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Effect of Sex on Fear Conditioning is Similar for Context and Discrete CS in Wistar, Lewis and Fischer Rat Strains

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PRYCE, C. R., J. LEHMANN AND J. FELDON. *Effect of sex on fear conditioning is consistent between context and discrete CS in Wistar, Lewis, and Fischer rats.* PHARMACOL BIOCHEM BEHAV **64**(4) 753–759, 1999.—Enhanced fear in males relative to females, both innate and conditioned, is a well-described characteristic of behavior in the laboratory rat. In the case of aversive conditioning to foot shock in Long–Evans rats, it has been described that conditioning to general (nondiscrete) contextual cues is greater in male rats relative to female rats, whereas conditioning to a discrete, predictive stimulis (CS) is not. These findings have been combined with evidence for greater levels of hippocampal LTP in males in Sprague–Dawley rats to derive a model of hippocampal–LTP-mediated contextual and not CS, fear conditioning. The present study reports on an analysis of the effect of sex in contextual and discrete CS conditioning to foot shock, assessed via measurement of freezing behavior in a novel automated paradigm, in three rat strains: Wistar, Fischer, and Lewis. In Wistar rats, there was a consistent but nonsignificant tendency for males to demonstrate both more contextual and more CS conditioning than females; in Fischer rats, males demonstrated both more contextual and more CS conditioning than females; in Lewis rats, a markedly enhanced acquisition of freezing in males did not translate into a sex difference in either context or CS conditioning at expression. Therefore, within each strain the effect of sex was consistent between context and CS conditioning. These findings, taken together with the hippocampal LTP evidence, suggest that the latter mediates both contextual and discrete CS aversive conditioning, and contributes to sex differences in both these forms of conditioning, in those strains where these sex differences exist. © 1999 Elsevier Science Inc.

Sex Strain Fear Conditioning CS Context Freezing Hippocampus LTP

IN the laboratory rat, male–female differences, or sexual dimorphism, in their respective behavioral responses to aversive situations are well described [e.g. (1,5)]. Generally, the evidence indicates that male rats demonstrate more spontaneous anxiety/fear in situations that are innately aversive; for example, males defecate more and locomote less in open fields, they require longer to enter and explore a novel environment such as an elevated plus-maze, and they freeze more in response to an unfamiliar sound, relative to females. Furthermore, the evidence also indicates that male and female rats perform differently in situations of aversive conditioning: Males are superior to females in acquisition on the passive avoidance paradigm, and males are inferior in acquisition on two-way shuttlebox avoidance and on operant avoidance. In terms of the information processing underlying these sex differences in conditioned fear in the rat, there are two main, competing hypotheses. First, it is possible that differences in response selection are responsible, with males being more likely to select and peform an inactive response, i.e., freezing, and females an active response, for example, escape attempts, despite both sexes acquiring equivalent levels of conditioned fear (1). Second, it is possible that differences in fear per se are responsible, with males more likely than females to perform freezing in response to an aversively conditioned cue or context because they acquire more conditional fear than females (4). There is a good deal of experimental evidence in support of this latter interpretation. For example, in the two-way shuttle avoidance paradigm, reduction of shock intensity and anxiolytic drug administration both enhance acquisition of avoidance, and presumably do so by reduced aversive conditioning to, and inhibition of performance in, the context of the shuttlebox (3). On this basis, it is parsimonious to assume that en-

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hanced female performance on this paradigm is also the direct outcome of a reduced level of context-conditioned fear (5,12).

Therefore, male–female sex differences in fear-motivated behavior, both spontaneous and conditioned, are pronounced in the rat and, the behavioral evidence suggests, have their basis in sex-specific mechanisms mediating the amount of fear evoked in any situation. Concentrating on conditioned fear, there are, of course, two types of stimuli that can become associated with an innate evoker of fear (unconditioned stimulus, US); namely discrete, predictive conditioned stimuli (CSs) and general (nondiscrete) context. A body of evidence, derived primarily from lesion studies, suggests that the amygdala and hippocampus function in an integrative manner in the mediation of aversive conditioning to such discrete and contextual cues; however, there is considerable debate concerning the relative roles of these two limbic structures in the mediation of these two types of conditioning. Some studies of the amygdala (basolateral amygdaloid complex) report that this limbic structure mediates the acquisition and expression of discrete CS fear conditioning specifically (18), while others report that it mediates discrete CS and contextual fear conditioning (13,16). For the hippocampus, the majority of the studies to date report mediation of fear conditioning to contextual (including spatial) cues specifically (8,16,18). Of direct relevance here are some recent and as-yet unpublished data from our own laboratory as obtained with hippocampal-lesioned subjects studied in the high-resolution aversive conditioning system described in this article: we are obtaining evidence that suggests hippocampal mediation of contextual and CS fear conditioning. Accepting that Pavlovian fear conditioning constitutes a CS-US or a context-US temporal association, then associative long-term potentiation (LTP) represents the most likely candidate in terms of the responsible synaptic level learning mechanism. LTP was first described and is most often associated with the hippocampus, but subsequently it has been identified in many other brain areas as well (2). Evidence has been obtained for LTP mediation of the acquisition of contextual fear conditioning (7) and CS fear conditioning (14), and it has been proposed that associative LTP is the synaptic mechanism for aversive conditioning in both the hippocampus and the amygdala (7,11,19).

