Behavioral Neuroscience
1992, Vol. 106, No. 5, 846-852

0735-7044/92/$3.00

Circadian Modulation of the Rat Acoustic Startle Response

Christopher C. Chabot and Douglas H. Taylor
Zoology Department, Miami University

The acoustic startle response (ASR) of male rats was measured during several sessions over a 24-hr period in both a light–dark cycle and a constant-dark condition. Each session consisted of 10 trials each at 80, 90, 100, 110, and 120 dB white noise. The results indicate robust daily and circadian modulation of ASR amplitude that consist of an approximately twofold nocturnal increase at eliciting-stimuli intensities above 80 dB. Similar results were observed in female rats in constant-dark conditions. To determine whether daily changes in auditory thresholds were responsible for the observed modulation, ASR reflex modification procedures were used. These procedures were designed to measure auditory thresholds at frequencies of 10 and 40 kHz at several times of day. The results suggest a lack of significant circadian differences in auditory thresholds at these frequencies. This study demonstrates a novel role of the rat circadian system in the modulation of ASR amplitude.

The mammalian acoustic startle response (ASR) consists of a stereotypical neuromuscular reflex in response to a loud acoustic stimulus. A direct neural pathway underlying this response has been elucidated (Davis, Gendelman, Tischler, & Gendelman, 1982). In addition, much is known about the physical parameters that are required to elicit a startle response (cf. Hoffman, 1984). For example, measurable responses persist after hundreds of exposures to eliciting stimuli (ES) with little decrement in amplitude (Davis, 1970). In addition, ASR amplitude can be modified by antecedent sensory stimuli: Sound (Hoffman & Wible, 1970), light (Ison & Hammond, 1971), or somatosensory stimuli (Pinckney, 1976) immediately before the ES can reduce ASR amplitude. Because this phenomenon occurs without prior training (Hoffman, 1984), it is known as reflex modification and, by presenting an appropriate range of auditory prepulses, can be used to quickly and accurately determine auditory thresholds in rats (Crofton, 1990). These characteristics, combined with the ease of ASR measurement using computer-controlled experiments, have made the ASR a favorite tool of study for many pharmacologists, toxicologists, and reflex and sensory physiologists.

Modification of ASR amplitude can also be effected by different internal behavioral states (e.g., fear; Davis, 1986) and by photoperiodic phase. Horlington (1970) and Davis and Sollberger (1971) found significant increases in ASR amplitude during the dark phase (D) versus the light phase (L) of the light–dark (LD) cycle in male rats. However, the rats in the Davis and Sollberger study were not only maintained on an LD cycle, but the testing was also performed in a lighted or a darkened startle chamber depending on the appropriate condition in the colony room. Subsequent experiments (conducted during L) indicated that the lighting conditions during ASR testing may affect ASR response amplitude (Ison, Bowen, & Kellog, 1991; Ison & Hammond, 1971). These findings suggest that the LD amplitude differences seen by Davis and Sollberger may have been due in part to the photic conditions during ASR measurement. However, the experiments by Ison and his colleagues (Ison et al., 1991; Ison & Hammond, 1971) indicated changes in response amplitudes due to illumination changes immediately (milliseconds to seconds) before presentation of the startle ES. In contrast, Davis and Sollberger found a peak elevation of startle several hours after the onset of D and a peak depression of startle several hours after the onset of L. Thus, the results from Davis and Sollberger clearly indicate temporal modulation of ASR amplitude during L and D. Horlington found significant LD ASR amplitude differences in two groups of female rats exposed to a single ES, one group during mid-L and the other during mid-D. Although the two groups were housed separately, the startle chamber photic conditions were reported as "controlled," and significant LD ASR amplitude differences were found. In addition, Chabot and Taylor (1992) found significant (50–100%) increases in female rat ASR nighttime versus daytime amplitudes measured in dark chambers. These findings demonstrate a robust daily modulation of ASR amplitude in female rats.

Daily modulation of other behavioral activities have also been described in rats, including wheel-running activity, feeding, and drinking (cf. Rusak, 1981). Many of these behavioral rhythms also persist in constant environmental conditions with periods of approximately 24 hr and thus are endogenously generated circadian rhythms. These rhythms are mediated by an internal oscillator or clock that can be entrained by environmental stimuli, especially photic conditions (Daan & Pittendrigh, 1976). Although the amplitude of the female rat ASR exhibits robust changes in an LD cycle (Chabot & Taylor, 1992; Horlington, 1970), its circadian control is an open question. In the experiments presented here, we measured

This research was supported by an Academic Challenge Grant from the State of Ohio to the Department of Zoology, Miami University.

We sincerely thank Lynn Johnson and John Morrow, who designed and assembled the apparatus; Kevin Crofton for his expert advice on measuring startle responses; Joe Simpson and Jon Patton for writing the data analysis software; and Kirk Larsen and Tilghman Hall for their critical review of the manuscript of this article.

Correspondence concerning this article should be addressed to Christopher C. Chabot, Zoology Department, Biological Sciences Building, Miami University, Oxford, Ohio 45056.

