy has no effect on the extinction rates of females but increases the extinction rates of males. Testosterone treatment restores extinction rate in gonadectomized males. When testosterone is administered to gonadectomized females, extinction is slowed to the rate found in males. Since females have the capacity to respond to testosterone and since testosterone must be present in order for slow extinction rate to be expressed, testosterone exerts activational effects on extinction. To determine whether the presence of testosterone during the perinatal period alters sensitivity to testosterone, the extinction of a CTA in perinatally androgenized females and control females and males was examined. The amount of testosterone required to produce the slow extinction rate is altered by the presence of testosterone during the perinatal period. Adult gonadectomized rats that had no testosterone present during the perinatal period (normal females) exhibited a fast rate of extinction when given a low dose of testosterone whereas adult gonadectomized rats that had testosterone present during the perinatal period (males and androgenized females) showed a slow extinction rate when given the same low dose. Thus, although the presence of testosterone during the perinatal period is not critical for the expression of a slow extinction rate, it does reduce the amount of testosterone required to produce the slow rate (see Appendix B, submitted for publication).

Effects of gonadectomy on extinction of a conditioned food aversion. Gonadal hormones alter the rates of extinction of conditioned food aversions in rats. Males have slower extinction rates than females. Gonadectomy increases the rates in males but has no effect in females. Testosterone treatment slows extinction in both males and females. We have observed that gonadectomy increases the extinction rates of males to those of females in Sprague-Dawley but not Fischer 344 rats. In studies of reproductive behavior, the decrease in sexual activity after gonadectomy occurs over a long period of time. One possible explanation for the difference in the effects of gonadectomy in Sprague-Dawley and Fischer 344 rats is that gonadectomy may take longer to show an effect in Fischer 344. The following experiment was designed to determine whether the extinction rates of Fischer 344 rats varies with the length of time after gonadectomy. A conditioned food aversion was induced in 20 males and 20 females one week or 5 weeks after gonadectomy. Following the first presentation of a 10% sucrose solution, the conditioned food aversion was induced by injection of LiCl (0.15 M, 10 ml/kg). Daily extinction trials began 2 days later and continued

until criterion for extinction (100% of first day consumption) was reached. The extinction rates of the 5-week males were significantly faster than those of the 1-week males ($p<0.05$). The extinction rates of the two groups of females did not differ. Both groups of males, however, still exhibited slower extinction rates than both groups of females ($p<0.05$). These results suggest that differences in the effects of gonadectomy on extinction rates in Sprague-Dawley and Fischer 344 rats may be accounted for, at least in part, by differences in the length of time the effects of gonadectomy are expressed (see Appendix C, submitted for presentation).

Experimental Series 2: Age-Related Effects on Conditioned Taste Aversions

Age-related changes in sensitivity to estradiol. In this experiment, the effects of estradiol on extinction of a CTA was examined in 20 young (3 months) and 18 old (19 months) females. Young and old females were either untreated or treated with estradiol. Whereas, estradiol increased the extinction rate in young females it had no effect on extinction rate in old females. These results suggest that for CTAs, aging females have a reduced sensitivity to estradiol.

Age-related changes in acquisition and extinction of conditioned taste aversions in males. There is an age-related decrease in the sensitivity of the target tissues mediating sexual behavior to testosterone. The following experiments were designed to determine whether the same was true for target tissues mediating testosterone modulation of CTAs. Some investigators have reported prolonged extinction which is opposite of what one would expect in aged males with decreased testosterone levels. Thus in the first experiment, the extinction rates of 10 young (3 months) and 10 old (18 months) males were examined. The old males had slower extinction rates. In the second experiment, the acquisition rates of 40 young (3 months) and 40 old (18 months) males were examined. The young and old males were given 4 different doses of illness-inducing toxin. Preliminary analyses of the results suggest that old males do not acquire a CTA as readily as young males. Thus the results from the extinction experiment are in the opposite direction of what one would expect in a male with reduced testosterone levels and the results of the acquistion experiment are in the same direction. At present we have no explanation for these seemingly contradictory results (see Appendix D, submitted for presentation)

COMPUTATIONAL RESEARCH

We have completed a paper that lays the groundwork for developing a computational model for CTAs (see Appendix E).

The determination of the neural substrates for CTAs should involve the identification of four pathways: the US pathway, the CS pathway, the pathway for the elicited response to the CS prior

to conditioning (UR_{cs} or unconditioned ingestive response, UIR), and the pathway for the elicited response to the CS after conditioning (CR_{cs} or unconditioned aversive response, UAR). Each taste is connected to both the ingestive and aversive patterns of responses. These connections are probably innate as hedonic reactions to taste have been observed in fetal and neonatal individuals (Pfaffman 1978, Steiner 1973, 1979).

The relative strengths of the two innate connections are dependent on the given taste. In the case of sucrose, the innate connection to the ingestive response is stronger than the innate connection to the aversive response. If exposure to sucrose is followed by illness, the connection to the ingestive response system will weaken and the connection to the aversive response system will strengthen. It is most likely that the illness-induced changes involve two rather than one process. Grill and Berridge (1985) have suggested that palatability processing involves two mechanisms and have provided evidence that the ingestive and aversive response systems can change independently. Thus, in order for the aversive response system to be expressed solely, a weakening of the ingestive response system would have to occur. If exposure to sucrose is not followed by negative consequences, a stronger connection to the ingestive response system will result. A stronger connection to the ingestive response system also will occur if a given taste is associated with positive reinforcement or if it is followed by recuperation from illness (Garcia et al 1977, Revusky 1967, 1974, Young 1966). So, experiential factors can alter the strengths of the innate connections to the ingestive and aversive response patterns. Thus, after a given taste is experienced, the relative strengths of the ingestive and aversive response systems are a function of the original innate connections, the number of exposures to sucrose with illness and the number of exposures to sucrose without illness. This hypothesis is supported by the findings that CTAs to nonpreferred tastes are stronger than to preferred tastes (Etscorn 1973), repeated pairings of a taste with illness strengthens an aversion and repeated pairings of a taste without illness reduces the strength of an aversion (Kalat & Rozin 1973).

There are other factors associated with the CS and US that can influence the strength of an aversion and therefore must be taken into account when developing a neural model for CTAs. The strength of an aversion has been found to be a function of the intensity of the taste as measured by concentration (Dragoin 1971) and the amount consumed on the first exposure (Bond & DiGuisto 1975), the intensity of the US (Revusky 1968) and prior experience with the US (Cannon et al 1975).

There are several factors which can modulate the development and strength of CTAs, but are not essential or critical for aversion learning. The development and strength of an aversion is dependent on the hormonal milieu and deprivation state of the animal. The presence of testosterone (T) increases the proportion of animals that develop a CTA (Chambers et al 1981) and the presence of dexamethasone attenuates the strength of an

aversion (Hennessy et al 1976). Water deprivation reduces the proportion of male rats that develop an aversion (Chambers et al 1981). It is interesting that deprivation can alter the hedonic value of tastes. Foods are reported to be more palatable with deprivation and less so with satiety (Cabanac 1971). Also, the number of ingestive responses decreases and the number of aversive responses increases as meal termination approaches (Grill & Berridge 1985). So, the relative strengths of the ingestive and aversive response systems are also a function of modulating factors. A complete understanding of the neural mechanisms controlling CTAs would include a determination of the neural circuitry for the modulating factors.

A neural model for extinction of a CTA can be outlined in a similar manner as acquisition. Extinction is a process by which connections to the aversive response system are weakened and connections to the ingestive response system are strengthened. Any information on the subsequent consequences of ingesting the CS is processed. If the consequences are neutral, that information serves to alter the relative strengths of the two response systems. Thus, after a CS has been experienced without negative consequences, the relative strengths of the ingestive and aversive response systems are a function of the relative strengths of these systems after the CTA, the number of exposures to the taste without illness, modulating factors, and probably the original innate predisposition.

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PUBLICATIONS

Chambers, K. C. A neural model for conditioned taste aversions; Annual Review of Neuroscience, 13, 373-385, 1990.

MANUSCRIPTS SUBMITTED OR IN PREPARATION

Sengstake, C. B. and Chambers, K. C. Sensitivity of male, female and androgenized female rats to testosterone during extinction of a conditioned taste aversion. Submitted.

Chambers, K. C. and Yuan, D. Temporal analysis of the blockage of testosterone-induced slow extinction of a conditioned taste aversion by estradiol. Submitted.

Yuan, D. and Chambers, K. C. Effects of estradiol on extinction of conditioned taste aversions. In Preparation.

Yuan, D. and Chambers, K. C. Changes in acquisition and extinction of conditioned taste aversions in aging male rats. In Preparation.

PRESENTATIONS AT MEETINGS

Yuan, D. and Chambers, K. C. Temporal analysis of estradiol blockage of testosterone effect on conditioned taste aversions. Poster presented at the first annual meeting of the American Psychological Society, Alexandria, VA, June 1989.

PRESENTATION REQUESTS SUBMITTED

Yuan, D. L., Hung, C. and Chambers, K. C. Effects of gonadectomy on extinction of a conditioned food aversion. Abstract submitted for the Western Psychological Society meeting, Los Angeles, 1990.

Yuan, D. L., Hung, C. and Chambers, K. C. Conditioned food aversion in young and old male rats. Abstract and summary submitted to the American Psychological Association meeting, Boston, 1990.

Appendix A

Temporal Analysis of the Blockage of Testosterone-Induced Slow Extinction of a Conditioned Taste Aversion by Estradiol

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Running Head: Estradiol Blockage of Testosterone

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ABSTRACT

CHAMBERS, K. C. and D. L. YUAN. Temporal analysis of the blockage of testosterone-induced slow extinction of a conditioned taste aversion by estradiol. PHYSIOL BEHAV. Experiments were conducted to determine whether estradiol (E) blocks the testosterone-induced slow extinction of a conditioned taste aversion by acting during acquisition or extinction. In the first experiment, gonadectomized males and females injected with estradiol dipropionate (EP) and testosterone propionate (TP) during extinction only had significantly faster extinction rates than those only injected with TP. The presence of T during acquisition or the post-acquisition/pre-extinction period did not alter the effectiveness of EP during extinction. When E capsules were implanted in gonadectomized males during acquisition, extinction, or both acquisition and extinction in Experiment 2, it was equally effective in blocking T-induced slow extinction. Thus, E does not have to be present concurrently with T during extinction to be effective. This suggests that E does not act on a T-related mechanism but rather acts independently of T.

conditioned taste aversion, estradiol, testosterone, acquisition, extinction

Adult male rats extinguish a conditioned taste aversion more slowly than adult female rats (Chambers & Sengstake, 1976). The ability of males to show this slow extinction is dependent on the presence of testosterone (T). Gonadectomized males show a rapid rate of extinction which is not different than that of females whereas intact males and gonadectomized males administered testosterone propionate (TP) exhibit a slow extinction rate (Chambers, 1976, 1980; Earley & Leonard, 1978). Gonadectomy has no effect on the extinction rate of females. However, gonadectomized females will exhibit a slow extinction rate if they are administered TP. The effectiveness of testosterone in decreasing the rate of extinction to the same level as that of intact males or TP-treated gonadectomized males is influenced by the presence of the ovaries or of estradiol (Chambers, 1976, 1980; Earley & Leonard, 1979). Intact females treated with TP and gonadectomized females administered estradiol dipropionate (EP) and TP show faster extinction rates than gonadectomized females only given TP. Estradiol also blocks the effect of T in males.

Estradiol could block the T-induced slow extinction by acting directly on a T related mechanism or by acting independently of T. Testosterone prolongs extinction only if it is present during the extinction phase of the conditioned taste aversion (Chambers & Sengstake, 1979). When female and male rats are administered TP during the acquisition phase of the conditioned taste aversion but not during the extinction phase, they show a rapid rate of extinction. However, when females and males are administered TP during extinction and either TP or vehicle during acquisition, they show a slow extinction rate. In order to understand the mechanism by which E blocks the effects of T, it is necessary to determine when E is acting. Since in the previous study, E was administered with T during both acquisition and extinction, it is not known whether E is blocking the effects of T during acquisition or extinction. The following experiments were conducted to determine when during the conditioned taste aversion process E acts to block the T-induced slow extinction rate.

GENERAL METHODS

Subjects

The subjects were adult female and male Sprague-Dawley derived rats obtained from Simonson Laboratories. The rats were group housed from weaning until the start of the experiment. They then were housed one per cage in Experiment 1 and two per cage in Experiment 2. When housed two per cage, they were separated by a wood divider during conditioned taste aversion testing. The total time of separtion was approximately 90 min. They were kept on ad lib food and a 12:12 hr light/dark cycle throughout the experiment. With the exception of the testing period, water was always available.