One approach to elucidating the roles of the amygdala and the hippocampus in discrete CS and contextual fear conditioning is to identify a factor that affects the level of LTP in either one of these structures and then to analyze the association between this factor and the two forms of fear conditioning. In a recent important study it was reported that Sprague–Dawley male rats demonstrate a significantly higher magnitude of hippocampal LTP (specifically, at perforant path synapses in the dentate gyrus) compared with females of the same strain (10,12). If the factor sex was found to be associated with one or both forms of fear conditioning, more specifically, should males demonstrate more contextual and/or CS conditioning than females, this would provide indirect evidence for the importance of the hippocampus (LTP) in contextual and/or CS conditioning. In the same study (12), it was reported that Long–Evans male rats demonstrate more contextual aversive conditioning than Long–Evans females, but that these males and females do not differ in terms of discrete CS conditioning (US = footshock, CS = tone, dependent measure = freezing). These data were interpreted as indirect evidence for (a) the mediation of contextual aversive conditioning by the hippocampus (hippocampal LTP), and (b) the absence of sex differences in the synaptic mediation of CS-US aversive conditioning in the amygdala, this latter interpretation being based on the assumption that CS conditioning is amygdala-mediated, i.e., nonhippocampal (12).

Against this background, the aim of the present study was to investigate whether sex differences in contextual and/or discrete CS aversive conditioning existed within any of the following three rat strains: the outbred Wistar strain, the inbred Fischer strain, or the inbred Lewis strain. The paradigm we used included repeated pairings of foot shock and tone and automated measurement of fear as freezing, i.e., species-specific defensive immobility, during development of conditioning and at subsequent tests of contextual and CS conditioning (17). Compared to the Maren et al. study (12), which reports enhanced male context conditioning in terms of both rate and at asymptote, we used a relatively large number of CS-US pairings. This is important, because it impacts on the suitability of our experimental design for detecting two different aspects of sex differences in conditioning: an increased number of pairings reduced the likelihood of our obtaining sex differences in expression due to dimorphism in rats of conditioning acquisition. However, at the same time it allowed us to analyze the actual development (acquisition) of freezing, and, as demonstrated in pilot studies, we were able to use a mild shock intensity to produce reproducible levels of both CS and contextual conditioning at acquisition and expression in the absence of ceiling effects. As such, for each strain, we were able to compare acquisition and expression aspects of male and female fear conditioning at stable asymptotic levels. According to Maren et al. (12), we would expect males to express more fear conditioning than females, but in terms of contextual conditioning only. If this was the case, then we would interpret this as further supportive evidence for the mediation of contextual aversive conditioning by the hippocampus and LTP. On the other hand, if males expressed more fear conditioning than females in terms of contextual and discrete CS conditioning, this would be consistent with the hypothesis that the hippocampus is involved in and underlies sex differences in both of these forms of aversive conditioning.

METHOD

Subjects

Six groups of animals are reported on in this study; namely, Wistar males ($n = 8$), Wistar females ($n = 8$), Fischer males ($n = 6$), Fischer females ($n = 6$), Lewis males ($n = 6$), and Lewis females ($n = 6$). The Wistar rats were studied in one experiment, and the Fischer and Lewis rats were studied together in a second experiment. The Wistar subjects were bred in-house (Zur: WIST [HanIbm], Animal Services, Swiss Federal Institute of Technology, Schwerzenbach), and were weaned at age 21 days and caged in isosexual groups of four per cage thereafter. Fischer and Lewis rats were obtained from Harlan Nederland (Fischer: F344/OlaHsd; Lewis: LEW/SsNHsd), and were aged 4–5 weeks on arrival in our laboratory, where they were caged in isosexual groups of three. All cages were Macrolon type IV measuring 59.0 × 38.5 × 20.0 cm. Husbandry comprised a reversed light/dark cycle, with a 0700–1900-h dark phase, temperature at 21 ± 1°C and humidity at 55 ± 5%, with pellet feed (Universal feed 3430, Moulin Kliba SA, Kaiseraugst, CH) and water available ad lib. Subjects were habituated to handling, and were then studied in terms of their conditioned freezing at age 8–12 weeks. Body weight (mean ± SEM) of subjects was as follows at week 10: Wistar males: 391 ± 16 g, Wistar females 229 ± 7 g,

Fischer males: 177 ± 5 g, Fischer females: 130 ± 2 g, Lewis males: 204 ± 8 g, Lewis females: 163 ± 5 g.

