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The Importance of Acclimation in Acoustic Startle Amplitude and Pre-Pulse Inhibition Testing of Male and Female Rats

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FARADAY, M. M., AND N. E. GRUNBERG. *The importance of acclimation in acoustic startle amplitude and pre-pulse inhibition testing in male and female rats.* PHARMACOL BIOCHEM BEHAV **66**(2) 375–381, 2000.—The acoustic startle reflex (ASR) and pre-pulse inhibition (PPI) of the ASR are used extensively to index drug effects in rodents. Important methodological issues exist, however, with regard to the specific procedures and equipment used. In particular, the effects of acclimation to the startle procedure on response stability and the effects of testing animals in groups vs. individually have not been examined but are relevant to data interpretation. The present experiment measured acoustic startle responses with and without a pre-pulse of 25 adult Sprague-Dawley rats (12 male, 13 female) tested individually and in same-sex groups at four time points. Individual testing increased startle responses and PPI of males at time 1 and altered PPI of females at times 1, 2, and 3 compared with group testing. Responses were indistinguishable in the two testing environments at time 4. Results indicate that testing environment may affect responses when subjects have not been acclimated to the testing situation and that there are sex differences in these effects. Because responses stabilized by the fourth testing point, repeated testing of subjects particularly females, may be an important methodological inclusion when evaluating effects of drugs and other manipulations on ASR and PPI. © 2000 Elsevier Science Inc.

Acoustic startle reflex Pre-pulse inhibition Testing environment Sex differences Sprague-Dawley rats

THE ACOUSTIC startle reflex (ASR) and pre-pulse inhibition (PPI) of the ASR are unconditioned behaviors widely used to index pharmacologic manipulations and pinpoint neurobiologic sites of drug action in rodents. Because these responses have robust cross-species validity, ASR and PPI also constitute critical, face-valid tools for animal models that seek to extrapolate to human conditions. For example, the ASR and PPI have been used to examine effects of cocaine and amphetamine (11,17), test anti-psychotic drugs in models of schizophrenia (31,32), examine withdrawal from anxiolytics (25,36), assess genotypic influences on drug responses (9,12,24), model possible attentional effects of nicotine (3,4,14,15), and index sequelae of prenatal drug exposure (28). Increasingly these behaviors also have been included as part of testing batteries in drug behavioral toxicity and neurotoxicity studies (26).

Potentially important methodological issues exist, however, with regard to appropriate testing paradigms that may affect the interpretation of results and the replicability of findings across laboratories. Methodological variables also may be relevant in the broader context of the extent to which findings in animals can be used to understand human conditions. In particular, investigators use varied procedures and different types of equipment to measure ASR and PPI responses. Some investigators report that animals are acclimated to the apparatus and paradigm before experimental testing occurs (3,14,15) whereas others do not report acclimation procedures (5,7,31). Acclimation may be a critical variable in startle testing of rodents because exposure to a novel situation may result in stress, and stress is known to alter ASR and PPI (3,14).

In addition, some investigators use equipment that evaluates subjects' responses in separate, sound-attenuating individual enclosures, whereas other equipment measures responses of individual subjects placed together in one sound-attenuating apparatus. Unlike subjects measured in an

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individual environment, subjects measured in a group environment may be able to see, smell, and hear conspecifics during the testing period. It has been reported, for example, that some rats emit ultrasonic vocalizations when tested in ASR paradigms, presumably as a result of fear (19,20). Whether these vocalizations alter the responses of tested animals is not known. However, if ultrasonic vocalizations are a form of communication, then it is possible that whether animals are tested in groups within the same apparatus vs. individually may affect responses. In addition, rats may react behaviorally and physiologically to the alarm substances emitted by other rats (1,13,18,22,23,35). To the extent that startle testing is a mildly stressful experience, especially in unacclimated animals, olfactory cues such as alarm substances may be present in the testing environment and may be a source of behavioral alterations.