Surgeries and Hormonal Manipulations

In Experiment 1, gonadectomies were preformed while the rats were under sodium pentobarbital anesthesia (36 mg/kg of body weight for females and 48 mg/kg of body weight for males). In Experiment 2, gonadectomies and silastic capsule implantations were performed while the rats were under ether (Part A) or halothane (Part B) anesthesia. The hormones were administered either by injection or implantation. For injection, TP (1 mg/rat) and EP (150 ug/rat) were dissolved in 0.05 ml of sesame oil and injected sc at the nape of the neck. For implantation, silastic capsules (0.157-cm i.d. and 0.318-cm o.d.) were either unfilled or were filled with hormone and each end was sealed with silicone type A Silastic adhesive. Testosterone was placed in a 30 mm capsule and E was placed in a 5 or 10 mm capsule.

Conditioned Taste Aversion Procedure

The experimental procedure was divided into three periods: preconditioning, acquisition, and extinction. During all of these periods, the animals were weighed daily just prior to the end of the light portion of the light/dark cycle. All solutions were stored under refrigeration for 24 hr before they were given to the animals and were introduced at the beginning of the dark portion of the cycle. The animals were given access to the solutions for 1 hr (Experiment 2) or 2 hr (Experiment 1). During the preconditioning period, each animal was given cold tap water. Animals housed two per cage also were adapted to the 90 min separation period during this time. The acquisition period started immediately after termination of the preconditioning period. On acquisition day all animals were given a cylinder of 10% (w/v; Experiment 1) or 9% (w/v; Experiment 2) sucrose solution. At the end of this period, the amount of solution consumed was recorded for each animal and the animals were irjected with a 0.30 M LiCl solution (20 ml/kg). The extinction period began at least two days after acquisition. Chilled tap water was given during the interval between acquisition and extinction. During the extinction tests the sucrose solution was given but no LiCl injections were given. Animals were tested until they regained the consumption level of acquisition day or until a specified number of tests were given.

Bleeding and Hormone Assays

Blood was collected by tail vein under vacuum (no anesthesia; Nerenberg & Zedler, 1975). The total blood collection time from each rat was approximately 2 min. The samples were allowed to clot at 4*C for at least 4 hr. They then were centrifuged (5000 rpm/4000 g for 20 min at 4*C) and serum was removed and stored in 0.5-ml portions at -20*C until assayed for E and T. The steroids were separated from the serum through extraction and chromatographic purification of LH-20 Sephadex columns and quantified by radioimmmunoassay. For Experiment 1, the mean percentages of recovery, water blanks, and intraassay coefficients of variation were as follows: 69.8%, 5.4 pg, and 5.7%, respectively, for E and 86.5%, 1.3 pg, and 3.0%,

respectively, for T. For Experiment 2, these values were as follows: 71.6%, 3.9 pg, and 12%, respectively, for E and 65.1%, 2.8 pg, and 9.6%, respectively, for T. Quantities calculated from standard curves were corrected for procedural losses and blank values.

Statistical Analyses

The number of days to extinction was computed for each animal. For those animal that did not reach criterion, one plus the maximum number of extinction tests given was used as the extinction score. For independent groups designs, the hormonal values and extinction scores were analyzed with a two-factor or one-factor analysis of variance (ANOVA). The Waller Duncan K-ratio T test was used to determine differences among treatments. For the repeated measures design, a two factor ANOVA with repeated measures on one factor was used. Single factor ANOVAs with repeated measures on one factor were used to determine whether each sex differed across time, t tests were used to determine sex differences at each time, and ANOVAs of contrast variables were used to determine which times differed.

EXPERIMENT 1

In this experiment, E was given only during extinction to determine whether it could block the effects of T during the extinction process. Further, the influence of the presence of T prior to extinction on the effectiveness of E in blocking the T-induced slow extinction was evaluated.

Methods

Part A. Twenty four females and 24 males (77-168 days old) were assigned in equal numbers to one of four groups: (1) oil during acquisition and extinction (O/O); (2) TP during acquisition and extinction (T/T); (3) EP and TP during acquisition and extinction (ET/ET); or (4) oil during acquisition and EP and TP during extinction (O/ET). All rats were gonadectomized 3 weeks before the experiment began. Injections of vehicle and hormone were given daily throughout the experiment. The injections and preconditioning period were initiated 7 days before acquisition. Twenty days after acquisition, the injections for the extinction period were initiated. Seven days later, extinction tests began and were continued until criterion was reached or until 42 tests were given.

An additional 6 females and 6 males were bled two weeks after gonadectomy. Injections of EP and TP were initiated one week after bleeding and were continued for 9 days. The rats were bled again 1 and 33 days after the last injection.

Part B. Thirty six males (66-143 days old) were assigned in equal numbers to one of six groups: (1) oil during acquisition and extinction and no treatment during the post-acquisition/pre-

extinction period (O/N/O); (2) TP during acquisition and extinction and no treatment during the post-acquisition/pre-extinction period (T/N/T); (3) EP and TP during acquisition and extinction and no treatment during the post-acquisition/pre-extinction period (ET/N/ET); (4) TP during acquisition, no treatment during the post-acquisition/pre-extinction period, and EP and TP during extinction (T/N/ET); (5) oil during acquisition, TP during the post-acquisition/pre-extinction period, and EP and TP during extinction (O/T/ET); (6) oil during acquisition, no treatment during the post-acquisition/pre-extinction period, and EP and TP during extinction (O/N/ET). All rats were gonadectomized 2-6 weeks before the start of the experiment. The preconditioning period and the injections for the acquisition period were initiated 7 days before acquisition. The injections continued daily until two days after acquisition. On the third day after acquisition and continuing for 6 days, the injections for the post-acquisition/pre-extinction period were given. On the day following the end of this period, injections for the extinction period were initiated and were continued until the end of the experiment. Extinction tests began 7 days after the injections were initiated. The tests were given daily until criterion was reached or until 42 tests were given.

Results

Part A. One female injected with EP and TP during acquisition and extinction died before the experiment was completed. The 8 groups of rats did not differ significantly in the amount of sucrose consumed on the day of acquisition. The extinction rates of the males and females did not differ ($F(1,45)=0.27$, $p>.05$) and the sex by hormone treatment interaction was not significant ($F(3,135)=2.07$, $p>.05$). There was a significant difference across treatments ($F(3,135)=36.35$, $p<.0001$; Figure 1). The T/T males and females had significantly slower extinction rates than the other 3 groups and the O/ET males and females had slower rates than the O/O or ET/ET males and females (Waller Duncan K-ratio T test, $p<.05$). The extinction rates of the O/O and ET/ET males and females did not differ significantly.

The E levels of the males and females did not differ ($F(1,10)=1.35$ and $F(2,20)=1.58$, $p>.05$). Mean (+SE) estradiol levels for males and females rose from 27.7 (+4.3) pg/ml to 2425.0 (+69.4) pg/ml after daily injections of EP and TP ($F(1,10)=1281.73$, $p<.0001$). Estradiol levels were still elevated 33 days after injections were terminated (mean (+SE) = 94.33 (+11.58) pg/ml; $F(1,10)=33.96$, $p<.001$). The T levels of the males and females differed significantly ($F(1,10)=7.31$, $p=.02$ and $F(2,20)=9.46$, $p=.001$). For both males and females, T levels rose significantly after daily injections of EP and TP (from means (+SE) of 0.13 (+0.04) and 0.16 (+0.06) ng/ml, respectively, to 25.50 (+3.13) and 35.95 (+1.66) ng/ml, respectively; $F(1,5)=66.82$ and 468.33, respectively, $p<.001$) and T levels were still elevated 33 days after injections were terminated (0.88 (+0.15) and 0.31 (+0.04) ng/ml, respectively; $F(1,5)=31.94$ and

7.65, respectively, $p<.05$). However, the T levels of the females were significantly higher than those of the males 1 day after termination of the injections ($t(10)=2.95$, $p<.02$) but were significantly lower 33 days after injections were terminated ($t(10)=3.68$, $p<.01$).

Part B. The 6 groups of males did not differ significantly in the amount of sucrose consumed on the day of acquisition. They did differ, however, in the rates of extinction ($F(5,30)=7.94$, $p<.0001$; Figure 2). The T/N/T males had slower extinction rates than the other 5 groups of males. The extinction rates of the T/N/ET, O/T/ET. and O/N/ET males did not differ. The extinction rates of each of these males was significantly slower than those of the ET/N/ET males but not the O/N/O males (Waller Duncan K-ratio T test, $p<.05$).

EXPERIMENT 2

The results of Experiment 1 clearly indicate that the presence of E during extinction can partially block the T-induced slow extinction. The presence of E during both acquisition and extinction, however, was more effective than its presence only during extinction. This suggests that E may also act during acquisition. Using EP to test this suggestion, however, is problematic since E in this form is long acting. Estradiol levels were still elevated 33 days after termination of EP injections. Legan, Coon and Karsch (1975) have found that when E is administered by implanting silastic capsules filled with E, serum E levels return to baseline levels within two hours after removal of the capsules. This suggests that administration of E by capsule is a viable means by which to assess the role of E in acquisition. Thus, in this experiment, E was administered by capsule during acquisition or during extinction to determine whether it would block the T-induced slow extinction.

Methods

Part A. Thirty two males (180 days old) were randomly assigned in equal numbers to one of four groups: (1) 10 mm and 30 mm blank capsules (0); (2) 30 mm T capsules (T); (3) 5 mm E and 30 mm T capsules (5ET); (4) 10 mm E and 30 mm T capsules (10ET). The preconditioning period was initiated three days after all rats were gonadectomized and implanted. Ten days after surgery, acquisition was induced. Two days later, extinction tests began. Tests were given until criterion was reached or until 45 tests had been given.

Part B. Fifty males (100 days old) were randomly assigned in equal numbers to one of five groups: (1) blank capsules in both acquisition and extinction (0/0); (2) T capsules in both acquisition and extinction (T/T); (3) E and T capsules in both acquisition and extinction (ET/ET); (4) T capsules in acquisition and E and T capsules in extinction (T/ET); (5) E and T capsules in acquisition and T capsules in extinction (ET/T). The blank capsules were 10 mm and 30 mm long, the T capsules were

30 mm long, and the E capsules were 10 mm long. The preconditioning period was initiated three days after all rats were gonadectomized and implanted. Nine days after surgery, acquisition was induced. Two days later, the capsules for acquisition were replaced with the capsules for extinction. All capsules were replaced in each group. Extinction tests began 9 days after capsule replacement. Tests were given until criterion was reached or until 30 tests were given. Blood samples were taken from all rats 2 days after the 30th test.

Results

Part A. The data from 7 rats (2 from blank group, 2 from T group, and 3 from 10 mm E group) were eliminated because these rats drank less than 2 ml of sucrose solution on the day of acquisition. The four groups of males did not differ significantly in the amount of sucrose consumed on the day of acquisition. They did differ, however, in extinction scores ($F(3,21)=3.81$, $p=0.025$). The T and 5ET groups did not differ and the 0 and 10ET groups did not differ. The extinction rates of the T and 5ET groups were significantly slower than those of the other two groups (Waller-Duncan K-ratio T test, $p<0.05$).

Part B. The data from 1 rat (ET/ET) was eliminated because of an incorrect capsule placement. The five groups of males did not differ significantly in the amount of sucrose solution consumed on the day of acquisition. They did differ, however, in rates of extinction ($F(4,44)=5.25$, $p=0.0015$; Figure 3). The 0/0, ET/ET, ET/T, and T/ET groups did not differ. These four groups extinguished faster than the T/T group (Waller-Duncan K-ratio T test, $p<0.05$).

The E data from 1 rat (0/0) was lost. The five groups of males differed significantly in the levels of E and T ($F(4,41)=7.85$ and $F(4,42)=119.38$, respectively, $p<0.001$). The E levels of the ET/ET and T/ET groups did not differ and those of the 0/0, T/T, and ET/T groups did not differ. Those males with E capsules during extinction had significantly higher E levels than those without E capsules (means (+SE) = 108.3 (+12.9) and 44.5 (+4.4) pg/ml, respectively; Waller-Duncan K-ratio T test, $p<0.05$). The T levels of the four groups of males that had T capsules during extinction did not differ but were higher than those of the males with blank capsules (means (+SE) = 3.31 (+0.07) and 0.16 (+0.04) ng/ml, respectively; Waller-Duncan K-ratio T test, $p<0.05$).