Aversive Conditioning Based on Freezing Behavior

The development of the fully automated system for measuring freezing behavior in the rat, as used in this study, has been recently described elsewhere (17). Four modular shock chambers each comprised an operant chamber (Habitest; Coulbourn Instruments, Allentown, PA) fitted with a parallel-grid shock floor (E10-10RF; Coulbourn Instruments) and with operant manipulanda withdrawn. Two sides of the chamber were aluminium, and two were clear Perspex. A white waste tray was situated below the shock floor. The house light remained off during the experimental sessions in the shock chambers. Each shock chamber was placed in a sound- and light-attenuating chamber (72 × 45 × 45 cm). Four modular no-shock chambers were positioned in an adjacent experimental room, and each comprised an operant chamber that differed from the shock chambers on the following contextual cues: the floor was a lattice grid (E10-18NS, Coulbourn Instruments), three of the sides were black and one was clear Perspex, the waste tray was brown, a house light (1.12 W) positioned near the top of one wall and directed upwards was on during experimental sessions, and the attenuating chamber measured 55 × 40 × 55 cm. Timed presentations of discrete auditory CS and of electric foot shock were controlled by a PC running dedicated Pascal software (S. Frank, Psychology Department, University of Tel Aviv, Israel) and connected via an internal mother board to four interfaces (Universal Environment Interface, Coulbourn Instruments). Each interface controlled the output from one of four electric shockers (E13-12; Coulbourn Instruments), each of which delivered a scrambled shock to the parallel-grid shock floor.

Attached to the center of the ceiling of each chamber (4× shock, 4× no-shock) was a monochrome minivideo camera equipped with a wide angle (100°) 2.5-mm lens (VPC-465B; CES AG, Zürich, CH). Four infrared (875-nm) light-emitting diodes (HSDL-4220; Hewlett Packard) positioned in the ceiling of each chamber provided sufficient illumination for camera function. The images from the four cameras were integrated into a four-quarter single image via a multiplexer (YS-Q430P, Sony) and the single image was captured to a video recorder (SVT1000; Sony). On-line activity analysis was performed as follows: the video image was transferred to a computer (7600/120 Power Macintosh) and analyzed second by second using a macroprogram written (P. Schmid, Behavioural Biology Laboratory, ETH Zürich) to customize the National Institutes of Health's public domain image processing and analysis program (*Image*; http://rsb.info.nih.gov/nih-image) to our in-house requirements. For each quarter of the single video image, each two consecutive 1 s of image were presented simultaneously on the computer monitor, each comprising 100,000 pixels. Depending on that part of the video image to which it corresponded, each pixel was given a "gray value" between 0 and 255, where 0 corresponds to absolute black and 255 to absolute white. The gray value of each pixel on the image from $time_{X+1}$ s was compared with that of the same pixel on the image from $time_X$ s, and a gray-value difference for each pixel pair was calculated. If the gray-value difference was greater than 5, then it was designated as a black pixel, and if the difference was less than 5, as a white pixel. Black and white pixels, produced as described, were used to prepare a third image for each pair of consecutive seconds, which provided a quantitative definition of activity vs. inactivity at 1-s intervals: if less than 50 of the 100,000 pixels (i.e., <0.05%) were black (i.e., derived from two consecutive 1-s images of the same pixel with a gray-value difference >5), then the subject was defined as being in a state of inactivity during that 1 s and given a score of 1. Simultaneously, the actual percentage of pixels that were black following comparison of two 1-s images was also recorded to provide an arbitrary but quantitative measure of general locomotor activity in the chamber. Finally, although the analysis was designed to be carried out on line, a backup video recording of each session was also made.

For validation, detailed comparison of inactivity scores with the actual behaviour of pilot subjects in the test chambers demonstrated that pilot subjects that had recent experience of foot shocks exhibited freezing, i.e., complete somatomotor immobility, and that this behavior pattern and only this behavior pattern was scored as inactivity, and on a second-by-second basis. Therefore, the system provided a validated, quantitative and fully automated measure of freezing for the study of the development and subsequent testing of aversive conditioning. It allowed for the measurement of the proportion of time spent freezing with a resolution of 1 s across specified time intervals.