To the extent that familiarity with the testing environment and social factors present in the testing apparatus potentially alter responses, acclimation procedures and testing environment may be critical variables because the manifestation of drug effects can depend on behavioral baselines. That is, drug actions hypothesized to enhance responses are best manifested when behavioral baselines are low, and vice versa. Behavioral drug effects also may depend on the extent to which the animal experiences the experimental situation as stressful (i.e., mild stressors can enhance or suppress drug effects) (14). In addition, within-group variability that might occur as a result of stress or other factors in the testing environment may obscure drug effects that are important but of small magnitude. Further, because investigators generally follow consistent behavioral testing procedures across experiments and may not explicitly examine the effects of alternative testing paradigms, it is relevant to know if commonly used behavioral assays are sensitive to variations in acclimation procedures and the testing environment. Quantifying the effects of acclimation and testing environment variations also is relevant when attempting to replicate work across laboratories and to interpret published work that employs different procedures.

The purpose of the present experiment was to investigate the effects of acclimation exposures to the startle paradigm as well as social factors in the testing environment (individual testing vs. group testing) on ASR responses with and without a pre-pulse in male and female rats using a crossover, within-subject design. The within-subject design was used to minimize error variance across testing points. Because olfactory cues and vocalizations that might influence startle responses have been reported to occur in response to fear or stress, a second purpose of the experiment was to determine if any differences produced by testing environment would disappear over time as subjects acclimated to the testing procedures.

A third purpose of the experiment was to determine whether males and females were differentially affected by acclimation and/or by individual vs. group testing. This purpose was undertaken for several reasons. First, in order to more fully understand gender differences in human responses to drugs and stress, some investigators who use animal models are more frequently including female as well as male rats in their experiments. As the use of female as well as male rats becomes more prevalent, it is important to establish procedures (i.e., acclimation to testing apparatus and protocols) that cleanly quantitate sex differences where they exist. Procedures that have been employed effectively with male rats may need to be altered when using female rats or when comparing male and female rats. In addition, it is possible that laboratory methodologies that potentially produce stress can obscure or, alternatively, exaggerate sex differences in responses. Second, we have previously reported that unconditioned behaviors such as ASR and PPI and locomotion are differentially altered in male and female rats by manipulation of the social environment (i.e., housing animals individually vs. in same-sex groups) (15,16). Whether the social environment is a relevant variable in the testing situation and whether its effects vary in males vs. females, however, is not known.

METHOD

Subjects

Subjects were 25 Sprague-Dawley (12 male, 13 female) rats (Charles River Laboratories, Wilmington, MA, USA). Animals were housed in the same room in same-sex groups of 2 or 3 throughout the experiment in standard polypropylene shoebox cages (42 × 20.5 × 20 cm) on hardwood chip bedding (Pine-Dri). Throughout the study subjects had continuous access to rodent chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. Housing rooms were maintained at 23°C at 50% relative humidity on a 12-h reversed light/dark cycle (lights on at 1900 h). Startle and PPI testing were performed during the dark (active) phase of the light cycle (between 0900 and 1600 h) following the procedures of several investigators (4,14,15,30,34). Startle amplitudes are greater and more stable at this time (8,10). At the beginning of the experiment, subjects were 50 to 55 days old. Average male weight was 284 g and average female weight was 188 g. The experiment was conducted as a 2 (male or female) × 2 (individual testing or group testing) within-subject crossover design.

Equipment

ASR amplitudes and PPI were measured in a Coulbourn Instruments Acoustic Response Test System (Coulbourn Instruments, Allentown, PA, USA) consisting of four weight-sensitive platforms inside a single sound-attenuated chamber. Platforms were arranged radially around central speakers in the chamber's floor and ceiling. For grouped measurements, 3 or 4 same-sex animals were tested simultaneously within the same chamber. For individual measurements, each animal was tested alone in the chamber.

Testing was accomplished by placing each subject individually in a 8 × 8 × 16 cm open air cage that rested on top of the weight-sensitive platform. The open air cages were small enough to restrict extensive locomotion but large enough to allow the subject to turn around and make other small movements. Subjects' movements in response to stimuli were measured as a voltage change by a strain gauge inside each platform and were converted to grams of body weight change following analog to digital conversion. Responses were recorded by an interfaced computer as the maximum response occurring within 200 msec of the onset of the startle-eliciting stimulus.