ASR amplitude four times over a 24-hr period in female rats exposed to constant darkness (DD). In addition, because most ASR research involves male rats, we measured the ASRs of males in both LD and DD. We also began to attempt to determine the site(s) in the ASR neural pathway that is modulated by the circadian system. Using a reflex modification procedure, we attempted to determine whether the thresholds of the sensory neurons in the cochlea are modulated by the circadian system.

Materials and Method

Environmental Conditions

Male (n = 12; 70–160 days old) and female (n = 12; 70-140 days old) Sprague-Dawley rats were housed in standard plastic laboratory cages (20 × 20 × 40 cm) in a light-tight, ventilated chamber on an LD cycle for at least 2 weeks before being exposed to startling stimuli. Males and females were not concurrently housed in the same chamber. Both females (lights on at 0600 hr, lights off at 1800 hr) and males (lights on at 0300 hr, lights off at 1500 hr) were held in a 12:12-hr LD cycle. All animals were housed 2 per cage except for females used in the threshold determination experiment (housed 3 per cage). Food (Purina rodent chow, Diet 5001) and water were available ad libitum except during the startle procedure.

ASR Apparatus

ASRs were measured in four identical, sound-attenuating chambers. A more complete description of the apparatus has been previously published (Chabot & Taylor, 1992). Briefly, each chamber contained a wire cage (8 × 4.5 × 4.5 cm) that was mounted on a force transducer (Coulborn Instrs., Model 45-15, Columbus, OH) and one speaker (Realistic Super Tweeter, Catalog No. 40-1310B) that was situated at the level of the animal's head for the delivery of ESs. The speakers were calibrated using a Bruel and Kjaer microphone (Model 4136) with a Type 2633 preamplifier (Bruel and Kjaer, Marlborough, MA). The ESs consisted of a white noise burst (42 ms in duration, 0.15 ms exponential rise–fall time). Background noise intensity in the startle chambers was 56 dB (A; sound level meter Model 215, Quest Electronics, Oconomowoc, WI). A Macintosh SE/30 computer with Labview software (National Instrs. Co., Austin, TX) was used to create virtual instruments that controlled the analog-to-digital converter, tone generator, digital output instruments (GW Instrs., Models MacADIOS 8ain, fg, and 8dio, Somerville, MA) and custom-designed electronic hardware. This system monitored and recorded the output from the force transducer at 1-ms intervals for 100 ms after ES onset. By convention, force was converted to grams (1 g = 0.01 N) and is presented as such in this article. The digitized output was converted to grams on the basis of calibration curves that were determined for each transducer. The baseline value of the animal on the transducer (in the absence of an ES) was calculated as the mean output sampled at 1-ms intervals for 1 s before ES onset (this value represented the body weight of the rat plus the weight of the wire cage). ASR amplitude was determined by subtracting the mean baseline value from the maximum force exerted on the transducer within a 125-ms window after ES onset that exceeded the mean baseline value by four standard deviations. If baselines were not exceeded by four standard deviations within the 125-ms data window, then a value of zero was recorded.

Procedure

At specific times during LD or DD, rats were removed from the light-tight chamber and placed into a wire cage. The cage was designed to allow the animal to orient in only one of two directions, both of which kept the animal's ears at a fixed distance from the speaker. The cage was placed on a force transducer next to a high-frequency speaker in a dark, sound-attenuating chamber. Ten minutes later, an ASR session was initiated. Rats were handled in the dark with the aid of an infrared viewer (Electrophysics Corp., Nutley, NJ).

Experiment 1: Amplitudes

A: Males in LD. The objective of this first experiment was to characterize ASR amplitude in an LD cycle. Rats were exposed to four ASR sessions over a 24-hr period. During each session the rats were exposed to 50 ES trials, the intensity of which varied in a semirandom but balanced fashion (10 trials each of 80, 90, 100, 110, and 120 dB; 50 trials total) and immediately placed back into their cages (because the rats responded measurably only 60% of the time to 80 dB, we do not report the data here). Because there is evidence that the first ASR session yields generally larger response amplitudes than do subsequent sessions (Davis, 1972), these rats were preexposed to startling stimuli 2 days before this experiment (this property could have caused an initial session bias independent of time of day). In the preexposure session, half of the rats were exposed to a startle session (50 trials as just described) just before lights off, and half were exposed to a session just after lights off. The ASRs of male rats housed in an LD cycle were first measured at 0700 hr (L) and then at 1100 hr (L), 1900 hr (D), and 2300 hr (D). One week later, this order was changed; ASRs were first measured at 1900 hr (D) and then at 2300 hr (D), 0700 hr (L), and 1100 hr (L). We performed the experiment in this way to ensure that the habituation or sensitization processes (or both) that may underlie the ASR (Davis, 1972) were not solely responsible for the LD amplitude differences observed. Because significant LD differences ($p < .05$) were observed in both of these experiments, the data for each animal from these two experiments were combined by time of day, and these data were analyzed as described in the Statistical Analysis section.