GENERAL DISCUSSION

Estradiol was effective in blocking the T-induced slow extinction of a conditioned taste aversion when administered during acquisition, extinction or both acquisition and extinction. In Experiment 2, E was equally effective when administered during each of these time periods. However, in Experiment 1, E was more effective when given during both acquisition and extinction than when given only during

extinction. The reason for this discrepancy is unclear although it may be associated with the differences in the doses of E or T used in the two experiments. The blood levels of E were 22 times higher in Experiment 1 than Experiment 2 and the levels of T were 9 times higher. Earley and Leonard (1979) have suggested that the ratio of estrogen to androgen rather than either hormone alone is the crucial factor in determining the rate of extinction. But the differences in the results of Experiments 1 and 2 cannot be accounted for by differences in the ratios of E to T since the ratio was higher in Experiment 1 than in Experiment 2.

Testosterone is not effective in prolonging extinction in intact females (Chambers, 1976). Thus E levels that are within the physiological range can effectively block T-induced slow extinction. In intact females, the mean (+SE) levels of E range from 28.0 (+1.4) pg/ml to 81.0 (+5.4) pg/ml during the estrous cycle (Huang, Steger, Bruni & Meites, 1978). Fifty three percent of the ET-treated males in Experiment 2 had E levels that were the same or lower than the mean level found in intact females. This suggests that, at least for some males, E levels that are within the physiological range for intact females can be effective in blocking the effects of T.

Testosterone is effective in slowing extinction of a conditioned taste aversion when it is present during extinction but not when it is present during acquisition. The results of these experiments clearly show that E does not have to be present concurrently with T during extinction to be effective since E can block the T-induced slow extinction when it is administered during acquisition or during extinction. This suggests that E does not act on a T-related mechanism but rather acts independently of T.

A number of different hypotheses have been proposed to account for the effects of T on extinction of a conditioned taste aversion, eg., decreases relearning, retards forgetting, increases persistence behavior (Chambers, 1985; Chambers & Sengstake, 1979; Earley & Leonard, 1978). Although E may also affect these processes, it is more likely that it affects processes that are common to both acquisition and extinction. It has been reported recently that E can be used as a toxin to induce a conditioned taste aversion in male and female rodents (Gustavson, Gustavson, Young, Pumariega & Nicolaus, 1989; Miele, Rosellini & Svare, 1988). It is possible that the effect of E on conditioned taste aversions can be explained in terms of its toxic effect. A number of investigators have shown that preexposure to a toxin can intefere with the subsequent learning of an aversion (Cannon, Baker & Berman, 1977; Domjan, 1978; Holman, 1976; Mikulka, Leard & Klein, 1977; Riley, Dacanay & Mastropaolo, 1984; Switzman, Fishman & Amit, 1981). Also, exposure to a toxin after acquisition but before extinction can attenuate the conditioned response and increase the rate of extinction (Holman, 1976; Mikulka, Leard & Klein, 1977; The toxin used prior to acquisition or extinction can be the same or

different from the toxin used to induce the aversion. In our studies, E was injected or implanted several days before acquisition or extinction. If the effect of E is mainly a toxic one, the increased rate of extinction that we found is consistent with the results described in the studies above. On would further expect that E would increase extinction rate regardless of the presence of T. Although we have not found significant increases in extinction in gonadectomized males treated with E alone, there were tendencies for these males to extinguish faster (Chambers, 1980). Also, Earley and Leonard (1979) have found that when gonadectomized males were exposed to sucrose 1 day before conditioning, the E-treated males extinguished faster than vehicle-treated males. We suggest, then, that E acts on the part of the neural substrates for conditioned taste aversions that mediates toxins.

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FIGURE CAPTIONS

Figure 1. Mean (+SE) days to extinguish a conditioned taste aversion in gonadectomized female and male rats given oil (O), testosterone propionate (T), or estradiol dipropionate (E) plus T (ET). Animals were given these treatments during acquisition (before slash) or during extinction (after slash). *Significantly different than the T/T females and males, $p<.05$. **Significantly different than the O/ET females and males, $p<.05$.

Figure 2. Mean (+SE) days to extinguish a conditioned taste aversion in gonadectomized male rats given oil (O), testosterone propionate (T), estradiol dipropionate (E) plus T (ET), or no treatment (N). Animals were given these treatments during acquisition (before first slash), during the post-acquisition/pre-extinction period (after first slash and before second slash), and during extinction (after both slashes). *Significantly different than T/N/T, $p<.05$. **Significantly different than T/N/ET, O/T/ET, and O/N/ET, $p<.05$.

Figure 3. Mean (+SE) days to extinguish a conditioned taste aversion in gonadectomized male rats implanted with blank (0), testosterone-filled (T), or estradiol-filled (E) and T-filled (ET) capsules. Animals were given these treatments during acquisition (before slash) or during extinction (after slash). *Significantly different than T/T, $p<.05$.

Fig 1

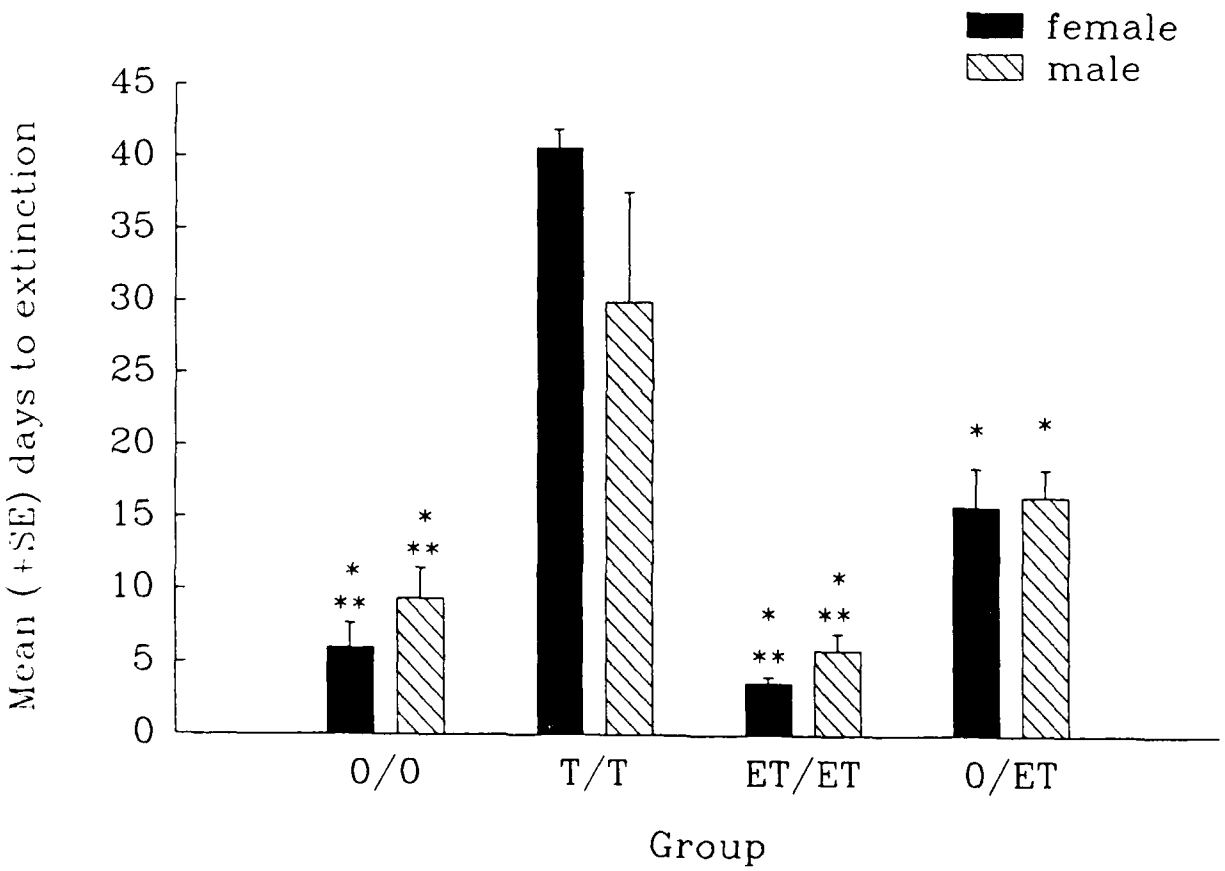
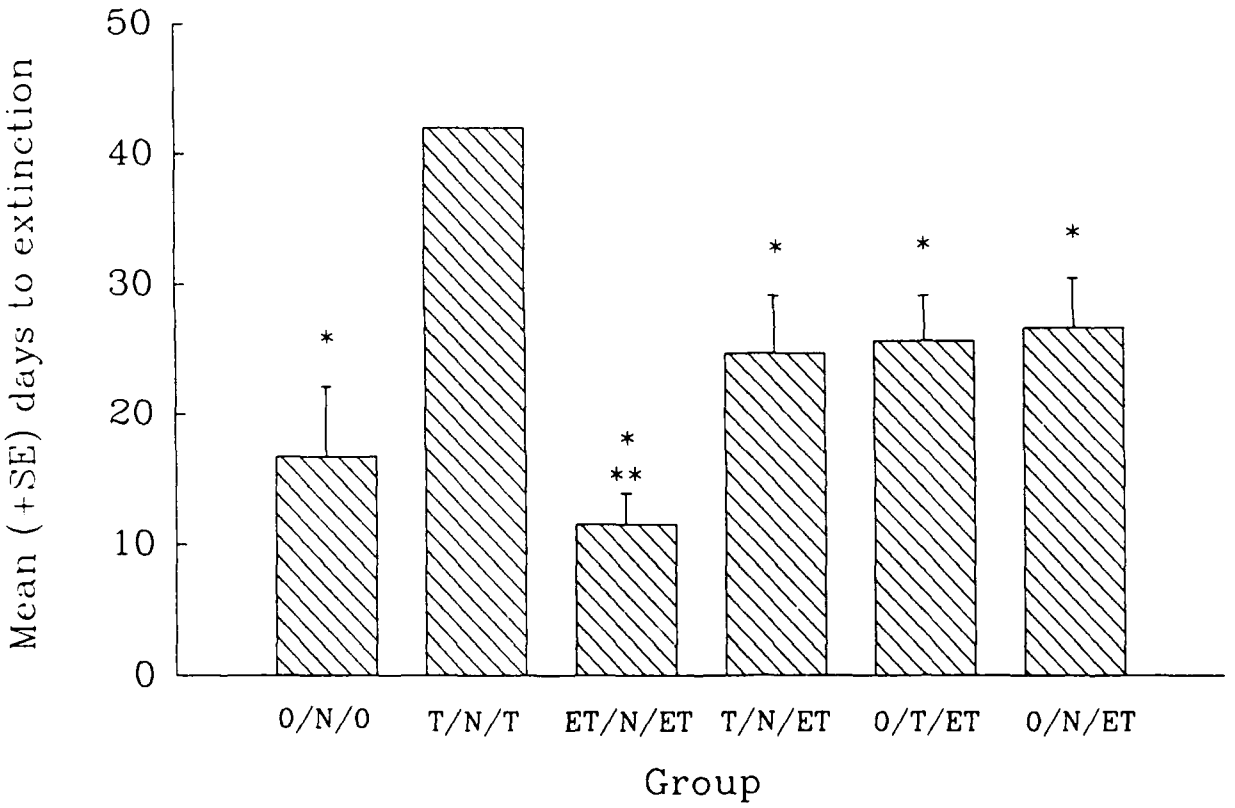
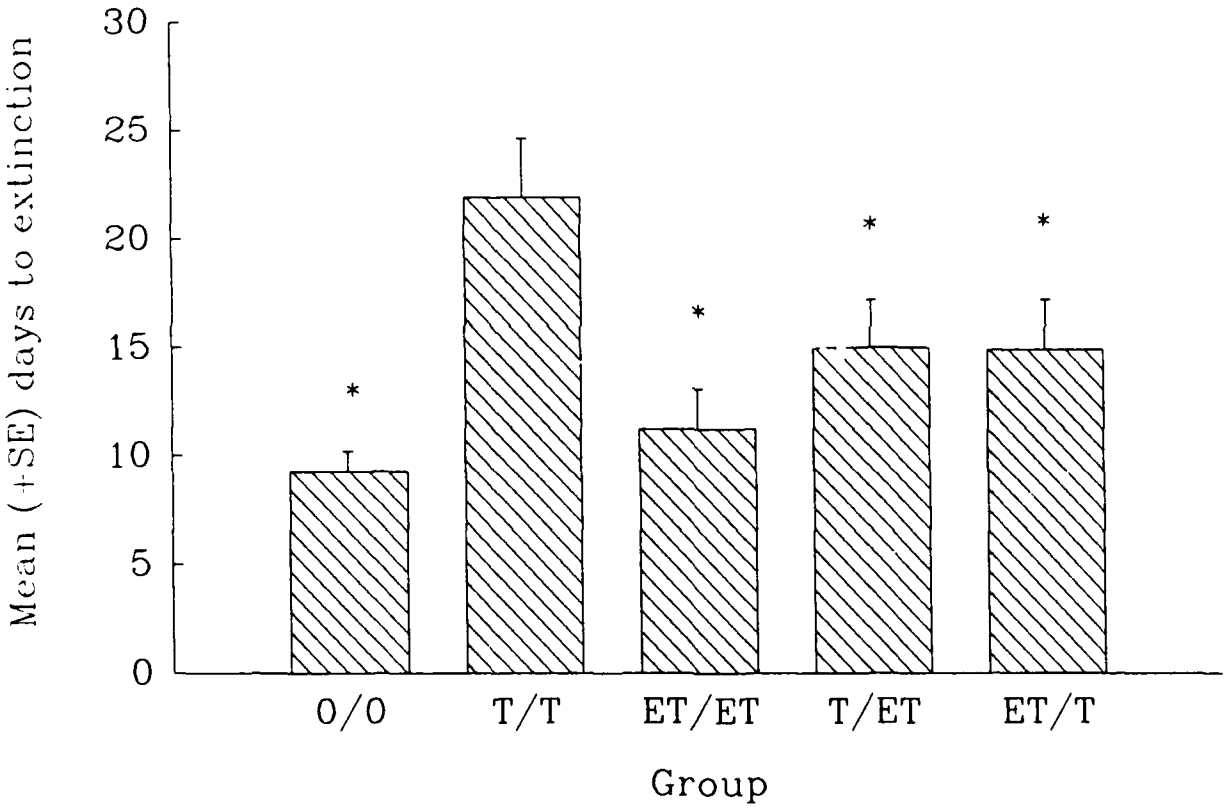