Following placement of subjects in the chamber, the chamber lid was closed, leaving the subjects in darkness. A 3-min adaptation period occurred in which no startle stimuli were presented. Startle stimuli consisted of 112 or 122 dB SPL (unweighted scale; re: 0.0002 dynes/cm^2) noise bursts of 20 msec duration sometimes preceded 100 msec by 68 dB 1 kHz pure tones (pre-pulses). Decibel levels were verified by a Larson-Davis Sound Pressure Machine Model 2800 (Provo, UT, USA). Each stimulus had a 2-msec rise and decay time such that onset and offset were abrupt, a primary criterion for startle. There were six types of stimulus trials, and each trial type was presented eight times. Trial types were presented in ran-

dom order to avoid order effects and habituation. Inter-trial intervals ranged randomly from 30 to 60 sec. Trial types included 1) 112 dB stimulus, 2) 112 dB stimulus preceded by pre-pulse, 3) 122 dB stimulus, 4) 122 dB stimulus preceded by pre-pulse, 5) pre-pulse only, and 6) no stimulus. The testing period lasted approximately 25 min. A ventilating fan provided an ambient noise level of 56 dB throughout the testing period in order to mask effects of noises from outside the sound-attenuating chamber. Open-air cages were washed with warm water and dried after each use. Males and females were brought into the testing room at the same time but were tested in separate test chambers. Each animal was tested in the same chamber across the four testing points.

Procedure

Subjects were allowed to acclimate to the animal housing facility for several days after arrival. During this period, subjects were handled once each day for 3 days in order to minimize any stress effects that might result from routine handling for behavioral testing. Subjects then underwent an exposure to the startle equipment but not to the startling sounds. This exposure consisted of placing animals in groups of 3 or 4 inside the sound-attenuating chamber in an open air cage for approximately 25 min. Open air cages were washed and dried after each use.

Over the next several weeks, all subjects were tested four times in the startle apparatus with a minimum of three days between each testing session. At each testing point, half of the subjects within each sex were tested alone in the chambers and half were tested in groups of three or four. Testing was done in a counterbalanced fashion (i.e., animals tested alone at time 1 were tested in groups at time 2, tested alone at time 3, and in groups at time 4). This procedure yielded four sets of startle measurements for each subject—two sets of measurements when tested individually and two sets of measurements when tested in a group.

Data Analysis

Each animal's responses were averaged within trial type. Trials during which no stimuli were presented were used to control for normal subject movements on the platform. Amplitudes to each trial type were derived by subtracting grams (g) of platform displacement on the no-stimulus trials (i.e., the body weight of each subject) from g of platform displacement in response to specific stimuli. The remainder from this calculation represented the amount of platform displacement related to the stimulus (e.g., 112 dB, 112 dB with pre-pulse, 122 dB, 122 dB with pre-pulse). Amount of PPI was calculated by subtracting amplitude to trials with a pre-pulse from amplitude to the same decibel level without a pre-pulse. Percent pre-pulse (%PPI) was calculated as [(amplitude of trial without pre-pulse) − (amplitude of trial with pre-pulse)/amplitude of trial without pre-pulse] × 100. The product was analyzed as % PPI. These calculations were based on established procedures of several investigators (3,4,14,15,34). Amount of PPI as well as %PPI were included as relevant variables because different investigators report different measures of PPI.

In order to be sure that variance accounted for by the factor of testing environment did not completely overlap with variance accounted for by the factor of repeated testing (i.e., test day), an initial analysis of variance (ANOVA) was run with factors of stimulus type, sex, testing environment, and test day. This analysis also was undertaken to ensure that different groups of stimuli (startle stimuli alone, amount of PPI, %PPI) were sufficiently different from one another that they could reasonably be analyzed in separate multivariate analyses of variance (MANOVAs). Then, at each time point separate global MANOVAs were performed on startle amplitudes, amount of PPI, and on %PPI with factors of sex and testing environment. Separate MANOVAs also were performed within each sex to assess for effects of testing environment. All tests were two-tailed with alpha < 0.05 unless otherwise indicated.

