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BRIEF COMMUNICATION

Daily Rhythmicity of the Rat Acoustic Startle Response

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CHABOT, C. C. AND D. H. TAYLOR. *Daily rhythmicity of the rat acoustic startle response.* PHYSIOL BEHAV **51**(4) 885–889, 1992.—We have measured the acoustic startle response (ASR) amplitude and latency in rats housed in a 12:12 light:dark (LD) cycle. The response amplitudes to eliciting stimuli (ES) of 110 dB or 120 dB (white noise) were significantly higher (nearly two-fold) during D than during L. Similar, but nonsignificant, trends were also observed at ES intensities of 90 dB or 100 dB. While some significant LD ASR latency differences were observed, we cannot ascribe them to the photoperiodic phase at this time. These findings conclusively demonstrate that the mammalian ASR amplitude exhibits daily rhythmicity.

Acoustic Startle Daily Rhythm Modulation Latency Light Dark Reflex

THE mammalian acoustic startle response (ASR) is a remarkably resilient reflex that persists after hundreds of trials with little decrement in amplitude (4). The primary neural pathway controlling this neuromuscular response to auditory eliciting stimuli (ES) has been identified (9), as have some of the important neurotransmitters (7). In addition, many of the physical parameters of the sensory stimuli necessary to elicit, modify, or eliminate the ASR have been extensively characterized (6). These well-characterized attributes, along with the ease with which the ASR can be measured, have made the ASR the focus of many investigators interested in pharmacology, toxicology, and reflex and sensory physiology.

While the ASR is being studied in many laboratories, few studies have examined the potentially important influence that time of day may have on this response. Davis and Sollberger (8) found significant light:dark (LD) increases in ASR amplitude during D versus L in rats. However, these rats were not only housed in LD, the testing was also performed in a lighted or a darkened startle chamber depending upon the appropriate condition in the colony room. Subsequent experiments (conducted during L) have demonstrated that the lighting conditions during ASR testing alone have effects of similar magnitude on ASR response amplitude (14,15). Thus, results from Davis and Sollberger (8) may have been directly attributable to the photic conditions during ASR measurement rather than time of day. In the only other study of which we are aware, Horlington (13) reported L versus D differences in the amplitude and latency of the ASR. Interpretation of this experiment is difficult, however, since the photic conditions during ASR measurement were not reported. In addition, the day and night groups of rats, between which Horlington (13) found ASR amplitude differences, were housed separately.

While direct evidence that the mammalian ASR exhibits daily modulation is lacking, there may be additional reason to suspect, based on the ubiquity of behavioral LD differences, that substantial LD ASR amplitude and/or latency rhythms do occur. Animals exhibit a wide variety of behaviors that are temporally synchronized to daily environmental cycles. General locomotor activity, wheel-running, feeding, drinking and a host of other behaviors in rats have been shown to be synchronized by daily photic changes (17). Along with these overt behavioral rhythms, there is evidence that simple reflexes such as the evoked electroretinogram (12) and visually evoked potentials (2) can also be synchronized by daily photic changes.

In our review of the ASR literature (>80 articles), we have found that many articles fail to mention the time of day during which startle was measured. In addition, of those which do cite the time of day, some ASR experiments have been conducted at night (19,20) and others during the day (10,16). Since the ASR is currently measured in many laboratories and since the previously reported day:night amplitude differences (8,13) are large (two-fold), we opined that a clear demonstration of LD ASR amplitude (or latency) differences, or lack thereof, would be of crucial importance to investigators in the ASR field. In the experiments reported here, we measured the ASR in individuals, at several phases of the light:dark (LD) cycle with the purpose of determining if there is daily modulation of the ASR of rats.

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MATERIALS AND METHOD

Animals and Environmental Conditions

Female Sprague–Dawley rats (n = 12) were housed two per cage in a light-tight, ventilated chamber on a 12:12 LD cycle for 3 weeks before being exposed to startling stimuli. Food (Purina Rodent Chow, 5001) and water were available ad lib except during testing.