Subjects were tested in the following 4-d procedure for activity, development of conditioning, test of contextual conditioning, and test of discrete auditory CS conditioning. Sessions were run between 1300 and 1830 h with chamber position counterbalanced across sex (Experiment 1 Wistar) or strain and sex (Experiment 2, Lewis and Fischer).

Day 1: activity monitoring in shock chamber. Subjects were placed in the shock chambers for 30 min in the absence of auditory stimulus and foot shock. The average percentage of black pixels was calculated for each 3-min time bin, to provide a measure of general activity in the context of the chamber.

Day 2: development of conditioned freezing. Subjects were placed in the shock chamber for 27 min and, in between an initial and a final interval of 120 s, were exposed to 10 pairings of 30-s auditory CS and 1-s foot shock (Experiment 1 = 0.5 mA, Experiment 2 = 0.3 mA; i.e., shock intensity adjusted to body weight) at 120-s intervals. The 1-s foot shock was contiguous with the final second of CS. The proportion of time spent freezing was calculated for each 30-s CS.

Day 3: test of contextual conditioning. Subjects were placed in the shock chamber for 8 min without foot shock and without specific CS. The proportion of time spent freezing was calculated for each 60-s bin.

Day 4: test of specific auditory CS. Subjects were placed in the no-shock chamber (i.e., "off baseline") for 11 min in total. After a 3-min habituation time subjects were exposed to 8 min of continuous auditory CS. The proportion of time spent freezing was calculated for each 60-s bin of auditory CS exposure.

Data Analysis

Freezing data for days 2–4 were transformed to a percentage of total time, thus providing a probability estimate amenable to parametric statistical analysis. These data were analyzed by analysis of variance procedures with a level of significance set at $p < 0.05$. For each strain separately, analysis took the form of a 2 (sex) × n (time blocks, repeated measure) ANOVA (SuperANOVA© General Linear Modelling Package, Abacus Concepts, 1991).

RESULTS

Wistar Males vs. Females

In terms of activity in the shock chamber without the auditory CS on day 1, there was a consistent decline in activity as confirmed by a significant main effect of the repeated measure of time, $F(9, 126) = 44.40$, $p < 0.001$. Males were more active than females overall, $F(1, 14) = 5.13$, $p < 0.04$, and a significant sex × time interaction confirmed that males demonstrated a more rapid attenuation in activity across time, $F(9, 126) = 2.77$, $p < 0.006$. The day 2 development of conditioned freezing, which described a significant increase across the repeated measure of 10 × 30-s CS blocks, $F(9, 126) = 12.37$, $p < 0.001$, is presented in Fig. 1(a). The average overall amount of freezing across the 10 CS presentations was not significantly different between males and females ($p > 0.36$); in addition, the development of freezing was not significantly different between the sexes, as indicated by the absence of a sex × time interaction ($p > 0.37$). During the test of contextual conditioning on day 3, there was a strong tendency for males to demonstrate more freezing than females overall (inset, Fig. 1b), and this approached the a priori level of statistical significance, $F(1, 14) = 4.29$, $p < 0.06$. There was no evidence of a sex difference in the time profile of expression of contextual conditioning (sex × time interaction: $p > 0.83$, Fig. 1b). During the auditory CS test on day 4 (Fig. 1c), there was no main effect of sex ($p > 0.14$; inset, Fig. 1c), but there was a strong trend towards a sex × time interaction, $F(7, 98) = 1.99$, $p < 0.07$: males and females expressed very similar levels of CS conditioning across bins 1–3, but thereafter freezing tended to extinguish more slowly in males than in females.

Comparing freezing to context and to discrete CS, the average amount of freezing to CS (40%) was approximately twice that of freezing to context (20%); the asymptotic level of expression of CS conditioning (50%) was very similar to the asymptotic level of freezing at acquisition (Fig. 1).