RESULTS

Figs. 1a and 1b present startle amplitudes to 112 and 122 dB without pre-pulses at times 1 and 2. Figs. 2a and 2b present startle amplitudes to 112 and 122 dB without pre-pulses at times 3 and 4. Table 1 presents amount of PPI to 112 and 122 dB at times 1, 2, 3, and 4. Table 2 presents %PPI to 112 and 122 dB at times 1, 2, 3, and 4.

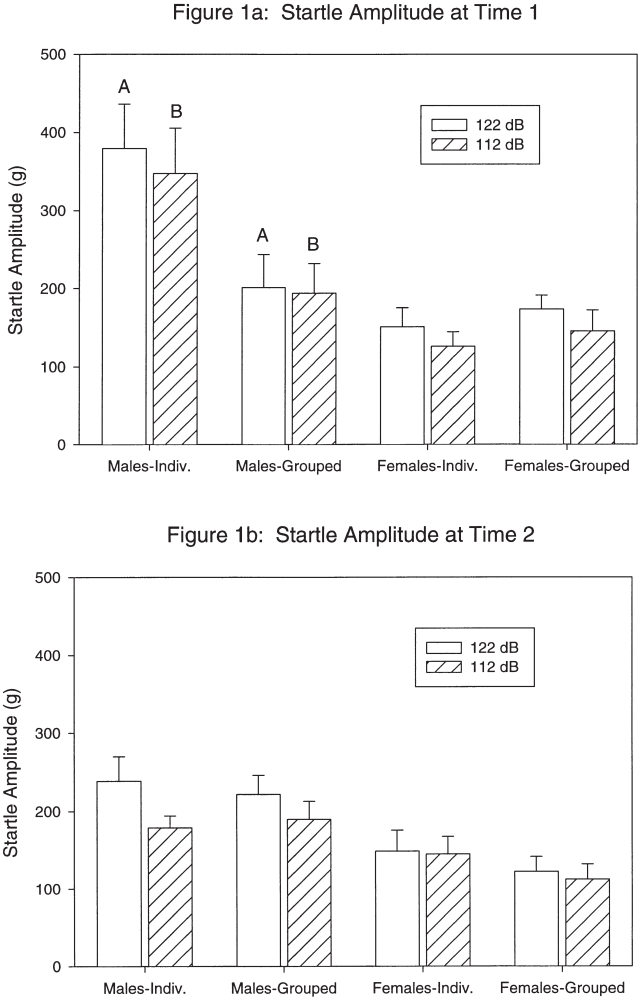
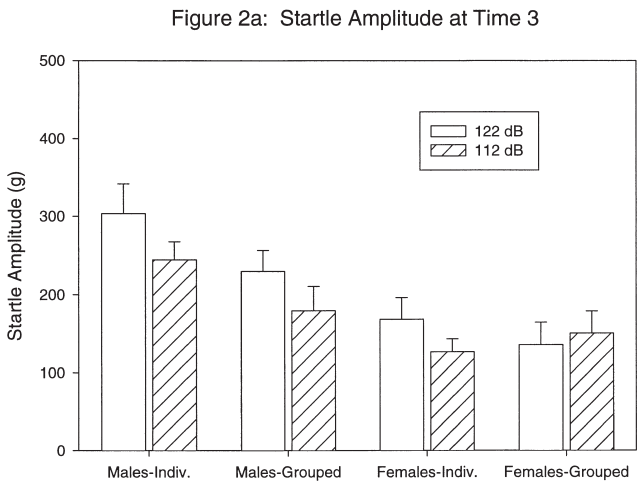


FIG. 1. (A) Startle amplitudes in g (group means ± SEM) to 122 and 112 dB without pre-pulses of male and female rats tested individually or in same-sex groups (n of 6 or 7 per group) at time 1. Letters indicate statistically significant differences between individual- and group-tested animals within-sex. (B) Startle amplitudes to the same stimuli following the same procedures at time 2.