female
male
Mean (+SE) days to extinction
45
40
35
30
25
20
15
10
5
0
0/0
T/T
ET/ET
0/ET
Group

Fig 2


Mean (+SE) days to extinction
50
40
30
20
10
0
*
*
**
*
*
*
0/N/0
T/N/T
ET/N/ET
T/N/ET
0/T/ET
0/N/ET
Group


Mean (+SE) days to extinction
30
25
20
15
10
5
0
*
*
*
*
0/0
T/T
ET/ET
T/ET
ET/T
Group

Appendix B

Sensitivity of Male, Female, and Androgenized Female Rats to Testosterone during Extinction of a Conditioned Taste Aversion

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Running Head: Testosterone Sensitivity

Abstract

The hypothesis that males and females differ in the amount of testosterone required to prolong extinction of a conditioned taste aversion was tested. Gonadectomized males and females were implanted with empty capsules or 30, 60, or 120 mm long testosterone-filled capsules. Blood samples were taken before conditioning and after extinction. Both males and females exhibited slow extinction rates when given a 120 mm capsule but when both were implanted with either a 30 mm or a 60 mm capsule, only males showed the slow extinction rate. The dimorphic sensitivity could not be attributed to differences in plasma testosterone levels since the levels for males and females with either 30 mm or 60 mm capsules were not different. In experiment 2 the hypothesis that the presence of testosterone in the male during the perinatal period results in a greater sensitivity to testosterone in adulthood was tested. Females exposed to testosterone propionate during the perinatal period showed prolonged extinction when given a 30 mm testosterone-filled capsule as an adult whereas unexposed females did not. These results support the hypothesis that the amount of testosterone required to activate the prolonged extinction is dependent on the perinatal presence of testosterone.

Sensitivity of Male, Female, and Androgenized Female Rats to Testosterone during Extinction of a Conditioned Taste Aversion

The influences of gonadal hormones on both reproductive and nonreproductive sexually dimorphic behaviors have been placed within the framework of organizational (perinatal) and activational (adult) effects (Goy & Goldfoot, 1973). Under this formulation, the various dimorphic behaviors were classified according to the relative importance of the two stages of hormonal action. Many dimorphic behaviors were seen as clearly dependent on both organizational effects and activational effects. In this case, adult gonadectomy would be expected to block the expression of the behavior and adult treatment of the opposite sex with the appropriate hormone should result in a diminished response at best. For example, adult castration of male mice results in a reduction in same sex aggression and exogenous testosterone (T) treatment results in a return to pre-castration levels. Injections of T in adult female mice, however, do not result in levels of aggression equal to those of normal males (Tollman & King, 1956; Simon & Whalen, 1987).

Other behaviors were seen as primarily dependent on the hormonal levels during a sensitive part of the perinatal period, i.e., organizational effects. In this case, the presence or absence of gonadal hormones would be expected to

determine whether the organism develops the neural system required for that behavior to be exhibited as an adult. Gonadectomy or exogenous hormone administration in the adult animal should have only minimal effects on the behavior. An example of an organizationally determined behavior is open-field behavior in the rat (Bengelloun, Nelson, Zent, & Beatty, 1976). Females are more active than males in open field tests. Adult gonadectomies in male and female rats have little effect on this behavior but neonatal castration of the male results in female levels of activity when tested as an adult; injections of testosterone propionate (TP) in the neonatal female results in male levels as an adult.

Activationally determined behaviors were thought to be dependent upon the presence of the appropriate gonadal hormone(s) in the adult regardless of the hormonal conditions during the perinatal period. Adult gonadectomy would result in a decrease in the behavior while injections of the appropriate hormone would result in the full expression of the behavior in both sexes. An example of an activationally determined behavior is the rate of extinction of a conditioned taste aversion in rats (Chambers, 1976). Castration of the adult male results in female-like behavior (i.e., a rapid rate of extinction); exogenous administration of TP in the adult rat of either gender results in male-like behavior (i.e., a slow rate of extinction).

A major difference, then, between behaviors that are activational and behaviors that are both activational and organizational is the relative sensitivity of the target tissues in the opposite sex to the activating hormone. In the case of behaviors that are both activational and organizational, the sensitivity of the adult to the hormone is clearly dimorphic; the issue is less clear for behaviors that are activationally determined.

In our studies of the extinction of a conditioned taste aversion (Sengstake, Chambers, & Thrower, 1978; Chambers & Sengstake, 1979), for example, we have not observed a sex difference in the responsiveness to T; the males and the females given daily injections of TP have not differed in their rate of extinction. However, only relatively high doses of TP were used; 1 mg per rat per day. Differences in the responsiveness of males and females to T may well have been obscured by these high doses. Thus, the following studies were designed to determine whether males and females differ in their sensitivity to T and whether perinatal hormones play a role in conditioned taste aversions.

General Method

Husbandry

The subjects were Sprague-Dawley-derived rats. After weaning, they were housed in same-sex colony cages until one week before conditioning. During this period and continuing

throughout the experiment, they were maintained on ad libitum food and water and were housed in a temperature-controlled (22^{o}-23^{o}C) vivarium with a 12h:12h light/dark cycle (lights on at 0230).

Implants

T-filled implants were made by a method derived from Legan, Coon, and Karsch (1975). Silastic tubing (0.062 in. ID, 0.125 in. OD) was cut into lengths 3 mm longer than the desired final length. Crystalline T was pushed into each tube with a 17-gauge stainless steel rod. Because the rod fit snugly into the tube, the tube was filled with no air spaces. When the appropriate length of the column had been obtained, the ends were sealed with a small amount of type A Silastic adhesive, and allowed to cure overnight. Prior to surgical placement, the tubes were incubated for 72 hr at 37^{o} C in 0.01 M phosphate-buffered (7.4 pH) normal saline; the buffered saline solution was replaced twice a day during this incubation.

Conditioned Taste Aversion

The conditioned taste aversion was induced as follows. At the beginning of the dark phase of the light/dark cycle, the water bottle of each rat was replaced with a chilled sucrose solution (10% sucrose in water, wt/vol) in a graduated cylinder for 2 hr. The amount consumed was recorded, the sucrose solution was replaced with the regular

water bottle, and the animal received an injection of a 0.3 M LiCl solution (20 ml/kg of body weight, intraperitoneally). Two days after the acquisition of the aversion, daily extinction trials began. The extinction trials were conducted in the same manner as the acquisition session except that no LiCl was injected.

Radioimmunoassay

T was separated from the plasma (Experiment 1) or serum (Experiment 2) through extraction and chromatographic purification on LH-20 Sephadex columns and quantified by radioimmunoassay (Resko, Malley, Begley, & Hess, 1973; Resko, Ellinwood, Pasztor, & Buhl, 1980). Percentage of recovery, water blank values, and intraassay and interassay coefficients of variations were determined and quantities calculated from standard curves were corrected for procedure losses and blank values.

Experiment 1

This experiment was designed to determine whether males and females differ in the amount of T required to prolong extinction of a conditioned taste aversion and to determine the circulating T levels required in both males and females for a slow extinction.

Method

Part A: The subjects were 30 male and 30 female rats. They were randomly assigned to one of five conditions (six

males (M) and six females (F) per condition): gonadectomized with one 30 mm (30), one 60 mm (60), or two 60 mm (120) T-filled Silastic capsule implants; gonadectomized with one 30 mm empty capsule implant (0); or sham-gonadectomized with one 30 mm empty capsule implant (S). When the rats were 90-120 days old, they were gonadectomized or underwent sham operations with sodium pentobarbital anesthesia (36-48 mg/kg of body weight, intraperitoneally). Fourteen days later each animal received the subcutaneous Silastic implant under ether anesthesia.

Two weeks after implantation, each rat was moved to an individual cage that was to serve both as the testing and the home cage for the duration of the experiment. Beginning the next day and continuing daily throughout the experiment, each rat was weighed at the end of the light portion of the light:dark cycle. Also for the first seven days the water bottle of each animal was replaced with chilled tap water in a graduated cylinder for the first 2 hr of the dark phase. After 1 week of this preconditioning, the conditioned taste aversion was induced. Extinction continued either until the rat regained the consumption level shown on the acquisition day or until there had been 41 extinction trials (which ever occurred first). The extinction score for each animal was the number of trials to extinction, or 42 if no extinction occurred.

Part B: The subjects were 30 male (M) and 30 female (F) rats. They were randomly assigned to one of 5 conditions (6 males and 6 females per condition): gonadectomized with one 30 mm (30), one 60 mm (60), or two 60 mm (120) T-filled capsule implants; gonadectomized with one 30 mm empty capsule implant (0); or sham-gonadectomized with 30 mm empty implant (S).

The procedure was identical to that of Part A with the following exceptions. Blood samples were taken from each animal 1 and 2 weeks after the implantation. The day after the second sampling, each rat was moved to an individual cage. Two additional blood samples were taken from each animal at the end of the extinction trials, 73 and 80 days after the initial implantation. The blood samples were taken from the tail veins of etherized animals with heparinized syringes and were stored on ice for no more than 30 min before being centrifuged (2,000 rpm/700 x g for 20 min at 4° C). The plasma was stored at -18° C until assayed for T. The mean (±SE) percentage of recovery and blank value, and the intraassay and interassay coefficients of variation were: 73.69(±2.40)%, 9.90(±4.64) pg/ml, 12.61% and 6.77% respectively.

Results

The data on two (one 60F and one 0F) animals that died during the experiment in Part B were excluded from all

calculations and analyses. The mean levels of T in blood samples 1 and 2 (preconditioning trials) and the mean level in samples 3 and 4 (postextinction trials) were computed for each animal. Both the days to extinction and the mean levels of T data were analyzed using a one factor ANOVA followed by selected linear contrasts.

For both Part A and B, the extinction rates of the 10 groups differed (Part A: $F(9,50) = 6.37$, $p < .001$; Part B: $F(9,48) = 3.79$, $p = .001$; see Figure 1). Neither the extinction rates of the 0M and the 0F (Part A: $F(1,50) = 0.01$; Part B: $F(1,48) = 0.76$), nor the 120M and 120F (Part A: $F(1,50) = 0.31$; Part B: $F(1,48) = 3.09$) differed but the extinction rates of the SM and SF (Part A: $F(1,50) = 8.27$, $p = .006$; Part B: $F(1,48) = 4.39$, $p = .04$) and the extinction rates of the 30M and the 30F (Part A: $F(1,50) = 7.18$, $p = .01$; Part B: $F(1,48) = 5.42$, $p = .02$) were different. The extinction rates of the 60M and the 60F differed in Part A ($F(1,50) = 8.59$, $p = .005$) but not in Part B ($F(1,48) = 0.90$). When the mean days to extinction were calculated for the combined data from Part A and Part B for the 60M and the 60F, however, the two genders were different ($F(1,108) = 6.33$, $p = .013$).

Insert Figure 1 about here.