Fischer Males vs. Females

In terms of activity in the shock chamber without the auditory CS on day 1, there was a consistent decline in activity as confirmed by a significant main effect of the repeated measure of time, $F(9, 90) = 24.49$, $p < 0.001$. Their was neither an overall sex difference ($p > 0.42$) nor a sex difference in the rate of reduction in activity across the session ($p > 0.92$). The day 2 development of conditioned freezing, which described a significant increase across the repeated measure of 10 × 30-s CS blocks, $F(9, 90) = 4.81$, $p < 0.001$, is presented in Fig. 2(a). The average overall amount of freezing across the 10 CS presentations was higher in males than in females, as indicated by the main effect of sex, $F(1, 10) = 6.98$, $p < 0.03$ (inset, Fig. 2a). That the development of conditioning was not significantly different in Fischer males and females is demonstrated by the absence of sex × time interaction ($p > 0.47$, Fig. 2a). During the test of contextual conditioning on day 3, males demonstrated significantly more freezing overall than females, $F(1, 10) = 10.93$, $p < 0.008$ (inset, Fig. 2b), whereas there was no interaction between sex and time ($p > 0.31$, Fig. 2b). During the auditory tone test on day 4, again overall freezing levels were significantly higher in males than in females, $F(1, 10) = 16.70$, $p < 0.002$ (inset, Fig. 2c). A significant sex × time interaction confirmed that both the onset and extinction of freezing expression were more pronounced in males than in females, $F(7, 70) = 2.77$, $p < 0.01$ (Fig. 2c).

Comparing freezing to context and to discrete CS, the average amount of freezing to CS (males: 40%, females: 15%) was approximately twice that of freezing to context (males: 23%, females: 6%); the asymptotic level of expression of CS conditioning (males: 70%, females: 30%) was very similar to

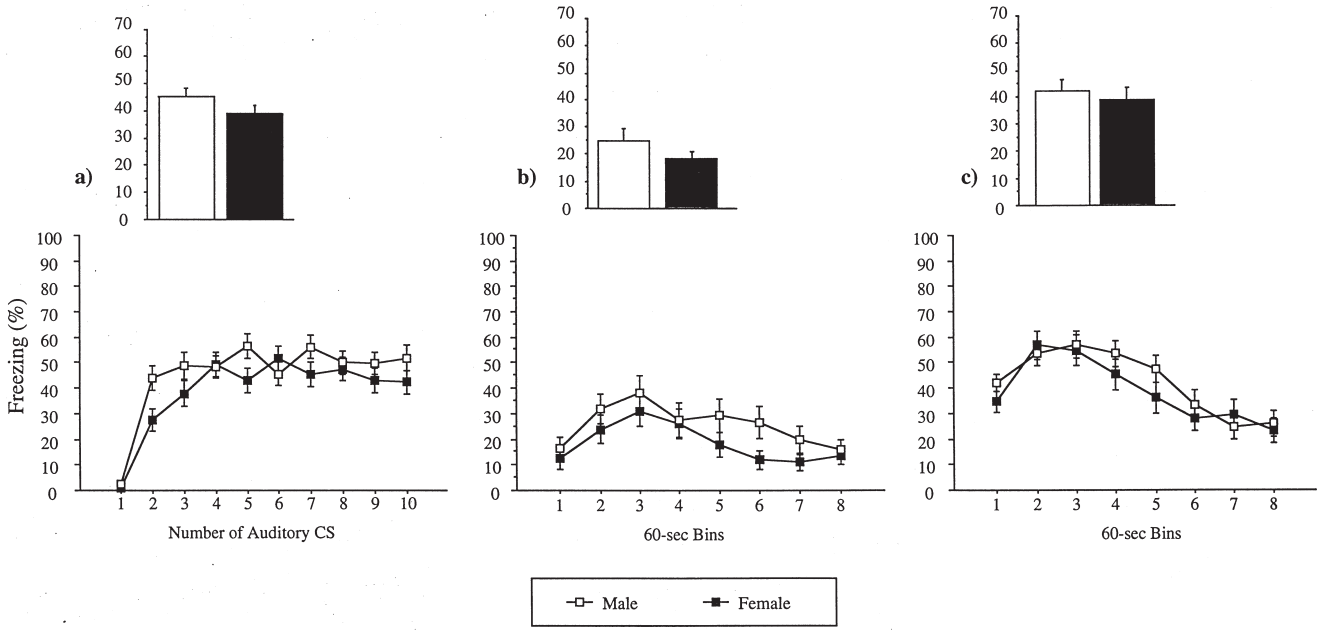

FIG. 1. Percentage time spent in freezing behavior (mean ± SEM) by male and female Wistar rats (n = 8,8) during: (a) day 2—acquisition of fear conditioning during 10 CS-US pairings at 2-min intervals; (b) day 3—8-min test of expression of contextual conditioning; (c) day 4—8-min test of expression of discrete CS conditioning. Inset figures show overall mean ± SEM obtained after collapsing data across time.