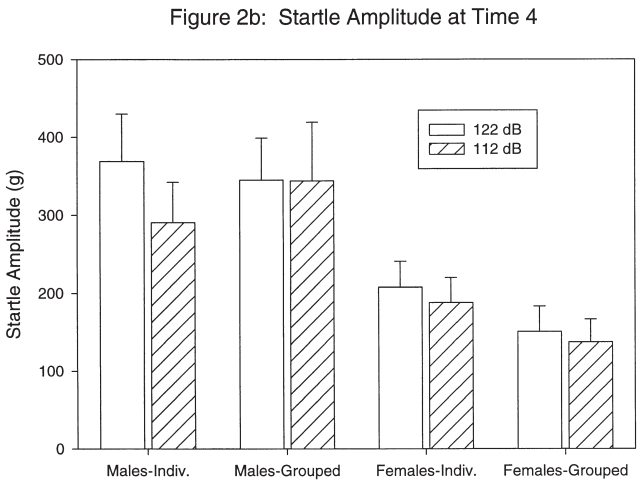


FIG. 2. (A) Startle amplitudes in g (group means ± SEM) to 122 and 112 dB without pre-pulses of male and female rats tested individually or in same-sex groups (n of 6 or 7 per group) at time 3. (B) Startle amplitudes to the same stimuli following the same procedures at time 4.

The initial ANOVA with factors of stimulus type, sex, testing environment, and test day revealed significant main effects for stimulus type [$F(5, 504) = 108.98$], sex [$F(1, 504) = 92.95$], and test day [$F(3, 504) = 9.64$]. In addition, there were significant interactions of sex X test day [$F(3, 504) = 3.05$], sex X testing environment [$F(1, 504) = 11.47$], sex X stimulus type [$F(5, 504) = 19.90$], test day X stimulus type {$F(15, 504) = 2.07$], and sex X test environment X stimulus type [$F(5,5\ 04) = 3.29$]. Because these significant main effects and interactions indicated that separate proportions of variance were accounted for by the factors of testing environment and test day, further specific MANOVAs on data from each test day were pursued.

Time 1

At the first testing point, global MANOVA on startle amplitudes revealed: main effects of sex on both stimuli [122 dB: $F(1, 21) = 11.58$; 112 dB: $F(1, 21) = 13.10$] with males startling more than females; main effects of testing environment on responses to the 122 dB stimulus [$F(1, 21) = 4.29$] with individually tested animals startling more than group-tested animals; and sex X Testing environment interactions in responses to both stimuli [122 dB: $F(1,21) = 7.13$; 112 dB: $F(1,21) = 5.40$] such that females responded similarly regardless of testing environment but individually tested males exhibited greater responses than group-tested males. Global MANOVA on amount of PPI revealed main effects of sex on both stimuli [122 dB: $F(1, 21) = 8.13$; 112 dB: $F(1, 21) = 5.60$] such that males exhibited greater amount PPI than did females; and a main effect of testing environment on the 122 dB stimulus [$F(1, 21) = 7.21$] such that individually tested animals exhibited greater PPI amounts than did group-tested animals with a trend for the same pattern to the 112 dB stimulus [$F(1, 21) = 3.25$; $p = 0.086$]. Global MANOVA on %PPI to the two stimuli did not reveal any differences.

Because males and females differed significantly on most measurements, further separate MANOVAs on males and females were performed. These analyses revealed at testing time 1: main effects for testing environment on startle amplitude of males to both stimuli [122 dB: $F(1, 10) = 6.33$; 112 dB: $F(1, 10) = 4.91$] such that males tested individually startled significantly more than did males tested in groups; a main effect of testing environment on amount PPI of males to the 122 dB stimulus [$F(1, 10) = 7.62$] such that individually tested males exhibited greater PPI amounts than did group-tested males; and a main effect of testing environment on % PPI of females to the 112 dB stimulus [$F(1, 11) = 4.94$] such that individually tested females had significantly greater % PPI than did group-tested females.

Time 2

At the second testing point, global MANOVA on startle amplitudes revealed main effects of sex on both stimuli [122 dB: $F(1, 21) = 13.85$; 112 dB: $F(1, 21) = 7.52$] with males startling more than females. Global MANOVA on PPI amounts revealed a trend for a main effect of sex to the 122 dB stimulus [$F(1,21) = 3.53$; $p = 0.074$] such that males tended to exhibit greater PPI amounts than did females. Global MANOVA on %PPI to the two stimuli did not reveal any differences.