Apparatus

ASRs were measured in four identical, sound-attenuated chambers. Each chamber (37 × 37 × 31 cm) contained a wire cage (8 × 4.5 × 4.5 cm) mounted on a force transducer (Coulborn Instruments, model No. 45-15, Columbus, OH) and one speaker (Realistic Super Tweeter, Cat. No. 40-1310B) situated at head-height of the animal for the delivery of the eliciting stimulus (ES). 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 duration, 0.15 ms exponential rise/fall time). A Macintosh SE/30 computer with Labview™ software (National Instruments Co., Austin, TX) was used to create virtual instruments that controlled the analog-to-digital converter, tone generator, digital output instruments (GW Instruments, 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 following ES onset. The digitized output was converted to grams based on a calibration curve 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 250 ms prior to 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 (determined from a 1-s window immediately prior to ES presentation) from the maximum force exerted on the transducer within a 125-ms window after ES onset which exceeded the average baseline value by 4 standard deviations. ASR latency (time from ES onset to a point 4 standard deviations above baseline) was also determined in each trial. When the criteria for startle were not exceeded (>4 standard deviations above baseline mean), a value of zero was recorded as the amplitude while the data were excluded from the latency analysis.

General Experimental Procedure

At specific times during the LD cycle (see below), 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 animals 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. During each ASR 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, 120 dB; 50 trials total) and immediately placed back into their cages. Rats were handled in the dark with the aid of an infrared viewer (Electrophysics Corp., Nutley, NJ).

EXPERIMENT 1A: L-FIRST

The objective of this experiment was to characterize ASR amplitude and latency in an LD cycle. The ASRs of rats (n = 12; aged 147–148 days) housed in an LD cycle (lights on 600 h; lights off 1800 h) were first measured at timepoint 1000 h (L), followed by 1400 h (L), 2200 h (D), and 0200 h (D). Because there is evidence that the first ASR session yields generally larger response amplitudes than subsequent sessions (5), these rats were preexposed to startling stimuli 14 days prior to this experiment (this property could have caused an initial session bias independent of time of day). In the preexposure sessions, ASRs were elicited as described above at four time points per 24-h period [two times during D (0200 h and 2200 h) and two times during L (1000 h and 1400 h)].

EXPERIMENT 1B: D-FIRST

Although we had preexposed the rats in Experiment 1A to startling sessions, we felt it necessary to determine if the first session (timepoint) during which we measured the ASRs would still yield a generally larger response. The objective of this experiment was to determine if the LD amplitude and latency differences observed (Fig. 1, left) were partly obscured by habituation or fully induced by sensitization processes known to affect ASR amplitude (5). To control for these possibilities, the same rats, housed as above, were used in this experiment as were used in Experiment 1A but the initial session time was changed. Seven days after Experiment 1A, the ASRs of the rats (n = 12; aged 154–155 days) were first measured at timepoint 2200 h (D), followed by 0200 h (D), 1000 h (L), and 1400 h (L).

EXPERIMENT 2

To provide better resolution of the time of day effect on amplitude, we measured ASRs at six time points during the LD cycle. Rats, aged 91–92 days, and housed in an LD cycle (lights on 700 h; lights off 1900 h), were split into two groups of six each: one group was first tested in the L portion of the LD cycle (2 h after lights on and every 4 h thereafter for 24 h); the other group was first tested in the D portion (2 h after lights off and every 4 h thereafter for 24 h). Since we observed significant LD amplitude differences in both Experiments 1A and 1B (Fig. 1) the amplitude data collected in this experiment were pooled by time of day.