B: Males and females in DD. Rats were held in DD for 24 hr before being exposed to ESs. On the 2nd day of exposure to DD, the rats were removed from the light-tight chamber and exposed to startling stimuli as described earlier. Because we did not have an independent measure of circadian phase (i.e., wheel-running activity or another behavior known to be controlled by the circadian system), our experimental design was based on the assumption that the circadian systems of this group of rats remained in phase with one another over the course of 2 days, an assumption generally valid in rats (Redman, Armstrong, & Ng, 1983). Thus, although the times reported here are Eastern Standard Time, we have assumed that these times are also approximately the same as the rats' internal (subjective) circadian time in relation to the preceding LD cycle. As in Experiment 1A, the rats were exposed to four sessions over a 24-hr period. These rats were also preexposed to startling stimuli 1 week before this experiment. The preexposure sessions exactly duplicated the first series of (four) DD sessions described here. (a) The ASRs of male rats previously housed in LD were first measured at 0700 hr (subjective light [SL]) and then at 1100 hr (SL), 1900 hr (subjective dark [SD]), and 2300 hr (SD). (b) One week later this order was changed; ASRs were first measured at 1900 hr (SD) and then at 2300 hr (SD), 0700 hr (SL), and 1100 hr (SL). Again, we performed the experiment in this way to ensure that the habituation or sensitization processes (or both) that may underlie the ASR (Davis, 1972) were not solely responsible for the DD amplitude differences observed. Because significant DD differences ($p < .05$) were observed in both of these experiments, the data from these two experiments were combined by time of day and analyzed as described in the Statistical Analysis section. Females were treated in exactly the same way and tested at the same phases in relation to the LD cycle (although at different times). Thus, females were initially tested at 1000 hr (SL) and then at 1400 hr (SL), 2200 hr (SD), and 0200 hr (SD).

One week later, females were first tested at 2200 hr (SD) and then at 0200 hr (SD), 1000 hr (SL), and 1400 hr (SL).

Experiment 2: Threshold Determination via ASR Amplitude Modification

A: Males in LD and DD. This experiment was designed to test the possibility that the significant amplitude differences observed in an LD cycle (males: Experiment 1A; females: Chabot & Taylor, 1992) and in DD (Experiment 1B) may be due, at least in part, to daily changes in auditory sensitivity. The experimental paradigm is based on the finding that audible sounds presented just before an ES will inhibit startle amplitude in an intensity dependent manner (Crofton, 1990). Thus, if the rat can hear the prepulse, then the response will be reduced in relation to an ES (blank) trial alone. This reflex modification of the ASR has been used as an accurate method to determine auditory thresholds in rats (Crofton). Threshold determination is accomplished by pairing prepulses of varying intensity at a given frequency with an unchanging ES. When responses are calculated as a percentage of the blank trial (ES only) and plotted against prepulse intensity, a segmented curve is generated. The breakpoint of the segmented line is defined as the threshold at that particular prepulse frequency.

Twelve male rats were used in this experiment and were exposed to four sessions over 24-hr period (0700 hr, 1100 hr, 1900 hr, and 2300 hr). During each session the rats were exposed to prepulses that varied by both frequency (10 and 40 kHz) and intensity (four trials each at 0, 15, 30, 45, 60, 75, and 90 dB) in a semirandom but balanced fashion, whereas the ES was of constant intensity (120 dB) and frequency (white noise). Both prepulses and ESs had rise–fall times of 0.15 ms. The rats were placed into the ASR chambers and handled in the dark as described earlier. The effects of time of day on auditory thresholds were first determined for rats held in an LD cycle. Five weeks later, the effects of circadian time on auditory thresholds were determined for the same rats 24 hr after exposure to DD.

B: Females in LD. This experiment was designed to test the possibility that auditory thresholds may be modulated in females at the same times of day as those tested previously in males. We also tested the possibility that auditory thresholds may be modulated at other times of the day in females. Sixteen female rats were used in this experiment and were tested on 3 separate days separated by at least 2 weeks as follows: Day 1–8 rats were tested at 0900 hr, 8 others were tested at 2100 hr; Day 2–8 rats were tested at 0900 hr, the same 8 were tested later at 1900 hr; 8 different rats were tested at 1200 hr, the same 8 were tested later at 2300 hr; Day 3–4 were tested at 1415 hr, 4 others at 1545 hr, 4 others at 0215 hr, and 4 others at 0345 hr. During each session the rats were exposed to prepulses that varied by both frequency (10 and 40 kHz) and intensity (five trials at each of 16 different decibels of prepulses [blank and 2–86 dB in approximately 6-dB increments]) in a semirandom but balanced fashion, whereas the ES and other prepulse parameters were the same as described earlier. The rats were placed into ASR chambers and handled in the dark as described earlier.

Statistical Analyses

Amplitudes

In Experiment 1, mean amplitude responses were determined for each animal for each block of 10 trials at a given decibel intensity level. These means were then averaged for stimulus intensity and time of day. A single-factor repeated measures multivariate analysis of variance (MANOVA) using Roy's greatest root ($p < .05$) was performed (SAS Institute, 1989) with ASR amplitude at the five ES intensity levels as the dependent vector. To interpret these results, we performed a univariate repeated measures analysis of variance (ANOVA) and a Bonferroni test (SAS Institute) to compare the differences between means ($p < .05$) with mean square error (MS_e) = subjects (time of day). We also examined the effect of ES intensity and the interactive effects of time of day and ES intensity on response amplitude using a two-factor repeated measures approach. Furthermore, we compared response amplitudes between males in LD and DD and between males and females in LD using paired and unpaired Student's t tests when appropriate.