Because males and females differed significantly on most measurements, further separate MANOVAs on males and females were performed. These analyses revealed that at time 2, differences as a result of testing environment remained in females but had disappeared in males. Specifically, MANOVAs revealed main effects of testing environment among females, with group-tested females having greater %PPI to the 112 dB stimulus than individually tested females [$F(1, 11) = 4.84$], with a trend for the same pattern to the 122 dB stimulus [$F(1, 11) = 3.82$, $p = 0.076$].

Time 3

At the third testing point, global MANOVA on startle amplitudes revealed: main effects of sex on both stimuli [122 dB: $F(1,2\ 1) = 14.07$; 112 dB: $F(1, 21) = 8.74$] with males startling more than females; and a trend for a sex X testing environment interaction in responses to the 112 dB stimulus [$F(1, 21) = 3.22$, $p = 0.087$] such that females startled similarly across testing conditions but individually tested males tended to startle more than group-tested males. Global MANOVA on PPI amounts revealed a main effect of testing environment on PPI amounts to the 122 dB stimulus [$F(1, 21) = 4.73$] such that individually tested amounts exhibited greater PPI

TABLE 1

AMOUNT PPI IN GRAMS (MEAN AND +/−SEM) TO 122 AND 112 DB WITH PRE-PULSE OF MALE AND FEMALE RATS AT TIMES 1, 2, 3, AND 4

			TIME 1	TIME 2	TIME 3	TIME 4
MALES	Indiv.	122 dB	181.23 ± 27.85	149.33 ± 23.38	175.10 ± 28.61	188.71 ± 41.68
		112 dB	165.50 ± 39.32	116.56 ± 13.73	144.69 ± 31.16	140.88 ± 39.98
	Group	122 dB	96.81 ± 12.67*	113.63 ± 31.57	118.69 ± 24.15	150.83 ± 29.47
		112 dB	92.85 ± 20.92	105.42 ± 24.06	119.02 ± 18.69	203.34 ± 50.46
FEMALES	Indiv.	122 dB	93.54 ± 16.82	85.06 ± 19.05	133.75 ± 23.67	121.02 ± 22.10
		112 dB	79.23 ± 12.78	93.05 ± 16.18	100.87 ± 12.70	123.77 ± 24.26
	Group	122 dB	72.11 ± 18.99	91.02 ± 17.43	84.37 ± 19.72	104.45 ± 24.39
		112 dB	64.67 ± 18.33	88.32 ± 18.84	110.84 ± 21.79	100.68 ± 22.41

*Asterisks indicate significant pairwise difference between responses of the designated cell and responses of same-sex individually-tested animals to the same stimulus (e.g., 112 dB or 122 dB).

amounts than did group-tested animals. Global MANOVA on % PPI revealed significant main effects of sex on responses to the two stimuli [122 dB: $F(1, 21) = 3.81$, $p = 0.06$; 112 dB: $F(1, 21) = 10.35$] such that females exhibited greater % PPI than did males.

Because of these sex differences, further separate MANOVAs on males and females were performed. These analyses revealed that at time 3, testing environment effects persisted among females and were absent among males. Specifically, individually tested females exhibited significantly greater % PPI to the 122 dB stimulus than did group-tested females [$F(1, 11) = 20.97$].

Time 4

At the fourth testing point, global MANOVA on startle amplitudes revealed main effects of sex on both stimuli [122 dB: $F(1, 21) = 14.92$; 112 dB: $F(1, 21) = 9.82$] with males startling more than females. Global MANOVA on PPI amounts revealed a trend for a main effect of sex on PPI amounts to the 122 dB stimulus [$F(1, 21) = 3.61$; $p = 0.07$] such that males tended to exhibit greater PPI amounts than did females. Global MANOVA on % PPI revealed a significant main effect of sex on responses to the 112 dB stimulus [$F(1, 21) = 4.06$; $p = 0.057$] such that females exhibited greater % PPI than did males. Further analyses conducted on males and females separately revealed no differences among males or females in ASR and PPI responses as a result of testing environment at time 4.