Statistical Analyses

Mean responses (ASR amplitudes and latencies) were determined for each animal for each block of ten trials at a given dB intensity level. These means were then averaged for stimulus intensity and time of day. A single factor multivariate repeated measures analysis using Roy's greatest root ($p < 0.05$) was performed (18) with ASR amplitude at the five ES intensity levels as the dependent vector. To interpret these results, we performed a univariate repeated measures analysis and a Bonferroni test (18) to compare the differences between means ($p < 0.05$) with mean square error = subjects (time of day).

RESULTS

Figure 1 shows that the ASR amplitudes were significantly higher during D than during L at ES levels of both 110 [left panel, $F(3,33) = 9.57$; right panel, $F(3,33) = 4.37$] and 120 dB [left panel, $F(3,33) = 10.47$; right panel, $F(3,33) = 5.22$]. Similar, but nonsignificant, results occurred at ES levels of 90 dB and 100 dB. Data in Fig. 1 also indicate a similar increase in ASR amplitude as ES intensity increases in both L and D. While we examined the possibility that the amplitudes of the startle trials during the first timepoint may have been larger than subsequent timepoints, the D amplitudes were significantly higher than L amplitudes whether rats were initially startled in L or D (Fig. 1). As a supplemental issue, we examined the effect of ES in-