The T levels of the 10 groups differed both before conditioning and after extinction ($F(9,48) = 24.29$ and 22.31, respectively, with $p < .001$ in both cases; see Figure 2). The SM had higher T levels than the SF both before and after conditioning ($F(1,48) = 13.14$, $p = .001$, and 4.47, $p = .04$, respectively. The T levels did not differ significantly between the sexes either before conditioning or after extinction in the following groups: the 0M and 0F ($F(1,48) = 0.64$ and 0.02 respectively), the 30M and 30F ($F(1,48) = 0.08$ and 0.61 respectively) or the 60M and 60F ($F(1,48) = 0.002$ and 0.96 respectively). Although the T levels of the 120M and 120F did not differ before conditioning ($F(1,48) = 1.57$), they were significantly higher in the 120F than in the 120M after extinction ($F(1,48) = 19.47$, $p < .001$).

Insert Figure 2 about here.

Experiment 2

In experiment 1, when males and females were given either 30 or 60 mm of T, the extinction rate of the males was slower than that of the females. This sex difference cannot be accounted for by different levels of circulating T since neither the males and females given 30 mm of T nor the males and females given 60 mm of T differed significantly in plasma T levels.

Although several other hypotheses can be offered to account for this dimorphic sensitivity to T, a very likely explanation is the presence of T in males during differentiation of the central nervous system. In support of this hypothesis is the study by Babine and Smotherman (1984) in which they found that female rats presumably exposed to T prenatally from neighboring male siblings show a prolonged extinction as adults when given low-dose injections of T, whereas females without neighboring male siblings (and less prenatal T) do not. The following experiment was designed to determine if sensitivity to T, as a factor in determining extinction rate, is established during the perinatal period. Under this hypothesis we predicted that the amount of T required to prolong extinction is less in females that have been exposed to exogenous T during the perinatal period compared to females not so exposed.

Method

Four females were injected daily with 2 mg of TP (dissolved in 0.1 ml of sesame oil) and another four were injected daily with 0.1 ml of the oil on days 16 through 20 of pregnancy. Twenty-four and 72 hr after delivery, the pups of the TP-injected females were injected with 1 mg of TP (dissolved in 0.02 ml of sesame oil) and the pups of the oil-injected females were injected with 0.02 ml of the oil. The pups were weaned when 22 days old.

When the offspring were 80 days old, all of the TP-treated females (n = 10) were selected and 16 oil-treated females and 8 oil-treated males were randomly selected to be subjects in this experiment. All of the subjects were gonadectomized and implanted with silastic capsules while under ether anesthesia. All of the males (OTM) and half of the TP-treated (TTF) and oil-treated (OTF) females were implanted with 30 mm T-filled capsules. The other half of the TP-treated (TOF) and oil-treated (OOF) females were implanted with empty 30 mm capsules. One week later, the rats were placed in single cages and 1 week after that the conditioned taste aversion was induced. Extinction continued either until the rat regained the consumption level shown on the acquisition day or until 29 extinction trials had been given (which ever occurred first). The extinction score for each animal was the number of trials to extinction, or 30 if no extinction occurred.

Two days after the last extinction trial, all of the animals were bled. Blood samples were taken from a tail vein under vacuum by the method of Nerenberg and Zedler (1975). The animals were unanesthetized. The blood was allowed to clot at 4° C. It was centrifuged at 5000 rpm (4000 x g) at 4° C for 30 min. The serum was removed and stored in 1 ml aliquots at -20° C until assayed for T.

Results

The extinction and T data were analyzed using a one factor ANOVA followed by selected linear contrasts. The five groups did not all have the same extinction rate ($F(4,29) = 9.09$, $p < .001$; see Figure 3). The extinction rates of the 00F and the T0F groups did not differ ($F(1,29) = 1.13$), and they did not differ from the 0TF animals ($F(1,29) = 2.33$). The rates of the TTF and 0TM animals also were not different ($F(1,29) = 0.79$). However, the extinction rates for the TTF and the 0TM animals were significantly slower than those of the 00F, T0F, and 0TF animals ($F(1,29) = 31.39$, $p < .001$).

Insert Figure 3 about here.

The five groups also did not all have the same serum level of T ($F(4,29) = 38.17$, $p < .001$; see Figure 4). The T levels of the 0TF and the 0TM animals did not differ ($F(1,29) = 2.12$) nor did they differ from the TTF animals ($F(1,29) = 0.84$). The T levels of the 00F and the T0F animals were not different ($F(1,29) = 0.12$). As expected, the T levels of the rats with T-filled capsules (0TF, 0TM, and TTF) were significantly higher than those with empty capsules (00F and T0F) ($F(1,29) = 143.36$, $p < .001$).

Insert Figure 4 about here.

General Discussion

The amount of T required to produce a slow extinction rate is altered by the presence of T during the perinatal period. Adult gonadectomized rats that had no exogenous T present during the perinatal period (normal females) exhibited a fast rate of extinction when given a 30 mm T-filled capsule whereas adult gonadectomized rats that had T present during the perinatal period (males and androgenized females) showed a slow extinction rate when given a 30 mm T-filled capsule. Thus, the presence of T during the perinatal period results in an increased sensitivity of the neural substrate to T for this behavior in the adult animal.

The presence of T during the perinatal period also has been found to influence sensitivity to T in adulthood for other behaviors dependent on the activational effects of gonadal hormones. Gonadectomized male mice require less T to activate intermale aggression than neonatally castrated males or gonadectomized females (Bartley & Goldman, 1977a, b; Simon & Whalen, 1987). However, although a higher dose of T activates aggression in adult females, it does not produce levels equal to those of normal males (Tolman & King, 1956; Simon & Wahlen, 1987). T prolongs extinction of a conditioned taste aversion in normal adult females but whether there is a dose of T that produces the same extinction rate in males and females remains unclear.

Although we failed to find a behavioral difference between males and females given 120 mm of T, this may have been due to our limiting the number of extinction trials to 41.

The results from Experiment 1 suggest that 60 mm of T is a near threshold dose of T for normal females as a group. A reexamination of the extinction data in terms of the percentage of animals with extinction scores of 20 or greater revealed that 83-100% of the animals from those groups with a slow extinction (sham males, gonadectomized males with 30, 60, or 120 mm capsules, and gonadectomized females with 120 mm capsules) and 8 - 23% of the animals from those groups with a fast extinction (sham females, gonadectomized males and females with no T and gonadectomized females with 30 mm capsules) extinguished after 20 or more days. The percentage of females with 60 mm capsules whose extinction was 20 days or longer (45%) fell in between the range of values of those groups with a slow and those with a fast extinction. It may be that at least part of the individual differences found in female sensitivity to 60 mm of T is due to their in utero environment. Babine and Smotherman (1984) have found that female rats with males on both sides in utero showed prolonged extinction as adults when given low-dose injections of T whereas female rats with females on both sides in utero did not.

The T levels of females with 60 mm capsules were not different from those of intact males ($t(9) = 0.79$). The range of T values found in intact males was 1.53 - 4.18 ng/ml and the range for those females with 60 mm capsules that extinguished after 20 or more days was 2.77 - 4.44 ng/ml. This indicates that extinction can be prolonged in at least some females by doses of T that produce T values that are within the range of values found in intact males.

In his review, Beatty (1979) stated that no sexually dimorphic behavior has been shown to be without some early organizational influence. The sexual dimorphism in the extinction of a conditioned taste aversion is no exception. The variation between behaviors seems to be in the relative dominance of the organizational and activational effects. Those behaviors that seem to be primarily organizational result from one gender being much more sensitive to the hormone than the other gender. To the extent that the behavior seems to have little organizational influence, the two genders are much more equal in their sensitivity to the hormone. Thus, the amount of disparity between genders in sensitivity to the activational effects of gonadal hormones for the different sexually dimorphic behaviors could be seen as a continuum; at one end we would find small differences in sensitivity, such as is the case for the extinction of a conditioned taste aversion, and at the other extreme we would

find large differences in sensitivity, such as is the case for aggression in mice and open-field behavior in rats.

From the results of Experiment 2, it is clear that the sexual dimorphic rate of extinction is dependent on T action during adulthood. Those adult gonadectomized rats that had T present during the perinatal period (males and androgenized females) did not exhibit a slow rate of extinction unless T was present during testing. Animals that had little or no T during the developmental period (females) displayed the slow rate of extinction if given sufficient T in adulthood. Thus, although the presence of T during the perinatal period alters subsequent sensitivity to T, it is the presence of sufficient T during adulthood, and not the presence of this hormone during the perinatal period that is critical for the expression of a slow extinction rate.

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Author Notes

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Figure Captions

Figure 1. Mean (±SE) days to extinguish a conditioned taste aversion in sham females (SF), sham males (SM), gonadectomized females (light shading) and gonadectomized males (dark shading) implanted with empty silastic capsules (0) or 30, 60, or 120 mm testosterone-filled capsules for Experiments 1A and 1B. *Significantly different than the males in the same treatment condition, $p < .05$.

Figure 2. Mean (±SE) serum testosterone levels (ng/ml) in sham females (SF), sham males (SM), and gonadectomized females (light shading) and gonadectomized males (dark shading) implanted with empty silastic capsules (0) or 30, 60, or 120 mm testosterone-filled capsules before acquisition of a conditioned taste aversion (Pre-Acquisition) and after extinction (Post-Extinction). *Significantly different than males in the same treatment condition, $p < .05$.

Figure 3. Mean (±SE) days to extinguish a conditioned taste aversion in males treated with oil perinatally and testosterone (T) during testing as an adult (OTM), females treated with oil perinatally and no T during testing (OOF), females treated with oil perinatally and T during testing (OTF), females treated with testosterone propionate (TP) perinatally and no T during testing (TOF), and females

treated with TP perinatally and T during testing (TTF). *Significantly different than OTM, $p < .05$.

Figure 4. Mean (±SE) serum testosterone levels (ng/ml) in males treated with oil perinatally and testosterone (T) during testing as an adult (OTM), females treated with oil perinatally and no T during testing (OOF), females treated with oil perinatally and T during testing (OTF), females treated with testosterone propionate (TP) perinatally and no T during testing (TOF), and females treated with TP perinatally and T during testing (TTF). *Significantly different than OTM, $p < .05$.

Fig 1

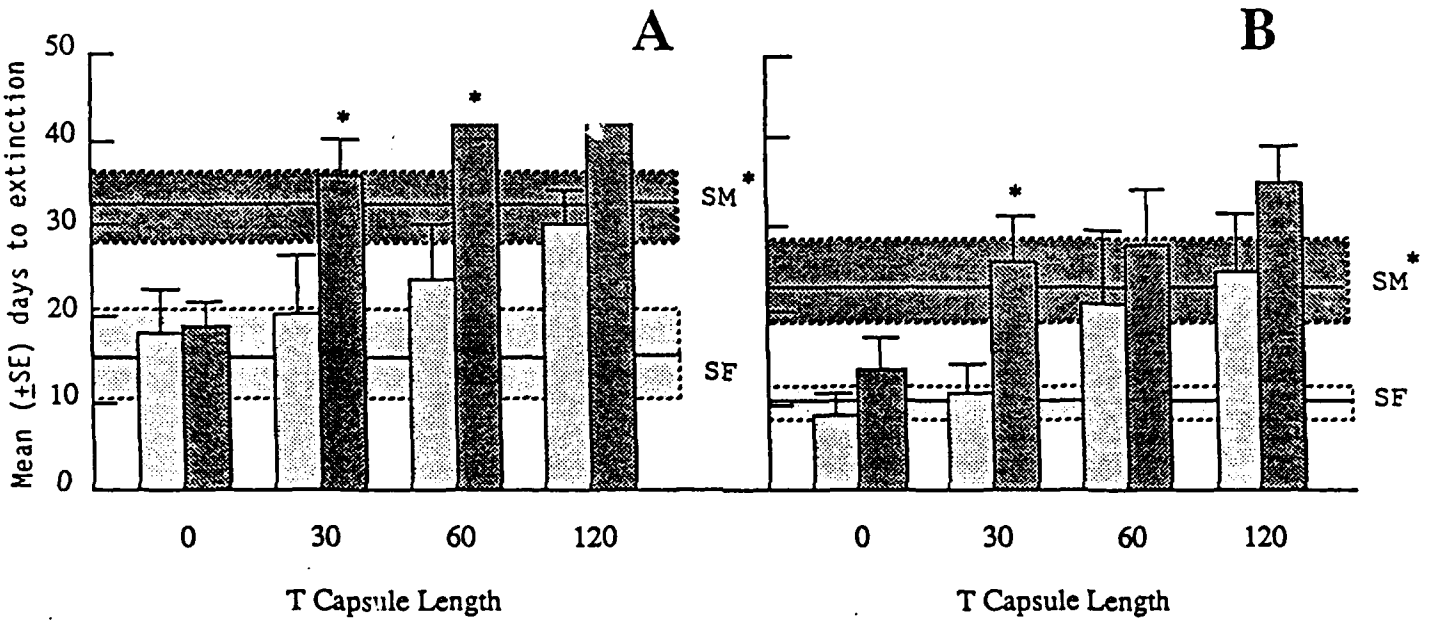

A
B
Mean (±SE) days to extinction
50
40
30
20
10
0
SM
SF
*
0
30
60
120
T Capsule Length