DISCUSSION

The present experiment examined the effects of progressive exposure to the startle paradigm as well as testing environment—individual vs. group testing—on acoustic startle and PPI responses of male and female rats over four time points. Testing environment altered responses differentially in males and females. These sex differences were evident in the time course of effects and in the particular variable (ASR or PPI) affected.

Male rats tested individually startled more and exhibited greater PPI amounts at time 1 than did males tested in groups. Male responses were not affected consistently by testing environment over the next three time points. It is worth noting, however, that an increase in male startle as a result of individual testing at time 3 was suggested by a significant sex X testing environment interaction. Although this finding was not supported by a significant main effect of testing environment when males were analyzed separately, greater statistical power (i.e., more than 6 subjects per group) might have revealed this pattern more clearly.

In contrast, female startle responses were not altered by testing environment throughout the experiment but female PPI variables were influenced by testing environment at times 1, 2, and 3. At times 1 and 3, individually tested females had greater %PPI than did group-tested females. At time 2, females tested in groups had greater %PPI than did females tested individually. By the fourth exposure to the testing situation, female re-

TABLE 2

PERCENT PPI (MEAN AND +/−SEM) TO 122 AND 112 DB WITH PRE-PULSE OF MALE AND FEMALE RATS AT TIMES 1, 2, 3, AND 4

			TIME 1	TIME 2	TIME 3	TIME 4
MALES	Indiv.	122 dB	51.91 ± 9.89	63.15 ± 6.73	58.80 ± 7.08	53.14 ± 7.39
		112 dB	49.21 ± 10.51	66.09 ± 6.78	57.01 ± 8.34	49.99 ± 10.44
	Group	122 dB	52.43 ± 5.38	52.23 ± 12.11	54.72 ± 11.29	48.70 ± 9.41
		112 dB	45.69 ± 11.68	59.31 ± 12.57	67.58 ± 2.04	59.17 ± 8.48
FEMALES	Indiv.	122 dB	61.02 ± 3.54	56.10 ± 6.51	79.15 ± 2.20	61.54 ± 8.34
		112 dB	63.39 ± 3.99	62.36 ± 5.74	80.45 ± 2.33	66.42 ± 6.67
	Group	122 dB	45.14 ± 12.07	71.95 ± 5.03	60.62 ± 3.55*	61.47 ± 11.14
		112 dB	43.90 ± 8.29*	77.10 ± 3.80*	74.90 ± 4.27	73.11 ± 3.97

*Asterisks indicate significant pairwise difference between responses of the designated cell and responses of same-sex individually-tested animals to the same stimulus (e.g., 112 dB or 122 dB).

sponses apparently had stabilized such that no differences were detected between animals tested alone or in groups.

These results indicate that progressive exposure to the startle paradigm as well as social factors in the testing environment can affect startle and PPI and that there are sex differences in the extent and nature of these effects. The methodological implications of these findings, therefore, may be different for male vs. female rats. In particular, for investigators who use male rats it is important to be aware that individual testing procedures produce a markedly enhanced startle response at the first testing exposure. This enhancement may be particularly relevant to interpretation of experimental findings when animals are tested repeatedly for responses to the same drug or to different drugs and when baseline procedures do not include an acclimation exposure to the testing protocol. Depending on the drug, an enhanced initial response produced by the testing situation may be erroneously attributed to drug actions or may result in failure to detect a drug action. In order to obtain stable startle responses in male rats, investigators should incorporate at least one acclimation exposure to the startle paradigm before beginning testing for experimental purposes.

The fact that male startle responses and PPI amounts were enhanced at time 1 by individual testing is consistent with the interpretation that animals were experiencing stress as a result of initial exposure in isolation to the procedure. Several investigators have reported that startle and PPI of male rats is enhanced by other stressors, such as immobilization (3,14). That male startle amplitudes when tested individually were less at time 2 than at time 1, despite the fact that the animals were larger and had greater muscle mass at time 2, also supports the interpretation that the time 1 startle enhancement was the result of stress.