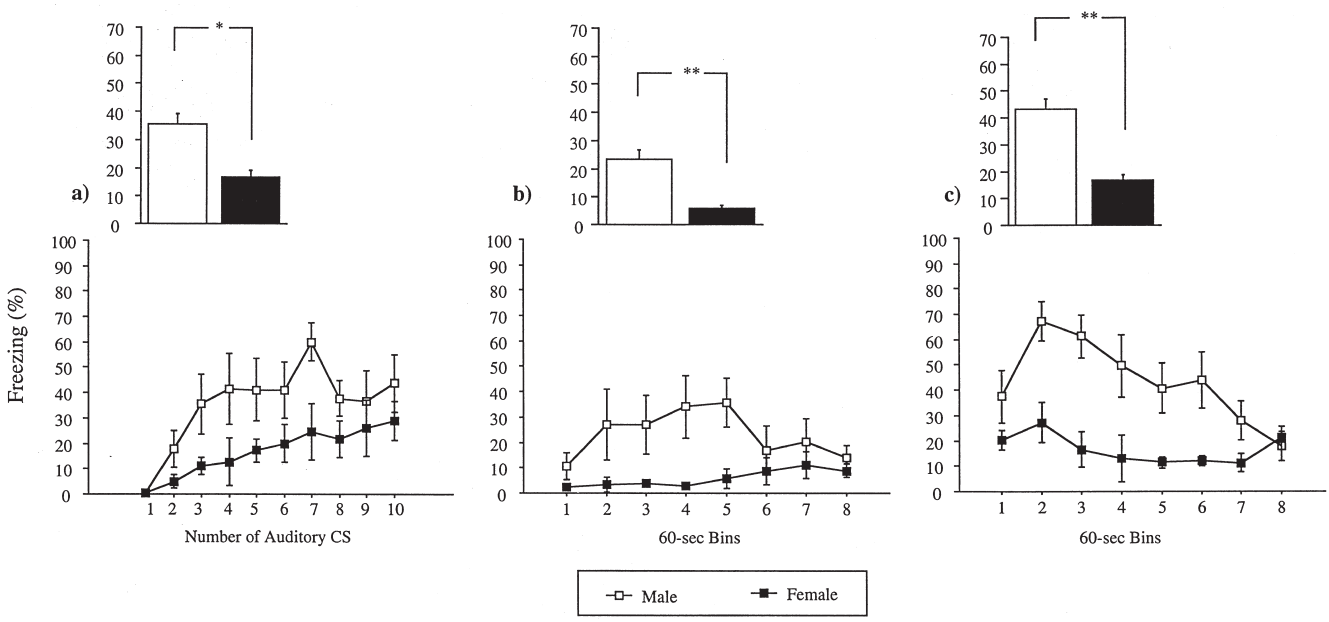

FIG. 2. Percentage time spent in freezing behavior (mean ± SEM) by male and female Fischer rats (n = 6,6) during: (a) day 2—acquisition of fear conditioning during 10 CS-US pairings at 2-min intervals; (b) day 3—8-min test of expression of contextual conditioning; (c) day 4—8-min test of expression of discrete CS conditioning. Inset figures show overall mean ± SEM obtained after collapsing data across time. *$p < 0.05$; **$p < 0.01$.

the asymptotic level of freezing at acquisition (males: 60%, females: 30%) (Fig. 2).

Lewis Males vs. Females

In terms of activity in the shock chamber without the auditory CS on day 1, there was a consistent decline in activity as confirmed by a significant main effect of the repeated measure of time, $F(9, 90) = 18.53$, $p < 0.001$. Their was neither an overall sex difference ($p > 0.18$) nor a sex difference in the rate of reduction in activity across the session ($p > 0.83$). The average overall amount of freezing across the 10 CS presentations was higher in males than in females, as indicated by the main effect of sex, $F(1, 10) = 19.11$, $p < 0.002$ (inset, Fig. 3a). A significant sex × time interaction confirmed that the development of freezing was also different in the two sexes, $F(9, 90) = 2.85$, $p < 0.006$). In male subjects, freezing developed rapidly across CS-US pairings 1–3 to a value of 85% time, reached an asymptote of 90% by pairing 5, and declined only gradually thereafter to an average of 60%; in female subjects, conditioned freezing was also close to asymptote (30–35%) by pairing 3, and remained close to this level until it declined at pairing 9. During the test of contextual conditioning on day 3, both male and female Lewis rats demonstrated low levels of freezing behavior (Fig. 3b). The sexes were almost equivalent in terms of their overall levels of freezing ($p > 0.87$; inset, Fig. 3b), although there was a close to significant sex × time interaction, $F(7, 70) = 2.07$, $p < 0.06$ (Fig. 3b), with females tending to freeze more than males during bins 4–5 and males during bins 6–7. During the auditory tone test on day 4, again, male freezing levels were low compared to conditioning. Also, as during the context test, there was no overall male–female difference ($p > 0.29$); furthermore, there was not a sex × time interaction ($p > 0.85$, Fig. 3c).