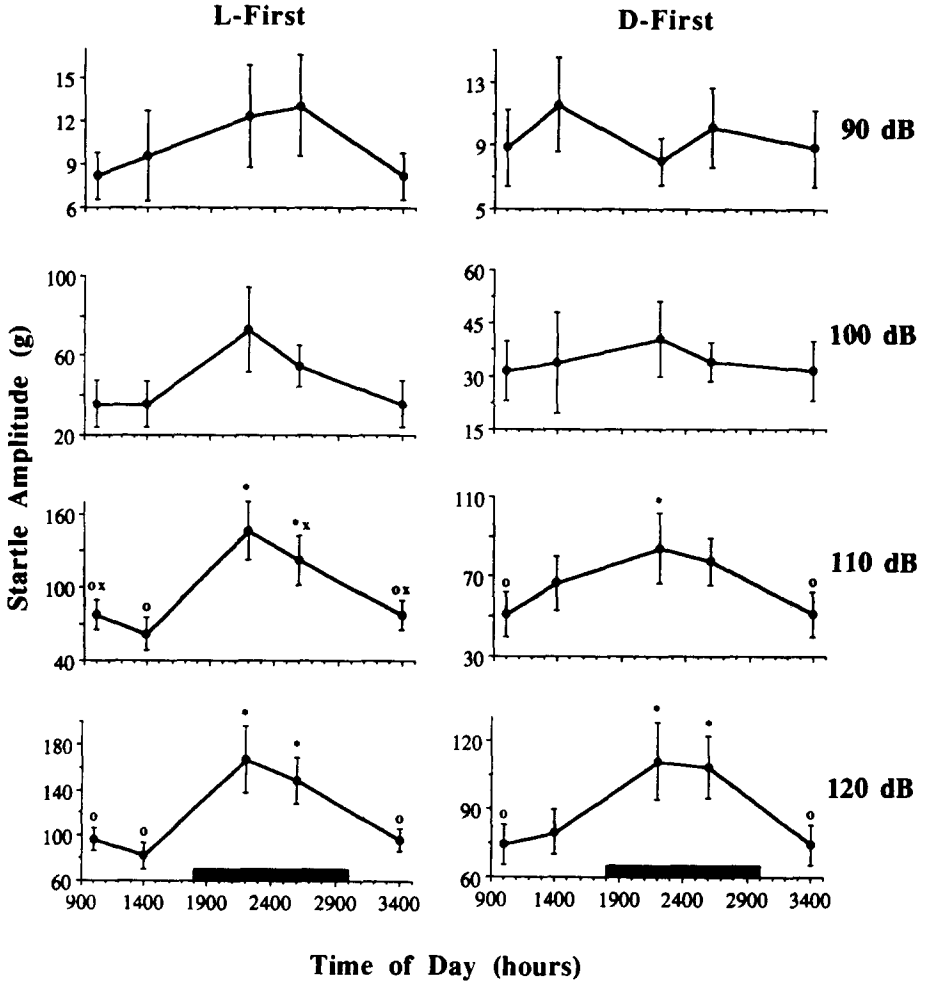

FIG. 1. The effects of time of day on rat ASR amplitude. Left panels: rats (n = 12) were first exposed to startling stimuli during L. Right panels: rats were first exposed to startling stimuli during D. Data points with different symbols are significantly different ($p > 0.05$). The first data points have been replotted to improve visualization of the rhythmic LD differences. Darkened bar = dark portion of LD cycle.

tensity on response amplitude and the interactive effects of time of day and ES intensity using a two-factor repeated measures approach. There was a significant effect of ES intensity on startle amplitude [left, F(4,209) = 94.13; right, F(4,209) = 110.09]. In addition, there was significant interaction between time of day and ES intensity in the L-first [F(12,209) = 3.34], but not in the D-first experiment [F(12,209) = 1.47].

While we found significantly shorter ASR latencies during D than L at ES intensities of 100 dB [F(3,11) = 7.49] and 110 dB [F(3,11) = 8.63] for those animals first startled in L, and a similar trend at 90 dB (data not shown) and 120 dB, no significant differences or trends were apparent when rats were first startled in D (data not shown).

Figure 2 shows amplitude data gathered from rats at six different times during a 24-h period. Mid-D amplitudes were found to be significantly larger than mid-L amplitudes at 100 dB [F(5,55) = 3.93], 110 dB [F(5,55) = 3.05] and 120 dB [F(5,55) = 3.97] ES levels. Furthermore, mid-D and mid-L amplitudes were generally higher and lower, respectively, than other D and L amplitudes at 100-, 110-, and 120-dB ES levels.

DISCUSSION

The results presented here clearly demonstrate a daily rhythmicity of rat ASR amplitude. In an LD cycle, the ASR amplitude is generally 50–100% higher during D than during L (Figs. 1 and 2). These findings underscore the importance of the daily, temporal organization of the mammalian ASR. The robust daily fluctuations in amplitude are of major import to researchers working on the ASR and should be taken into account in comparing results from experiments conducted at different times of day.

Our results are important to the design of future ASR experiments. While Horlington (13) found LD differences in rats exposed to only one startling stimulus, our study shows that significant daily ASR amplitude differences persist even after repeated measurements. This finding has important implications for pharmacologists, toxicologists, and reflex and sensory physiologists currently studying the mammalian ASR. Our results (Fig. 2), like those of Davis and Sollberger (8) suggest there is continual modulation of ASR amplitude over the course of a day even during L or D. Clearly the measurements of ASRs of different experimental groups must be balanced by time of day, especially when ASRs are measured close to photic transition times. This consideration is generally lacking in previous ASR experiments.

The trend of continual modulation of ASR amplitude seen in Fig. 2 also suggests that ASR amplitude changes may be endogenously modulated. If the daily changes in ASR amplitude