Fig 2

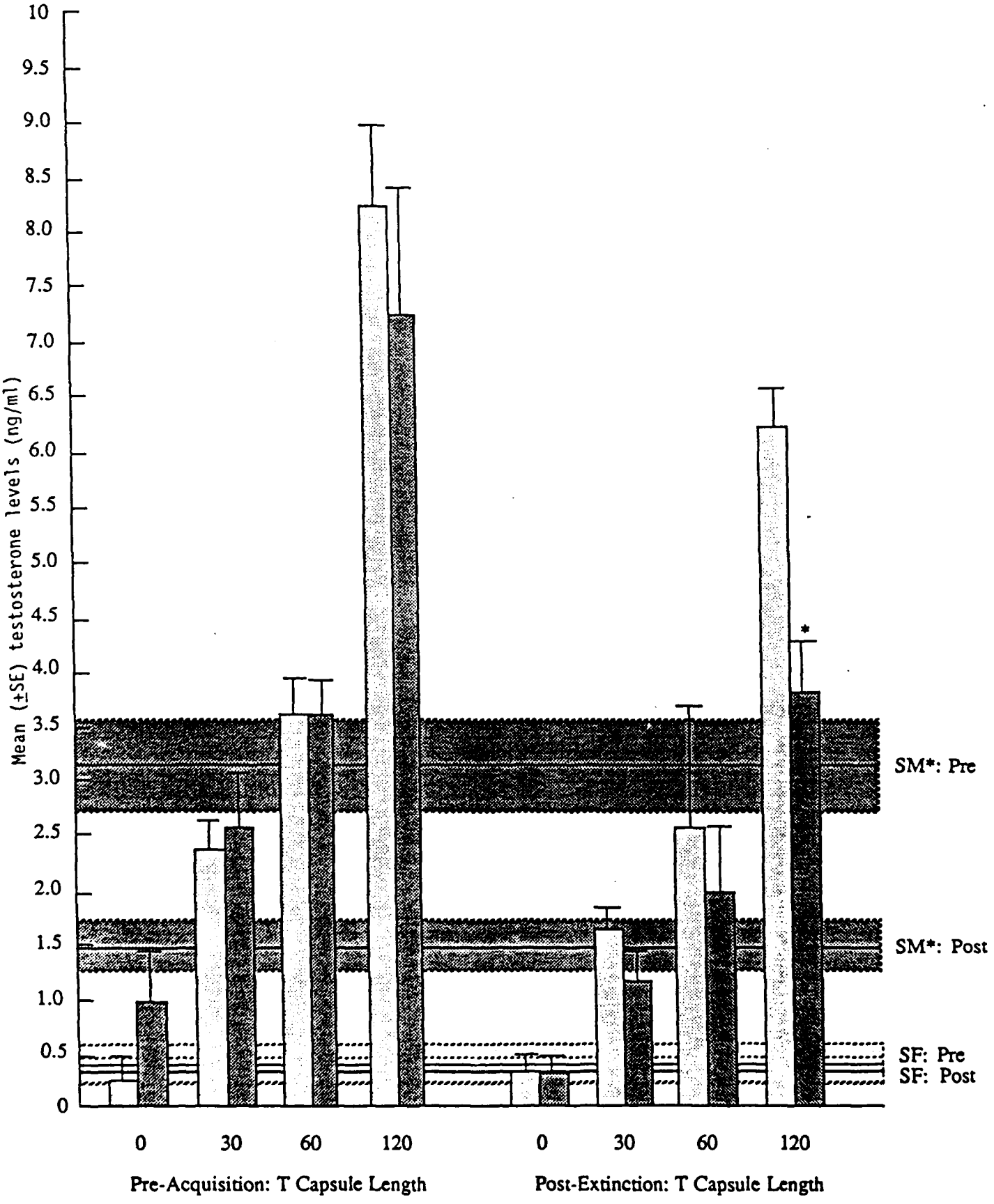

Mean (±SE) testosterone levels (ng/ml)
10
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
*
SM*: Pre
SM*: Post
SF: Pre
SF: Post
0
30
60
120
0
30
60
120
Pre-Acquisition: T Capsule Length
Post-Extinction: T Capsule Length

Fig 3

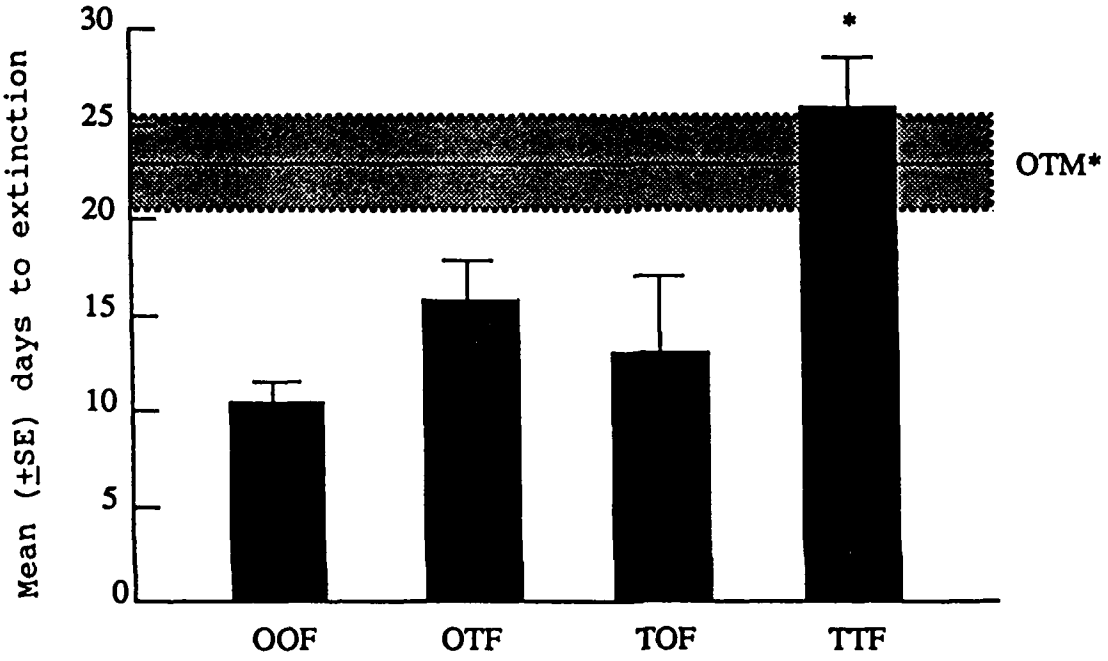
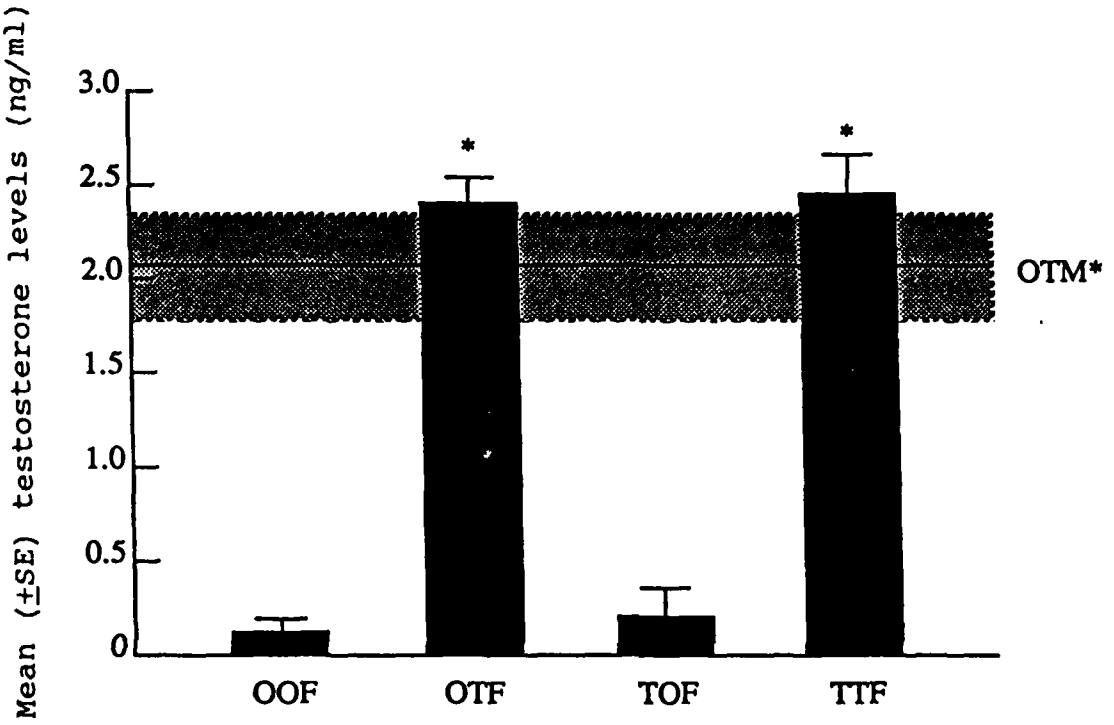


Fig 4


Mean (±SE) testosterone levels (ng/ml)
3.0
2.5
2.0
1.5
1.0
0.5
0
*
*
OTM*
OOF
OTF
TOF
TTF

Appendix C

Submitted for presentation at Western Psychological Association meeting, Los Angeles, 1990.

EFFECTS OF GONADECTOMY ON EXTINCTION OF A CONDITIONED FOOD AVERSION. D.L. Yuan, C. Hung, and K.C. Chambers. Department of Psychology, USC, Los Angeles, CA 90089

Gonadal hormones alter the rates of extinction of conditioned food aversions in rats. Males have slower extinction rates than females. Gonadectomy increases the rates in males but has no effect in females. Testosterone treatment slows extinction in both males and females. We have observed that gonadectomy increases the extinction rates of males to those of females in Sprague-Dawley but not Fischer 344 rats. In studies of reproductive behavior, the decrease in sexual activity after gonadectomy occurs over a long period of time. One possible explanation for the difference in the effects of gonadectomy in Sprague-Dawley and Fischer 344 rats is that gonadectomy may take longer to show an effect in Fischer 344. The following experiment was designed to determine whether the extinction rates of Fischer 344 rats varies with the length of time after gonadectomy. A conditioned food aversion was induced in 20 males and 20 females one week or 5 weeks after gonadectomy. Following the first presentation of a 10% sucrose solution, the conditioned food aversion was induced by injection of LiCl (0.15 M, 10 ml/kg). Daily extinction trials began 2 days later and continued until criterion for extinction (100% of first day consumption) was reached. The extinction rates of the 5-week males were significantly faster than those of the 1-week males ($p<0.05$). The extinction rates of the two groups of females did not differ. Both groups of males, however, still exhibited slower extinction rates than both groups of females ($p<0.05$). These results suggest that differences in the effects of gonadectomy on extinction rates in Sprague-Dawley and Fischer 344 rats may be accounted for, at least in part, by differences in the length of time the effects of gonadectomy are expressed. Supported by grants HD 20970 and ONR N00014-89-J-1296.

Appendix D

Submitted for presentation at the American Psychological Association meeting, Boston, 1990.

ABSTRACT

CONDITIONED FOOD AVERSION IN YOUNG AND OLD MALE RATS. David L. Yuan, Charles Hung, and Kathleen C. Chambers. Department of Psychology, USC, Los Angeles, CA 90089-1061

Previous studies have shown that old male rats exhibit greater resistance to extinction of a conditioned food aversion than young males. This study was designed to determine the nature of the age-related difference 1) by varying the time interval between acquisition and extinction and 2) by varying the dosages of the illness-inducing agent, LiCl. The results indicate that the retention and extinction processes of old male rats are more resistant to the effects of the passage of time. However, young rats are more sensitive to a lower dosage of LiCl. Supported by grants NIH-HD20970 and ONR-N00014-89-J-1296.