Comparing freezing to context and to discrete CS, the average amount of freezing to CS (males: 20%, females: 12%) was approximately twice that of freezing to context (males: 7%, females: 7%); the asymptotic level of expression of CS conditioning (males: 35%, females: 25%) was considerably lower than the asymptotic level of freezing at acquisition in males (90%), but almost equivalent to the acquisition level in the case of females (30%) (Fig. 3).

DISCUSSION

This comparative study of sex differences in aversive conditioning to context and discrete tone CS in Wistar, Fischer, and Lewis rats, carried out using a novel, fully automated apparatus, has revealed some interesting effects, both between males and females and between strains. In the outbred Wistar strain there was no sex difference in the development of conditioning, either in terms of its rate or asymptotic level. Although not statistically significant in either case, there was a marked trend toward increased context conditioning and CS conditioning (slower extinction) in Wistar males relative to Wistar females. In the inbred Fischer strain there were sex differences in the development of conditioning, in contextual conditioning and in CS conditioning. In each case, the asymptotic level of conditioning was enhanced in males relative to females. In the inbred Lewis strain there was a marked sex difference in the rate and asymptote of conditioning development, being greater in males than in females. However, the subsequent expression of conditioning was attenuated markedly in males, resulting in the absence of a sex difference in conditioning at test; this was the case in contextual and CS conditioning. Therefore, in contrast to Maren et al. (12), we did not find any evidence that enhanced expression of fear conditioning in males is limited to contextual conditioning only. In Wistar subjects, males demonstrated strong trends to more context and CS conditioning; in Fischer subjects, males conditioned more to context and CS; and in Lewis subjects, the attenuation of fear conditioning at expression applied to

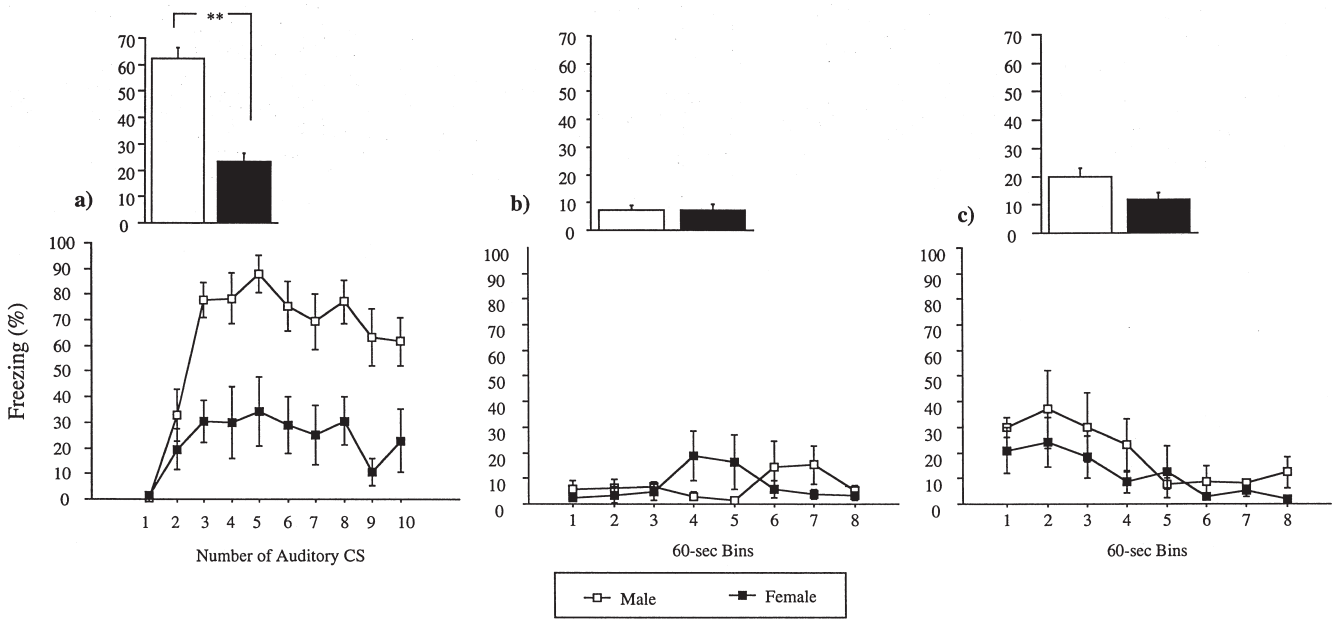

FIG. 3. Percentage time spent in freezing behavior (mean ± SEM) by male and female Lewis rats (n = 6,6) during: (a) day 2—acquisition of fear conditioning during 10 CS-US pairings at 2-min intervals; (b) day 3—8-min test of expression of contextual conditioning; (c) day 4—8-min test of expression of discrete CS conditioning. Inset figures show overall mean ± SEM obtained after collapsing data across time. $^{**}p < 0.01$.