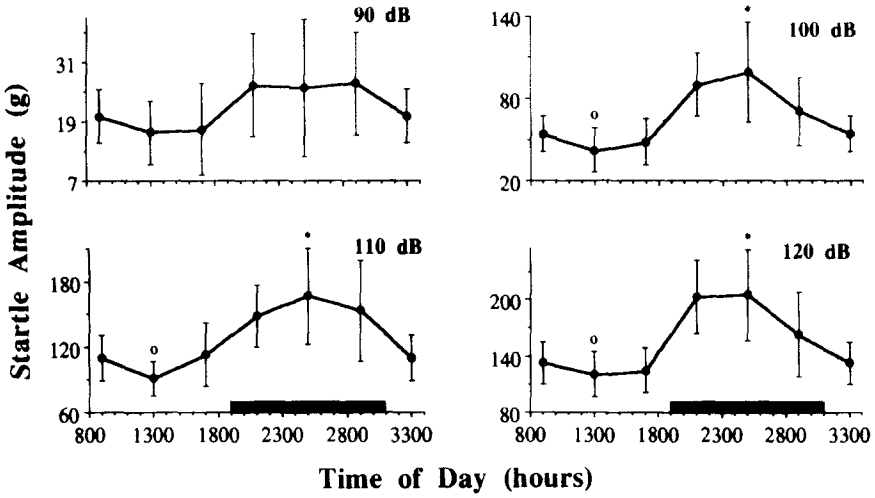

FIG. 2. The effects of time of day on rat ASR amplitude.

were passively driven by the diurnal cycle, then one would expect the amplitudes measured during D (or L) to be roughly equivalent. However, the maxima and minima of these data occur in mid-D and mid-L, respectively, while ASR amplitudes assayed at times of day closer to the D-to-L and L-to-D transition times are intermediate. This trend, similar to that seen by Davis and Sollberger (8) is suggestive of an internally driven ASR amplitude modulation, perhaps of circadian-system origin. Many mammalian behaviors exhibiting daily modulation are also modulated by the circadian system under constant conditions (17). While we measured the ASR in constant conditions (darkness), our animals were housed in an LD cycle. ASR amplitude variations could have been prompted by the LD conditions in which the animals were housed. Evidence that the rat ASR amplitude is modulated by the circadian system requires measurement in rats housed and tested in constant conditions.

That major daily ASR modulation exists suggests that the neuromuscular pathway involved in this reflex (9) is also subject to daily modulation. While identification of the specific sensory, motor, or interneuronal component(s) of the pathway involved in this modulation was beyond the scope of our experiment, we have established a framework that should allow investigators using currently available ASR reflex-modification procedures (3) to determine if modulation occurs at the level of the auditory system.

While we measured significant ASR amplitude differences independent of the LD phase in which the rats were initially tested, some variation in response between the two experiments were observed. For example, amplitudes were generally higher (Fig. 1) in Experiment 1A (L-first) versus Experiment 1B (D-first). Also, while there were significant differences in latency to threshold measurements in rats first measured in L, there were no significances or apparent trends in rats first measured in D (data not shown). Thus, the daily phase during which the rat ASR is first measured may have large effects on subsequent ASR amplitudes and latencies. Since the ASR does exhibit habituation and sensitization (4,5), this finding may be based on different underlying rates of habituation or sensitization during L and D. This possibility can be experimentally addressed by examining rates of habituation and sensitization during L and D phases. Alternatively, the decreased amplitudes and lack of significant latency differences observed during the D-first experiment could have been due to habituation from the previous L-first experiment performed 7 days previously. Davis (5) reported ASR habituation aftereffects which lasted at least 6 days. Since there are multiple possible explanations for these differences, we are unable to, at the present time, identify the factor(s) causing the amplitude and latency differences between Experiments 1A and 1B.

While we originally designed this series of experiments to examine the possibility that there is daily variation in the ES intensity required to elicit startle (startle threshold), significant interactive effects were observed only in the L-first experiment. However, the results do raise some interesting questions. Fleshler (11) found that ES intensity had to be approximately 90 dB in order for a measurable reaction to occur. We observed measurable reactions greater than 65% of the time (data not shown) at ES values of only 80 dB. This threshold value is considerably lower than the generally accepted startle threshold of 90 dB (11). Since recent data suggests that startle occurs at even lower ES levels in humans (1), the startle threshold may be much lower in rats as well.

Overall, the results presented in this paper demonstrate that the mammalian ASR amplitude exhibits clear daily modulation. These daily fluctuations have important bearing on the design of ASR experiments: the experiments should be balanced by time of day to avoid biasing the results. In addition, our results suggest that ASR amplitude may be modulated endogenously, perhaps by the circadian system. These findings provide further evidence of the importance of an organisms temporal organization.