SUMMARY

CONDITIONED FOOD AVERSION IN YOUNG AND OLD MALE RATS. David L. Yuan, Charles Hung, and Kathleen C. Chambers. Department of Psychology, USC, Los Angeles, CA 90089-1061

Previous studies have shown that old male rats exhibit greater resistance to extinction of a conditioned food aversion than young males. This study was designed to determine the nature of the age-related difference 1) by varying the time interval between acquisition and extinction and 2) by examining the difference in old and young male rats in their sensitivity to the illness-inducing agent, LiCl, and therefore their ability to acquire a conditioned food aversion.

In the first experiment, twenty young (3.3-4.3 months) and 26 old (18 months) Fischer male rats were randomly assigned to four groups: young rats with 1 day delay between acquisition and extinction (young 1-day-delay group), young rats with 1 month delay between acquisition and extinction (young 1-month-delay group), old rats with 1 day delay between acquisition and extinction (old 1-day-delay group), and old rats with 1 month delay between acquisition and extinction (old 1-month-delay group). The rats were housed two per cage and were kept on a 12:12 hr light/dark cycle. On the day of acquisition (Day 1), the rats were given access to a chilled 10% sucrose solution

(wt/vol in water) at the beginning of the dark phase of the cycle. One hour later, the conditioned food aversion was induced by injection of LiCl (0.15 M, 10 ml/kg). Acquisition for the 1-day-delay and 1-month-delay groups was given at different times. For all groups the first extinction trail was given at the same time. Daily extinction trials were continued until criterion for extinction (100% of first day consumption) was reached.

The extinction rates of the young 1-month-delay group were significantly faster than those of the young 1-day-delay group and the two old groups ($F(3,39)=3.95$, $p=0.15$). The extinction rates of the two groups of old males and the young 1-day-delay males did not differ statistically. Clearly, the retention and extinction processes of old male rats are less subject to the effects of the passage of time than are those of young males.

The extinction process is affected by the strength of acquisition. In the aging studies, differences in the retention or extinction processes could be accounted for by differences in the initial level of acquisition in old and young animals. In the previous studies, old and young males were given the same dosage (per body weight) of illness-inducing agent. It may be that old males have an increased sensitivity to the agent. We tested this hypothesis in the second experiment by pairing several different dosages of LiCl repeatedly with a sucrose solution. We then compared the proportion of old and young animals that acquired an aversion at each dosage level.

Forty young male rats (3 months) and 38 old male rats (16 months) were randomly assigned to 1 of 4 groups: 1.00 (Group 1), 0.625 (Group 2), 0.250 (Group 3), and 0.125 (Group 4) mg/kg of body weight of LiCl. On the first acquisition day, immediately after the one hr sucrose solution presentation, rats received intraperitoneal injections of LiCl according to their group assignment. On Day 2 the rats were given access only to the chilled tap water for 1 hr. For the next 22 days, there was an alternation of acquisition and recovery days with the same experimental procedure used on days 1 and 2, respectively. The experiment was terminated on Day 24. Once a rat reached a complete aversion (consumption of 2 ml or less of the sucrose solution during any 1-hr test period) it was no longer given LiCl injections.

The results indicate that all old and young rats in Group 1 acquired an aversion. Eight out of ten young rats in Group 2 acquired an aversion but only 2 old rats did. There were 4 and 1 young rats in Groups 3 and 4, respectively, that acquired an aversion but none of the old rats in these group did. The difference in the proportion of animals in the four groups of young and old males that acquired an aversion was significant ($Chi^2(3)=57.06$, $p<0.01$). Young male rats tend to be more sensitive to a lower dosage of LiCl. These results do not support the hypothesis that the slower extinction observed in old male rats can be accounted by the increased sensitivity to LiCl in the old rats.

Appendix E

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Annu. Rev. Neurosci. 1990. 13:373–85

A NEURAL MODEL FOR CONDITIONED TASTE AVERSIONS

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From the time the parameters defining conditioned food aversions (CFAs, learned aversions to a food or fluid when consumption of that substance is followed by illness) were determined, this learning situation has fallen outside the main conceptualizations of the traditional forms of classical and instrumental learning (Garcia & Koelling 1966, Garcia et al 1966). The two main characteristics that distinguish CFAs from the traditional learning paradigms are learning after a long delay between the food stimulus and the illness (up to several hours) and strong and persistent learning after a single pairing of the food stimulus and illness. It is history, now, that these differences altered the theoretical framework of learning and memory. After the publication and acceptance of the seminal papers of Garcia (Garcia & Koelling 1966, Garcia et al 1955, 1966), there was a flurry of research on CFAs (Riley & Baril 1976). But as is apparently true of all things novel, habituation set in and interest in this area waned. Now with the growth of the field of behavioral neuroscience and the successful application of neurobiological techniques to the study of learning and memory, interest in this maverick of learning is again increasing.

NEURAL MODEL FOR CLASSICAL CONDITIONING: A POOR MODEL FOR CONDITIONED TASTE AVERSIONS

The most significant progress in identifying and characterizing the neuronal substrates of learning and memory has been made for classically conditioned situations, e.g. autonomic conditioning of heart rate (Cohen 1982, Kapp et al 1982) and eye blink conditioning (Thompson 1986). In

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these Pavlovian paradigms, a stimulus (unconditioned stimulus, US) that elicits a response (unconditioned response, UR) is paired with another stimulus (conditioned stimulus, CS) that does not elicit the UR. The two stimuli are paired so that they are contiguous and so that the CS can provide information about the US (Rescorla 1988). Learning is inferred when presentation of the CS produces a response similar to the UR. The determination of the neural substrates for this learning situation has involved the identification of four pathways: the US, UR, CS, and CR pathways.

Although CFA learning is thought to be a form of classical conditioning, it does not fit within this four-pathway model. The food stimulus has been identified as the CS, the illness as the US, and the avoidance of the food as the CR. There is, however, no clearly identifiable UR; a CFA can occur without an overt UR (Garcia et al 1972). A three-pathway conceptualization has been implicit in most discussions of the neural basis of CFAs. Discussions have focused on how the food stimulus and illness are integrated neurally to produce a new response to the food. But a detailed analysis of the learning situation for one form of CFA learning, conditioned taste aversion (CTA), suggests that this conceptualization is not adequate.

Taste stimuli have been known to elicit behavioral responses prior to food absorption in addition to the well-known physiological responses, salivation and increased insulin release (Fischer et al 1972, Grill & Berridge 1985, Pavlov 1927, Steiner 1979). The most preferred tastes, such as sweet, evoke increased consummatory responses and the least preferred tastes, such as bitter, evoke reduced consummatory responses and food spillage (Carpenter 1956, Rozin 1967). More recently, Grill & Norgren (1978) have described more complex behavioral responses elicited by different novel taste stimuli in rats. The rats were fitted with an intraoral catheter and the tastes were delivered directly into the mouth. The animals exhibited essentially two different patterns of stereotyped mouth, tongue, head, paw, and forearm movements that reflected hedonic responsiveness to taste. Preferred substances elicited a series of rhythmic mouth movements and alternations between tongue protrusions and tongue retractions that resulted in swallowing and paw licking (ingestive responses). Nonpreferred substances elicited mouth gaping with tongue retraction followed by long duration tongue protrusion and then tongue retraction and mouth closure. This sequence of responses was repeated several times, resulted in a reduction in swallowing, and was often followed by a sequence of fixed action patterns, which included chin rubbing, head shaking, paw wiping, and forelimb flailing (aversive responses). Other tastes elicited a mixture of these two redundant patterns.

When rats are poisoned after consuming a preferred sweet taste such as sucrose, their subsequent behavioral responses to sucrose resemble those exhibited after consumption of nonpreferred bitter tastes such as quinine. They exhibit decreases in consumption levels, spillage of food and stereotyped aversive responses (Berridge et al 1981, Garcia & Koelling 1966, Rozin 1967). Illness, then, alters the response elicited by taste.

In most classical conditioning situations, the response elicited by the CS is altered because of its association with the US. In some cases the CR resembles the UR quite closely. The CR generally does not, however, become identical to the UR (Holland 1984, Rescorla 1988). The CR can lack the intensity and some of the response repertoire observed for the UR and can include some responses that are not part of the UR. In some cases the CR produced by a given CS is opposite that of the UR, e.g. increases in activity and heart rate elicited by a shock US and decreases in activity and heart rate elicited by a tone CS (de Toledo & Black 1966, Rescorla 1988). Holland (1984) has suggested that the CR is composed of two behavioral elements: one that is similar to or at least in some way appropriate to the US and one that is similar to the response elicited by the CS prior to conditioning (Figure 1).

What distinguishes the CTA learning situation from many other forms of classical conditioning is that the CR is entirely part of the repertoire of elicited responses for the CS sensory modality, in this case, taste. Although the response is appropriate to the US in that illness often produces decreases in food consumption, the decrease in consumption in a CTA situation is not general as in the case of illness, but is specific to the CS. The CR is not similar to the response elicited by the CS prior to conditioning but is opposite that response. Consequently, the function of the US also is different in CTAs than in traditional classical learning. The US does not act as the elicitor of what will become the essential characteristics of the CR. Instead it changes the response elicited by the CS from one form (ingestive) to another (aversive).

PROPOSED NEURAL MODEL FOR ACQUISITION OF CONDITIONED TASTE AVERSIONS

The determination of the neural substrates for CTAs, then, should involve the identification of the following pathways (Figure 1): the US pathway, the CS pathway, the pathway for the elicited response to the CS prior to conditioning (UR_u), and the pathway for the elicited response to the CS after conditioning (CR_c). Each taste is connected to both the ingestive and aversive responses. These connections are probably innate, since

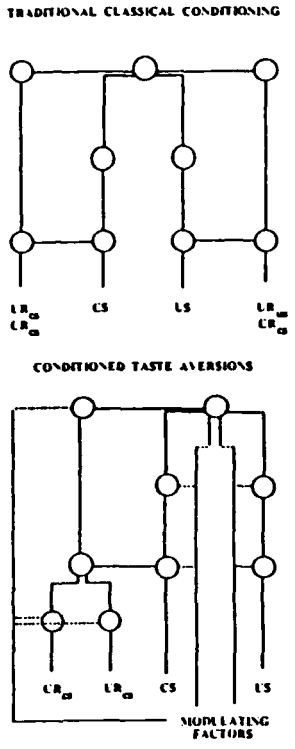

Figure 1 Simplified schematic of a neural model for traditional classical conditioning and conditioned taste aversion. Abbreviations: CR_{cs}, conditioned response to the CS; CS, conditioned stimulus; UR_{cs}, unconditioned response to the CS; UR_{us}, unconditioned response to the unconditioned stimulus; US, unconditioned stimulus.

hedonic reactions to taste have been observed in prematurely born and full-term neonatal individuals (Steiner 1973, 1979). The relative strengths of the two innate connections are dependent on the given taste. In the case of sucrose, the innate connection to the ingestive response is stronger than the innate connection to the aversive response.

If exposure to sucrose is followed by illness, the connection to the ingestive response system will weaken and the connection to the aversive response system will strengthen. It is most likely that the illness-induced changes involve two processes rather than one. Grill & Berridge (1985) have suggested that palatability processing involves two mechanisms and

have provided evidence that the ingestive and aversive response systems can change independently. Thus, in order for the aversive response system to be expressed solely, a weakening of the ingestive response system would have to occur. If exposure to a taste is not followed by negative consequences, a stronger connection to the ingestive response system will result. A stronger connection to the ingestive response system also will occur if a given taste is associated with positive reinforcement or if it is followed by recuperation from illness (Garcia et al 1977, Revusky 1967, 1974, Rozin 1969). So, experiential factors can alter the strengths of the innate connections to the ingestive and aversive response patterns. Thus, after a given taste is experienced, the relative strengths of the ingestive and aversive systems are a function of the original innate connections, the number of exposures to the taste with illness, and the number of exposures to the taste without illness. This hypothesis is supported by the findings that CTAs to nonpreferred tastes are stronger than to preferred tastes (Etscorn 1973), that repeated pairings of a taste with illness strengthens an aversion, and that repeated pairings of a taste without illness reduces the strength of an aversion (Kalat & Rozin 1973).

Other factors associated with the CS and US can influence the strength of an aversion and therefore must be taken into account when developing a neural model for CTAs. The strength of an aversion has been found to be a function of the intensity of the taste as measured by concentration (Dragoin 1971) and the amount consumed on the first exposure (Bond & Di Guisto 1975), the intensity of the US (Revusky 1968), and prior experience with the US (Cannon et al 1975).