In Experiment 2, mean amplitude responses were determined for each animal for each block of four (males) or five (females) blank trials. These means were then averaged by time of day. A single-factor MANOVA using Roy's greatest root ($p < .05$) was performed (SAS Institute, 1989) with ASR amplitude as the dependent vector. To interpret these results, we performed a univariate ANOVA and a Bonferroni test (SAS Institute) to compare the differences between means ($p < .05$) with MS_e = time of day. A repeated measures design was used when appropriate.

Reflex Modification

Amplitude data were converted to the percentages of blank (no prepulse) trial. From these data, psychometric functions were constructed for each animal at each prepulse frequency, day, and time of day. Auditory thresholds were determined from these data using a nonlinear regression analysis method for segmented lines (SAS Institute, 1989). Resultant threshold values that were less than 1 dB or greater than 90 dB were excluded from the analysis. A similar procedure has been used to produce a reliable measure of auditory thresholds in rats (Crofton, 1990). Mean time-of-day comparisons were made using an ANOVA, and significance-of-means separations were determined using a Bonferroni test ($p < .05$). A repeated measures design was used when appropriate.

Results

A MANOVA of male LD amplitudes indicated significant time-of-day effects, $F(5, 31) = 11.8, p < .0001$. The ASR amplitudes of male rats housed in LD conditions and startled at different times of day are presented in Figure 1. Startle amplitudes were significantly higher during D than during L at ES intensities of 90, $F(3, 33) = 7.48, p < .0006$, 100, $F(3, 33) = 15.37, p < .0001$, 110, $F(3, 33) = 14.45, p < .0001$, and 120, $F(3, 33) = 16.47, p < .0001$, dB. There was a significant effect of ES intensity on startle amplitude, $F(4, 209) = 321.66, p < .0001$. In addition, although the graphs do not clearly illustrate this effect, there was significant interaction between time of day and ES intensity, $F(12, 209) = 5.79, p < .0001$.

The ASR amplitudes of male and female rats housed in DD conditions and startled at different times of day are presented in Figures 2 and 3, respectively. In males, a MANOVA of startle amplitudes indicated significant time-of-day effects, $F(5, 31) = 15.66, p < .0001$. Startle amplitudes were significantly higher during SD than during SL at ES intensities of 90, $F(3, 33) = 4.43, p < .01$, 100, $F(3, 33) = 7.80, p < .0005$, 110, $F(3, 33) = 15.18, p < .0001$, and 120, $F(3, 33) = 10.51, p < .0001$, dB. In females, a MANOVA of startle amplitudes also indicated significant time-of-day effects, $F(5, 31) = 9.96, p < .0001$. Startle amplitudes were significantly higher during SD than during SL at ES intensities of 100, $F(3, 33) = 5.67, p < .0001$, 110, $F(3, 33) = 10.24, p < .003$, and 120, $F(3, 33) = 10.49, p < .0001$, dB. Similar, but statistically nonsignificant,

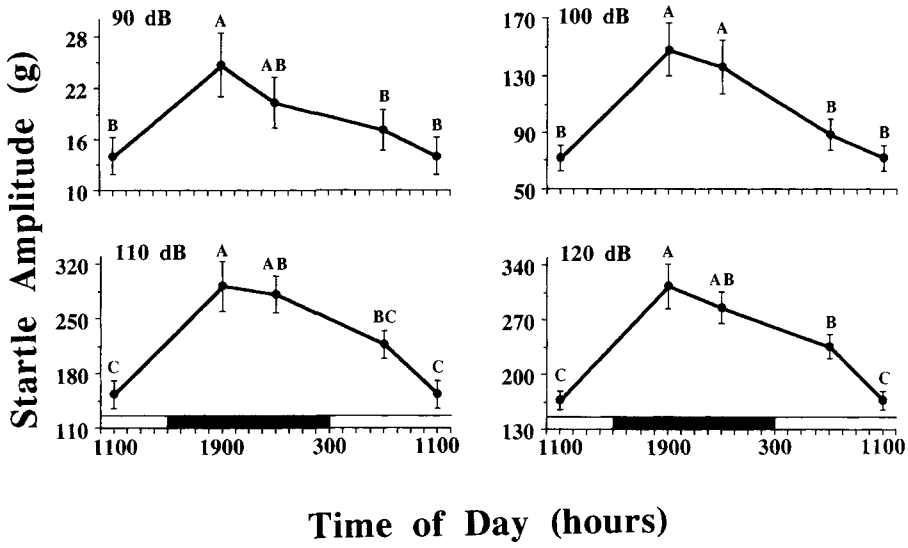

Figure 1. Effects of time of day and eliciting stimulus intensity on the acoustic startle response amplitudes of male rats in a light–dark (LD) cycle. (Values with different letters are significantly different [$p < .05$]. The first data points have been replotted to improve visualization of the rhythmic the LD differences. Values are $M \pm SE$. Filled portions of the lower abscissas indicate the dark period of LD cycle.)

results occurred at ES levels of 90 dB, $F(3, 33) = 2.33, p < .1$. In males, there was a significant effect of ES intensity on startle amplitude, $F(4, 209) = 246.24, p < .0001$, and a significant interaction between time of day and ES intensity, $F(12, 209) = 3.21, p < .0003$. In females, there was a significant effect of ES intensity on startle amplitude, $F(4, 209) = 127.73, p < .0001$, and a significant interaction between time of day and ES intensity, $F(12, 209) = 2.13, p < .02$.