both context and CS. On the basis of the existing evidence that hippocampal LTP is enhanced in males relative to females, the most parsimonious interpretation of our findings is that the hippocampus mediates both contextual and discrete CS aversive conditioning, and contributes to sex differences in both these forms of conditioning, in those strains where these sex differences exist. In line with this suggestion, we have demonstrated recently, using Lister hooded rats and the same automated freezing system (17), that excitotoxic lesions to the hippocampus attenuate the development of conditioning and the expression of both contextual and CS conditioning (Richmond, Pouzet, and Feldon, unpublished data). To the best of our knowledge, sex differences in hippocampal LTP have not yet been investigated in the rat strains reported on here. Furthermore, there are as yet no rat data available on whether or not there are sex differences in amygdaloid LTP. Until this information is available, our interpretation, while parsimonious, is speculative.

Within strain and sex, it is interesting to compare freezing levels across acquisition and the two subsequent days of expression/extinction, in terms of what this reveals about similarities between these strains and between the sexes. In Wistar males and females, Fischer males and females, and Lewis females (but not males, see below), two consistent patterns emerged: first, expression of discrete CS conditioning was approximately twice the level of expression of conditioning to context; second, asymptotic expression of CS conditioning was approximately equal to asymptotic development, or acquisition, of aversive conditioning. This consistency provides strong evidence that the automated freezing system with which this study was performed [see also (17)] permits a high level of consistency and reproducibility across studies. This should prove invaluable for future lesion studies aimed at advancing understanding of the roles of the amygdala and hippocampus in CS and contextual aversive conditioning.

In the Wistar rats studied here there was a strong trend toward enhanced male contextual and CS fear conditioning. Interestingly, a recent study that compared Wistar male and female freezing in the home cage following saline injection as a stressor also demonstrated increased freezing in males (9). In both Fischer and Lewis rats, there was clear evidence for enhanced acquisition of conditioned freezing in males; these findings are in line with the evidence accumulated for outbred strains [e.g., (6,12)] but, to our knowledge, represent the first demonstration of such a sex difference in these two inbred strains. In the case of the Fischer subjects, the tests of conditioning demonstrated sex differences in both contextual and, to a greater extent, CS expression of learning [see also (15)], whereas in the Lewis male subjects conditioning tests demonstrated both low expression and a consequent absence of a sex difference in context and CS learning. Such an apparent combination of highly efficient acquisition but highly deficient compilation of aversive conditioning, as displayed by males of the Lewis inbred strain, is worthy of further investigation. Interestingly, a recent study from this laboratory has described a pronounced deficit in the acquisition of active avoidance (CS = tone) in Lewis rats relative to Fischer rats and, within the Lewis strain, a further pronounced deficit in males relative to females (20). In light of this and of the high levels of freezing acquired by Lewis males in the present study, we can postulate that Lewis rats and Lewis males in particular are highly predisposed to select and perform an inactive response in various aversive situations. Furthermore, Lewis males appear to be highly nonadaptive to aversion: they are apparently deficient both in acquiring an adaptive avoidance response (20) and in storing information acquired about an aversive environment (present study).

In this study we failed to find evidence from any of three strains that supported the results obtained by Maren et al. (12) in Long–Evans rats, i.e., that enhanced fear conditioning

in males is limited to contextual fear conditioning. The most contradictory evidence is provided by the Fischer subjects in which, using a relatively high number of CS-US pairings at a mild shock level [cf. (12)], a distinct enhancement of contextual conditioning in males is accompanied by an equally distinct enhancement in CS conditioning. The evidence from the Wistar subjects is also in this direction as, in statistical terms, a strong trend. The Lewis strain is also nonsupportive in that it fails to identify enhanced male, relative to female, context conditioning. The most parsimonious interpretation of these findings, when taken together with the physiological evidence for sexually dimorphic levels of hippocampal LTP, is that the latter mediates both contextual and discrete CS aversive conditioning, and therefore, contributes to sex differences in both these forms of conditioning in those strains where these sex differences exist. Validation of this interpretation will require studies aimed at comparing LTP in the hippocampus and amygdala, in males and females, in different rat strains.

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