Several factors that are not essential or critical for aversion learning can modulate the development and strength of CTAs. The development and strength of an aversion are dependent on the hormonal milieu and deprivation state of the animal. The presence of testosterone (T) increases the proportion of animals that develop a CTA (Chambers et al 1981), and the presence of dexamethasone attenuates the strength of an aversion (Hennessy et al 1976). Water deprivation reduces the proportion of male rats that develop an aversion (Chambers et al 1981). It is interesting that deprivation can alter the hedonic value of tastes. Foods are reported to be highly palatable with deprivation and unpleasant with satiety (Cabanac 1971). Also, the number of ingestive responses decreases and the number of aversive responses increases as meal termination approaches (Grill & Berridge 1985). So, the relative strengths of the ingestive and aversive response systems are also a function of modulating factors. A complete understanding of the neural mechanisms controlling CTAs would have to include a determination of the neural circuitry for the modulating factors (Figure 1).

KNOWN NEURAL CIRCUITRY FOR CONDITIONED TASTE AVERSIONS

The US Pathway

A number of reviews have examined the US pathways (Ashe & Nachman 1980, Borison & Wang 1953, Coil & Garcia 1977, Kiefer 1985). The vagus nerve conveys information from the gastric-intestinal mucosa primarily to the caudal region of the nucleus of the solitary tract (NST; Torvik 1956). It is then conveyed to the pontine parabrachial nucleus (PBN; Norgren 1978) and the insular cortex (Cechetto & Saper 1987). The area postrema, an area of the brain on the floor of the fourth ventricle that lacks a blood-brain barrier, detects chemicals in the blood. As there are reciprocal neural connections between the area postrema and the NST (Morest 1960, 1967), information about these blood-borne chemicals is probably conveyed to the NST.

A wide variety of substances can be used as the US. The route by which information about these substances is conveyed to the brain varies with the particular chemical and the route of administration. LiCl, a widely used illness-inducing agent, appears to act primarily by way of the area postrema. Lesions of the dorsolateral region of this area attenuate or abolish the learning of taste aversions induced by LiCl (Ritter et al 1980). Vagotomized rats, however, develop essentially normal taste aversions (Martin et al 1978). The vagus nerve mediates copper sulfate-induced aversions when this substance is administered intraperitoneally or intragastrically, but when it is given intravenously the area postrema mediates the aversion (Coil & Norgren 1981, Coil et al 1978).

Although the vagus nerve and the area postrema are important routes for many different chemicals, they may not be the only means by which information is conveyed to the brain. The area postrema is an important structure for the induction of emesis when apomorphine is administered, but neither lesions of this area nor vagotomy has an effect on the ability of an animal to learn CTAs (Kiefer et al 1981, Van der Kooy et al 1983). It is not known what effect disruption of both systems has on CTA learning.

The CS Pathway

The CS for CFA learning involves stimuli that are normally used by a given species for the identification of food. For many species taste is the primary stimulus for identification. But it must be noted that other stimuli such as odor can serve as weak cues (Kiefer 1985) and some species use other senses, such as vision in birds, as the primary stimulus (Gaston 1977).

The gustatory pathway has been reviewed recently by Norgren (1984) and Travers, Travers & Norgren (1987). In summary, taste receptor cells are located primarily in the tongue and hard and soft palates. Taste information is transmitted primarily to three peripheral taste nerves: the chorda tympani branch of the facial nerve, the lingual branch of the glossopharyngeal nerve, and the greater superficial petrosal branch of the facial nerve. Gustatory afferent fibers from the facial and glossopharyngeal nerves terminate in the ipsilateral NST. Ascending axons from the NST terminate in the ipsilateral PBN in rodents and lagomorphs and in the ventroposteromedial nucleus of the thalamus in primates. The PBN sends projections ipsilaterally to the parvicellular division of the nucleus ventralis posteromedialis (VPMpc) and also projects extensively to the ventral forebrain, in particular, the lateral hypothalamus, central nucleus of the amygdala, and bed nucleus of the stria terminalis. The VPMpc projects to the insular cortex, which projects back to the VPMpc, central amygdala, PBN, and NST.

The UR_{cs} and CR_{cs} Pathways

Neural areas rostral to the PBN are not critical for hedonic reactions to taste. Hedonic responsiveness remains in rats with supracollicular decerebrate preparations that leave only the first (NST) and second (PBN) central gustatory relay nuclei. Intraoral taste stimulation of these rats elicits the same ingestive and aversive patterns of taste responsiveness at the same concentrations as it does in intact rats (Grill & Berridge 1985).

Some neurons in the PBN project to oro-motor nuclei (Travers & Norgren 1983) and respond to both the hedonic dimensions of taste and oro-lingual movement (Schwartzbaum 1983). It seems likely that the ingestive and aversive behaviors are organized entirely in the brain stem and that the control of these behaviors involves the NST and PBN and their polysynaptic connections to the motor neuron pools controlling the behaviors. As the ingestive and aversive movements to taste stimuli are stereotyped, repetitive, and rhythmic, the neural circuits for these behaviors may function as pattern generators, with higher brain systems acting only as modulators. In this sense, the control of the behaviors may resemble that of vertebrate locomotion (Grillner 1985).

Since the behavioral response elicited by bitter tastes is similar to that elicited by a taste that has been paired with illness, one would expect that at least at the level of the behavioral pattern generators, the neural code for the CS would be similar to that for quinine. Chang & Scott (1984) have found that the pattern of activity of sucrose-best NST neurons in response to a sweet taste changes after rats acquire an aversion to this taste so that the activity pattern more closely resembles that of bitter tastes.

CS-US Integration

Possible sites of taste-illness integration have been discussed in a number of recent reviews (Ashe & Nachman 1980, Gaston 1978, Grill 1985, Kiefer 1985). The search for such sites has been plagued by inconsistent findings, and unequivocal candidates have not emerged. The neural control of this primitive form of learning is clearly complex, and there are likely a number of neural routes that can be used for laying down the trace. Despite the contradictory endeavors, some findings have come out of the search that should provide more insight into the neural organization of CTAs.

One of the more consistent findings has been that lesions of the basolateral amygdala disrupt taste aversion learning and retention of an aversion learned prior to lesioning (Simbayi et al 1986, Nachman & Ashe 1974). After finding that cutting the connections between the amygdala and the temporal cortex produced the same deficits as lesions of the basolateral amygdala, however, Fitzgerald & Burton (1983) suggested that it is the destruction of the fibers of passage that produces the deficits after lesions of the basolateral amygdala, not the destruction of the nucleus itself. Recently, Dunn & Everitt (1988) found that ibotenic induced lesions that spare the fibers of passage had no effect on aversion learning. Anna Brownson, Richard Thompson, and I have confirmed and extended this finding in a preliminary study. Electrolytic lesions attenuated both the acquisition of an aversion and the retention of an aversion induced prior to lesioning; neurotoxic lesions (NMDA-induced), which also spare the fibers of passage, had no effect on either acquisition or retention (Figure 2). Clearly the issue of axons of passage is critical to an understanding of neural mechanisms.

Lesions of the PBN disrupt acquisition of a CTA when there is a delay between the CS and US (Schulkin et al 1986, Di Lorenzo 1988), but if there is no delay, animals can learn an aversion (Di Lorenzo 1988). Similar results have been found for lesions of the gustatory cortex (Lorden 1976). These findings suggest that there are different neural mechanisms for learning when CS-US intervals are short and long. Any neural model must include both pathways.

BEYOND ACQUISITION OF CONDITIONED TASTE AVERSIONS

Although I have focused only on the acquisition process, a complete neural model of CTA should include retention or memory storage processes and extinction processes as well. There probably are neural areas that are part of the pathways for all three processes, but the pathways are different.

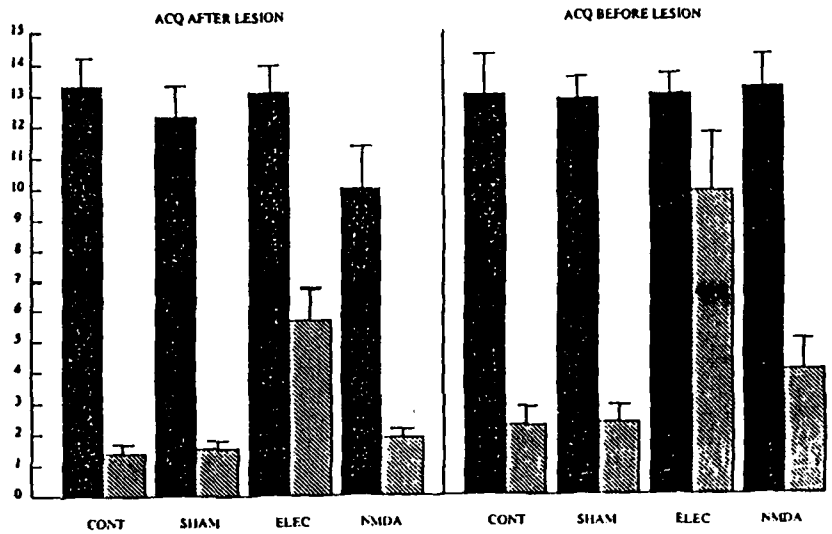

Figure 2 Mean (+SE) sucrose consumed by control (CONT), sham, electrolytic lesioned (ELEC), and neurotoxic lesioned (NMDA) male rats on the day of acquisiton (ACQ; *dark bars*) and the first day after acquisition (*hatched bars*) when acquisition was given after and before lesions of the basolateral amygdala.

Each process has its own set of modulating factors that influence that process independently of the other.

Retention

Little is known about the neural mechanisms for CTA, but the data so far suggest that the neural mechanisms for acquisition and retention differ. Although the gustatory neocortex is involved in acquisition, it is not essential (Braun et al 1972, Lasiter & Glanzman 1982, Lorden 1976). It is, however, critically involved in retention of aversions, as animals with these lesions do not retain a previously learned aversion (Braun et al 1981, Yamamoto et al 1981).

Extinction

Extinction has been regarded merely as a reflection of the acquisition process. Indeed, in much of the early Pavlovian and Skinnerian literature, the strength of acquisition was indexed by the rate of extinction. If extinction was slow, acquisition was said to be strong, and if extinction was fast, acquisition was asserted to be weak. Extinction, however, at least of a

CTA, is far more complex than this. Some evidence suggests that the neural processes mediating acquisition and extinction of a CTA are *different*. If animals are anesthetized or under cortical spreading depression when they are given exposure to a taste, they do not acquire a CTA but they do extinguish a previously learned aversion (Burés & Burešova 1979). The vagus nerve plays a role in extinction that is independent of its involvement during acquisition (Coil et al 1978, Kiefer et al 1981). Rats that are vagotomized prior to or after acquisition of a CTA exhibit a faster extinction than nonvagotomized animals, even when a US (apomorphine) that is not vagally mediated is used. A number of other factors modulate extinction independenly of acquisition. The rate of extinction is altered when adrenocorticotropin (ACTH) levels are elevated, when T is present, and when animals are under water deprivation (Chambers 1985, Chambers & Sengstake 1979, Kendler et al 1976, Sengstake & Chambers 1979). ACTH and T decrease and water deprivation increases the rate. It is the presence of these factors during extinction that alters the rate of extinction. Their presence or absence during acquisition of the aversion has no effect.

As suggested decades ago by Clark Hull (1943), extinction is a learning process. Simply stated for CTAs, it is unlearning that the taste predicts illness and learning that it predicts safety (Chambers 1985, Kiefer et al 1981). With respect to the neural model for CTAs outlined above, extinction is a process by which connections to the aversive response system are weakened and connections to the ingestive response system are strengthened. Any information on the subsequent consequences of ingesting the CS is processed. If the consequences are neutral, that information serves to alter the relative strengths of the two response systems. Thus, after a CS has been experienced without negative consequences, the relative strengths of the ingestive and aversive response systems are a function of the relative strengths of these systems after the CTA, the number of exposures to the taste without illness, modulating factors, and probably the original innate predisposition.

CONCLUSION

Since its discovery, students of learning have argued whether CTA fits best in a classical or instrumental learning framework. The fit seemed poor in either case. Although most have placed it within classical conditioning, the issue is still unresolved. Taste aversion learning seems to share the following with traditional kinds of Pavlovian conditioning: a neural integration of the CS and US, a change in the meaning of the CS as a result of this integration so that the CS becomes a signal that predicts the occurrence of the US, and an elicitation of a CR by the CS that is an

anticipation of the occurrence of the US. There are characteristics of CTAs, however, that traditional Pavlovian conditioning does not share, i.e. the ease with which the US and CS are integrated, the ability to integrate despite the long delay between the US and CS and despite intervening CSs from the same sensory modality, the context independence of the CS, and the origin, stemming from the CS, of the CR. It is unclear how critical these differences are, but they certainly alter how one would develop a neural model of learning.

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