Significant differences were also observed between male and female response amplitudes and between the response amplitudes of males startled in LD and DD. Mean response amplitudes of males in LD (142.4 ± 8.1 g) were significantly different, paired $t(11) = 4.14, p < .002$, than mean responses of males in DD (105.2 ± 11.6 g). In addition, the average response amplitudes from males exposed to startling stimuli in DD was significantly different, unpaired $t(22) = 3.38, p < .003$,

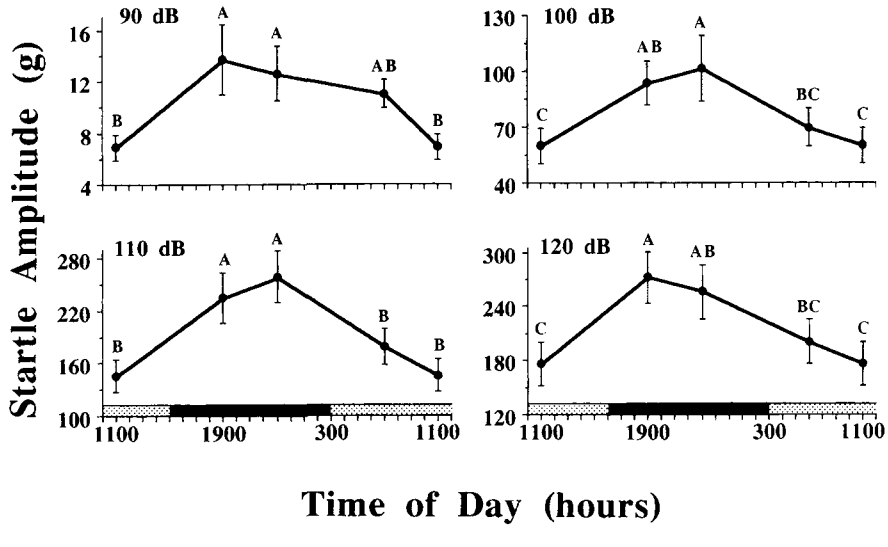

Figure 2. Effects of time of day and eliciting stimulus intensity on the acoustic startle response amplitudes of male rats in constant darkness conditions. (Filled portions of the lower abscissas indicate subjective dark period of the light–dark cycle, and the stippled portions indicate the subjective light period.)

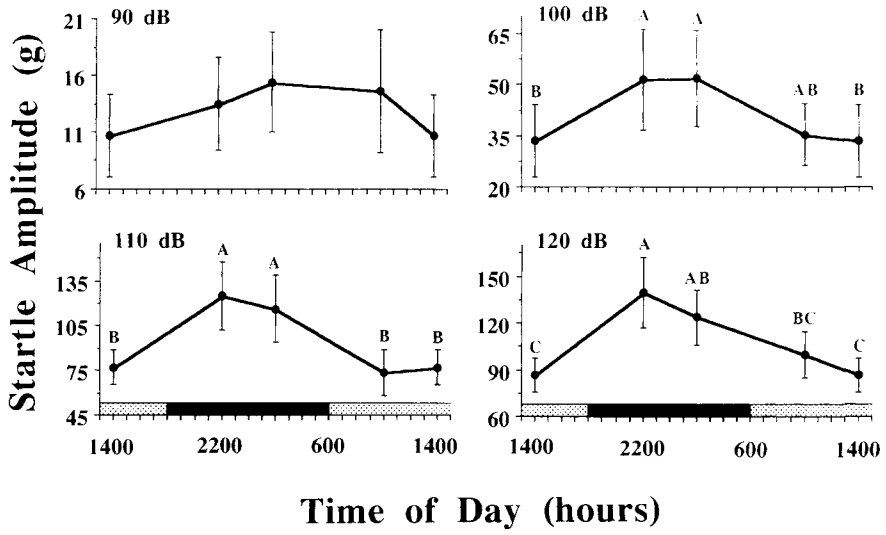

Figure 3. Effects of time of day and eliciting stimulus intensity on the acoustic startle response amplitudes of female rats in constant dark conditions.

than the mean response amplitudes of females (54.3 ± 9.6 g) exposed to the same conditions.