For females, effects of testing environment were manifested as changes in PPI at times 1, 2, and 3. These findings indicate that when using female rats, at least three exposures to the full testing situation are required for PPI to stabilize. These changes in PPI are not as easily interpreted, in part, because the directionality of effects varied (i.e., at times 1 and 3 individual testing increased PPI but at time 2 individual testing decreased PPI) and because it has been reported that female rat startle and PPI are not altered by mild stressors (14,27). It is possible that females were more sensitive than males to the presence or absence of social cues in the testing environment during the first three testing exposures and that these cues altered responses inconsistently. It also is possible, however, that these alterations in female PPI responses over the course of the experiment interacted with changes in responsivity that occur with changes in the estrous cycle (21). Specifically, it has been reported that PPI is reduced in females during the proestrus phase (21). We have observed that females housed in the same housing room over extended periods of time tend to cycle together (unpublished data). It may be that by the fourth measurement point females were largely cycling in synchrony, consequently eliminating possible estrous-cycle induced differences in PPI and resulting in PPI consistency among females across testing environments. It also may be relevant that because males and females were housed and tested in the same rooms, male responses may have been affected by the estrus cycle stage of females. Future studies will examine this possibility.

As with males, the effects of testing environment on females may be relevant when interpreting behavioral changes as the consequence of drug actions. If female PPI responses are altered during early testing exposures by the testing environment or an interaction of testing environment with estrous cycle, then it may be important to control for these potential sources of variability.

It also is worth noting that males and females exhibited similar %PPI at times 1 and 2 but that females exhibited greater %PPI than did males at times 3 and 4. This result contrasts with reports that male and female rats exhibit similar %PPI (30) except during the proestrus phase when female %PPI is reduced compared with males (21). It is possible that differences in acclimation procedures are at the root of these contrasting findings. In the cited study (30), animals appear to have been tested only once and without acclimation to the experimental situation. These results would have been obtained, therefore, under circumstances analogous to our time 1 findings—when no sex differences in PPI were noted.

The issues of startle and PPI stability and possible sex differences in PPI also are relevant when extrapolating from animal startle and PPI to human startle and PPI. Several studies in humans have revealed that although individuals vary widely in startle and PPI, these responses are stable over time within individuals and constitute a neurobiological marker that is reliable and sensitive to individual differences (2,6,29). Although reliability was not specifically assessed in the present experiment (because of the crossover design), findings suggest that maximum ASR and PPI stability is obtained in animal subjects after multiple exposures to the testing situation. This possible difference between animals and humans may occur because of factors inherent in the use of animal vs. human subjects: experimental procedures can be explained to humans to minimize stress and novelty, but "explaining" procedures to animals can be accomplished only by familiarizing the animal with the experimental situation.

With regard to human and animal sex differences, the human literature indicates that men generally exhibit greater %PPI than do women (30) and that this difference is most pronounced during the luteal phase of the menstrual cycle (33). The present experiment revealed that PPI differences between male and female rats emerged after two exposures to the testing situation, with females exhibiting greater % PPI than males—a pattern that contrasts with reports in humans regardless of possible estrus cycle effects. It may be relevant that studies reporting that men exhibited greater PPI than women have used a single testing session. Further, studies assessing human response stability over multiple sessions either have not used female subjects (2,6) or have used male and female subjects but did not assess for sex differences (29). It is not clear, therefore, whether this difference in humans is stable over time, disappears, or reverses with repeated testing. The extent to which species-specific differences exist in ASR and PPI—where rats diverge from humans—however, is important for methodological issues (i.e., how should studies be designed and run) as well as conceptual reasons (i.e., how should data obtained in rats be used to inform the human condition, and vice versa).

Taken together, the findings from the present experiment indicate that when evaluating published data, the sex of rat tested and whether or not an experiment included acclimation exposures to the startle procedures before experimental testing are critical pieces of information. Further, these findings suggest that investigators should routinely report acclimation procedures. These findings also indicate that repeated testing of subjects during a nontreatment baseline period may be an important methodological inclusion for the reliable evaluation of drug effects and other manipulations on

ASR and PPI. In addition, these results suggest that comparability across laboratories that employ different types of equipment can be obtained by incorporating at least one acclimation exposure to the full testing procedure (including the stimuli planned for experimental use) for male rats and at least three acclimation exposures to the full testing procedure for female rats.

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