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REFERENCES

1. Blumenthal, T. D.; Goode, C. T. The startle eyeblink response to low intensity acoustic stimuli. Psychophysiology 28:296–306; 1991.
2. Brandenburg, J.; Bobbert, A. C.; Eggelmeyer, F. Circadian changes in the response of the rabbit's retina to flashes. Behav. Brain Res. 7:113–123; 1983.
3. Crofton, K. M. Reflex modification and the detection of toxicant-induced auditory dysfunction. Neurotoxicol. Teratol. 12:1–8; 1990.
4. Davis, M. Effects of interstimulus interval length and variability on startle-response habituation in the rat. J. Comp. Physiol. Psychol. 72:177–192; 1970.
5. Davis, M. Differential retention of sensitization and habituation of the startle response in the rat. J. Comp. Physiol. Psychol. 78:260–267; 1972.
6. Davis, M. The mammalian startle response. In: Eaton, R. C., ed. Neural mechanisms of startle behavior. New York and London: Plenum Press; 1984:287–351.
7. Davis, M. Pharmocological and anatomical analysis of fear conditioning using the fear potentiated startle paradigm. Behav. Neurosci. 100:814–824; 1986.
8. Davis, M.; Sollberger, A. Twenty-four-hour periodicity of the startle response in rats. Psychonom. Sci. 25:37–39; 1971.
9. Davis, M.; Gendelman, D. S.; Tischler, M. D.; Gendelman, P. M. A primary acoustic startle circuit: Lesion and stimulation studies. J. Neurosci. 2:791–805; 1982.
10. Dean, K. F.; Sheets, L. P.; Crofton, K. M.; Reiter, L. W. The effect of age and experience on inhibition of the acoustic startle response by gaps in background noise. Psychobiology 18:89–95; 1990.
11. Fleshler, M. Adequate acoustic stimulus for startle reaction in the rat. J. Comp. Physiol. Psychol. 60:200–207; 1965.
12. Fowlkes, D. H.; Karwoski, C. J.; Proenza, L. M. Endogenous circadian rhythm in electroretinogram of free-moving lizards. Invest. Ophthal. Vis. Sci. 25:121–124; 1984.
13. Horlington, M. Startle response circadian rhythm in rats: Lack of correlation with motor activity. Physiol. Behav. 5:49–53; 1970.
14. Ison, J. R.; Hammond, G. R. Modification of the startle reflex in the rat by changes in the auditory and visual environments. J. Comp. Physiol. Psychol. 75:435–452; 1971.
15. Ison, J. R.; Bowen, G. P.; Kellog, C. Potentiation of acoustic startle behavior in the rat (*Rattus norvegicus*) at the onset of darkness. J. Comp. Psychol. 105:3–9; 1991.
16. Leitner, D. S.; Powers, A. S.; Stitt, C. L.; Hoffman, H. S. Midbrain reticular formation in the inhibition of acoustic startle. Physiol. Behav. 26:259–268; 1981.
17. Rusak, B. Vertebrate behavioral rhythms. In: Aschoff, J., ed. Handbook of behavioral neurobiology, vol. 4: Biological rhythms. New York and London: Plenum Press; 1981:183–213.
18. SAS Institute Inc. SAS User's Guide: Statistics, Version 5 Edition. Cary, NC; 1989.
19. Swerdlow, N. R.; Mansbach, R. A.; Geyer, M. A.; Pulvirenti, L.; Koob, G. F.; Braff, D. L. Amphetamine disruption of prepulse of acoustic startle is reversed by depletion of mesolimbic dopamine. Psychopharmacology 100:413–416; 1990.
20. Young, J. S.; Fechter, L. D. Trimethyltin produces an unusual form of toxic auditory damage in rats. Toxicol. Appl. Pharmacol. 82:87–93; 1986.