The threshold values of rats determined at several times of day in both LD and DD are presented in Table 1. Threshold values at different times of day were not statistically distinguishable either in females in LD or in males in LD or DD ($p < .05$). Response amplitudes from the blank trials (no prepulse) of these experiments were also analyzed by time of day. A significant effect of time of day was observed in male rats in LD (56 total trials/session), $F(3, 33) = 3.32$, $p < .04$, but the means were not statistically distinguishable. In the DD experiment, significant time-of-day differences of blank trial amplitude were not observed (56 total trials/session). There was also a lack of significance of time of day on female rats in DD (256 total trials/session).

Table 1
Auditory Thresholds (in Decibels) and Blank Trial Amplitudes (in Grams) at Different Times of the Day for Male Rats in a Light–Dark Cycle (LD) and in Constant Darkness (DD) and for Female Rats in DD as Determined by an Acoustic Startle Response Reflex Modification Procedure

Time of day (hr)	Auditory threshold 10 kHz *M* ± *SE*	*n*	Auditory threshold 40 kHz *M* ± *SE*	*n*	Blank trial amplitude *M* ± *SE*	*n*
			Males			
LD						
0700	48.4 ± 7.2	8	41.1 ± 8.1	7	171.8 ± 29.7	12
1100	32.4 ± 4.6	10	35.0 ± 7.3	11	140.2 ± 25.5	12
1900[a]	30.3 ± 3.5	12	44.9 ± 5.4	11	215.4 ± 37.2	12
2300[a]	32.4 ± 6.5	8	43.2 ± 8.3	10	220.7 ± 32.1	12
DD						
0700	33.6 ± 6.6	11	47.2 ± 6.5	9	252.6 ± 48.9	12
1100	28.4 ± 8.4	8	40.1 ± 6.7	8	189.0 ± 26.2	12
1900[a]	30.8 ± 5.8	10	35.6 ± 6.7	10	246.6 ± 52.7	12
2300[a]	36.4 ± 7.9	9	35.6 ± 5.6	9	218 ± 35.5	12
			Females			
DD						
0345	32.6 ± 10.0	3	52.6 ± 6.4	2	197.9 ± 65.1	4
0900	31.2 ± 6.0	11	40.2 ± 7.2	10	117.0 ± 13.3	16
1200	21.8 ± 5.1	6	28.6 ± 9.6	5	133.4 ± 17.7	8
1415	22.0 ± 5.2	3	53.4 ± 4.8	2	114.4 ± 40.2	4
1545	51.1 ± —	1	40.2 ± 17.4	3	99.2 ± 9.1	4
1900[b]	40.6 ± 6.3	7	40.4 ± 10.1	8	138.0 ± 13.9	8
2100[b]	28.1 ± 5.5	6	47.1 ± 12.1	5	153.3 ± 22.2	8
2300[b]	19.1 ± 9.5	5	26.2 ± 10.0	6	154.6 ± 22.0	8
0215[b]	28.4 ± 9.3	4	40.4 ± 17.8	2	155.5 ± 27.2	4

[a]Dark phase of LD. [b]Subjective dark phase.

Discussion

Our results are the first to demonstrate that rat ASR amplitude exhibits robust modulation over a 24-hr period in DD. This modulation consists of an approximately twofold increase in SD versus SL amplitudes (Figures 2 and 3). Because these differences persist in constant conditions, are of similar magnitude, and show similar phase changes as those measured in LD cycles (Figure 1; Chabot & Taylor, 1992), the results strongly suggest that ASR amplitude is endogenously modulated by the rat circadian system. Alternatively, one might hypothesize that the observed circadian ASR amplitude differences were due to different body postures affected by the rats at different times of day. Indeed, we have observed that hamsters (*Mesocricetus auratus*) placed into our startle chambers during L and exposed to startling stimuli curl up into a ball and, presumably because of this behavior, have resultant auditory thresholds (as determined by ASR reflex modification) much higher than rats (Chabot & Taylor, 1991a). However, rats do not appear to affect this body posture and tend to remain relatively immobile after initiation of a startle session regardless of whether they are startled during L or D (Chabot & Taylor, 1991a). Thus, it is unlikely that the ASR amplitude differences that we observed were due to gross

changes in body posture. Therefore, the results presented in this article document yet another mammalian behavior that is modulated on a circadian basis. In addition, to our knowledge, this is the first direct demonstration that a reflexive response can be modulated by the circadian system. Wheel-running activity, feeding, drinking, sexual behavior, and many other behaviors (cf. Rusak, 1981) have all been found to be modulated in the circadian range. The emerging ubiquity of behavioral circadian modulation emphasizes the importance of remaining synchronized to daily environmental changes.

Our findings of significant circadian ASR amplitude modulation provides the groundwork from which to determine the level of the circadian input into the ASR neural circuit. We have begun to address this question by using an ASR reflex modification procedure, which has been shown to be an accurate indicator of hearing thresholds in rats (Crofton, 1990), to determine whether auditory thresholds are modulated by time of day or circadian time. Our data indicate a lack of significant time-of-day or circadian differences of auditory thresholds at frequencies of 10 and 40 kHz (Table 1). These results suggest the following tentative conclusions. First, the significant LD and DD amplitude differences that were measured probably cannot be accounted for by auditory threshold changes. The broad spectrum (white noise) ES that we used in these experiments was composed of both 10- and 40-kHz frequencies. Significant amplitude differences were observed in both the ES-only experiments (Figures 1, 2, and 3) and in an analysis of the blank trial data (data similar to those presented in Figures 1, 2, and 3 that do not directly indicate sensory thresholds) in the reflex modification experiments (Table 1). Second, rat auditory thresholds are not modulated by time of day or by circadian time. This conclusion is based solely on our findings at only two frequencies, and because of this, it is extremely tentative. Although the two frequencies we tested span a significant portion of the rat's hearing range (Crofton, 1990), it is difficult to draw any firm conclusions from two, or even a dozen, data points. However, although it seems unlikely to us that only a narrow range of audition would be modulated in this way, additional frequencies should be tested to add credence to this conclusion.

If our experimental paradigm is an adequate test of circadian modulation of auditory thresholds, then the circadian modulation shown in Figures 2 and 3 must impinge on the pathway upstream from the cochlea and presumably the cochlear nucleus. Out of many mammalian behaviors known to be regulated by the circadian system (Rusak, 1981), the ASR is an ideal behavior in which to investigate the interaction between the circadian system and a specific behavior. Unlike the more complex circadian controlled behaviors such as locomotion, feeding, and drinking, the ASR is a relatively simple reflexive behavior in which the direct neural pathway has been identified (Davis et al., 1982). Our findings indicate that future experiments designed to identify the initial ASR neural nucleus receiving circadian input should focus on the nuclei downstream from the cochlear nucleus. In addition, study of the circadian system's interaction with the ASR may yield important insights into the mechanism through which the circadian system modulates behavior.

Our results bring up another question, dealing with the endogenous source of the circadian modulation. Because there is strong evidence that the hypothalamic suprachiasmatic nuclei (SCN) are the sites of the mammalian circadian clock(s) controlling other circadian rhythms such as wheel running (Ralph, Foster, Davis, & Menaker, 1990), drinking, sleep, and body temperature rhythms (Stephan & Nunez, 1977), these nuclei are good candidates for the source of circadian ASR amplitude modulation as well. However, the persistence of one behavioral circadian rhythm (wheel running in a restricted feeding paradigm) has been documented in rats with complete SCN ablations (Stephan, Swann, & Sisk, 1979). Therefore, it would be premature to conclude that the SCN are the source of modulation driving ASR amplitude modulation.

We also found significant LD ASR amplitude differences in male rats (Figure 1). Similar results have been previously observed in males housed and tested under different experimental conditions (Davis & Sollberger, 1971). Thus, male ASR amplitude, like female ASR amplitude (Chabot & Taylor, 1992), exhibits robust daily modulation. In fact, modulation of amplitude was observed at ES intensities of 90 dB and above in males in both LD (Figure 1) and DD (Figure 2) but only at 110 and 120 dB in LD (Chabot & Taylor, 1992) and DD (Figure 3) in females. These findings suggest that circadian modulation of ASR amplitude is especially significant in male rats. In addition, we observed a significant interaction between ES intensity and time of day in males in LD and DD but not in females in DD (the significant interactions are not immediately obvious from inspection of Figures 1 and 2 because the data were plotted to emphasize the temporal patterns at each intensity). Because most ASR studies involve male rats, these findings have important implications for pharmacologists, toxicologists, and reflex and sensory physiologists currently measuring the mammalian ASR. From these and previous (Chabot & Taylor, 1992; Davis & Sollberger, 1971) results, it is clear that the measurements of ASRs of different experimental groups need to be balanced by time of day. Possible exceptions to this point are ASR reflex modification experiments: We found no threshold differences in our reflex modification experiments at prepulse frequencies of 10 and 40 kHz (Table 1).

We have found that changes in experimental procedure can have a large effect on the robustness of circadian amplitude modulation. These are important findings for researchers currently measuring ASR. The data presented in Table 1 indicate circadian modulation of ASR (in a prepulse–ES paradigm) amplitude when the number of trials is low (56) but not when the number of trials is high (256). In addition, rats that have not been previously exposed to DD conditions display a greatly potentiated response during the first two startling sessions in DD (Chabot & Taylor, 1991b). Furthermore, the number of sessions to which rats are exposed may also affect the magnitude of the circadian differences. Although significant circadian amplitude differences were observed in these rats exposed to four startling sessions in a 24-hr period (Figures 2 and 3), we observed only a trend when the rats were exposed to six startle sessions (Chabot & Taylor, 1991b). These differences could have been due to the increased frequency of disturbance in the six-startle-session experiment. Although the timing of mammalian circadian

rhythms such as wheel-running activity is usually not greatly perturbed by handling (Redman, 1988), the effects of startling stimuli on the circadian system is not known. In addition, perturbations such as cage changes (Mrosovsky, 1988) and short periods of immobilization (Van Reeth, Hinch, Tecco, & Turek, 1991) can cause circadian behavioral disturbances in rodents.

We also observed a significant decrease in the average response amplitude in males tested in DD versus LD conditions (Figures 1 and 2). Although these response amplitude decreases may appear similar to the "damping" of other circadian rhythms when exposed to constant conditions, we cannot exclude other factors such as senescence of the animals or the increased number of startling sessions to which the animals were exposed. We have observed similar response amplitude decreases as the age of the animal or the number of startling sessions to which the animals were exposed increased (Chabot & Taylor, 1991b). Likewise, the significant differences between response amplitudes of males and females tested in LD are difficult to interpret because these animals were of different ages and had been exposed to different numbers of startling sessions.

Overall, our results demonstrate that the mammalian ASR amplitude exhibits clear daily and circadian modulation. These fluctuations are extremely important to consider when designing ASR experiments, which should be balanced by time of day to avoid biasing the results. These results provide another example of the ubiquity of circadian modulation of behavior and provide further support that the temporal organization of animals must be considered when addressing problems in behavior.

References

Chabot, C. C., & Taylor, D. H. (1991a). [The acoustic startle response in rats and hamsters: Behavioral correlates of amplitude responses and auditory thresholds]. Unpublished raw data.

Chabot, C. C., & Taylor, D. H. (1991b). [Effects of age and experience on acoustic startle response amplitudes in rats]. Unpublished raw data.

Chabot, C. C., & Taylor, D. H. (1992). Daily rhythmicity of the acoustic startle response. *Physiology and Behavior, 51,* 885–889.

Crofton, K. M. (1990). Reflex modification and the detection of toxicant-induced auditory dysfunction. *Neurotoxicology and Teratology, 12,* 1–8.

Daan, S., & Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents: II. The variability of phase response curves. *Journal of Comparative Physiology, 106,* 291–331.

Davis, M. (1970). Effects of interstimulus interval length and variability on startle-response habituation in the rat. *Journal of Comparative and Physiological Psychology, 72,* 177–192.

Davis, M. (1972). Differential retention of sensitization and habituation of the startle response in the rat. *Journal of Comparative and Physiological Psychology, 78,* 260–267.

Davis, M. (1986). Pharmacological and anatomical analysis of fear conditioning using the fear potentiated startle paradigm. *Behavioral Neuroscience, 100,* 814–824.

Davis, M., Gendelman, D. S., Tischler, M. D., & Gendelman, P. M. (1982). A primary acoustic startle circuit: Lesion and stimulation studies. *Journal of Neuroscience, 2,* 791–805.

Davis, M., & Sollberger, A. (1971). Twenty-four-hour periodicity of the startle response in rats. *Psychonomic Science, 25,* 37–39.

Hoffman, H. S. (1984). Methodological analysis in the behavioral analysis of startle. In R. C. Eaton (Ed.), *Neural mechanisms of startle behavior* (pp. 267–285). New York: Plenum.

Hoffman, H. S., & Wible, B. L. (1970). Role of weak signals in acoustic startle. *Acoustic Society of America, 47,* 489–497.

Horlington, M. (1970). Startle response circadian rhythm in rats: Lack of correlation with motor activity. *Physiology and Behavior, 5,* 49–53.

Ison, J. R., Bowen, G. P., & Kellog, C. (1991). Potentiation of acoustic startle behavior in the rat (*Rattus norvegicus*) at the onset of darkness. *Journal of Comparative Physiology, 105,* 3–9.

Ison, J. R., & Hammond, G. R. (1971). Modification of the startle reflex in the rat by changes in the auditory and visual environments. *Journal of Comparative Physiology and Psychology, 75,* 435–452.

Mrosovsky, N. (1988). Phase response curves for social entrainment. *Journal of Comparative Physiology, 162,* 35–46.

Pinckney, L. A. (1976). Inhibition of the startle reflex in the rat by prior tactile stimulation. *Animal Learning and Behavior, 4,* 467–472.

Ralph, M., Foster, R. G., Davis, F. C., & Menaker, M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. *Science, 247,* 975–978.

Redman, J., Armstrong, S., & Ng, K. T. (1983). Free-running activity rhythms in the rat: Entrainment by melatonin. *Science, 219,* 1089–1091.

Redman, J. R. (1988). *The effect of exogenous melatonin on rat circadian rhythms.* Unpublished doctoral dissertation, La Trobe University, Bundoora, Victoria, Australia.

Rusak, B. (1981). Vertebrate behavioral rhythms. In J. Aschoff (Ed.), *Handbook of behavioral neurobiology: Vol. 4. Biological rhythms* (pp. 183–213). New York: Plenum.

SAS Institute (1989). *SAS user's guide: Statistics, Version 5 edition.* Cary, NC: Author.

Stephan, F. K., & Nunez, A. A. (1977). Elimination of circadian rhythms in drinking, activity, sleep and temperature by isolation of the suprachiasmatic nuclei. *Behavioral Biology, 20,* 1–16.

Stephan, F. K., Swann, J. M., & Sisk, C. L. (1979). Entrainment of circadian rhythms by feeding schedules in rats with suprachiasmatic lesions. *Behavioral Neural Biology, 25,* 545–554.

Van Reeth, O., Hinch, D., Tecco, J. M., & Turek, F. W. (1991). The effects of short periods of immobilization on the hamster circadian clock. *Brain Research, 545,* 208–214.

Received February 26, 1992
Revision received April 16, 1992
Accepted April 16, 1992 ■