ABSTRACT
The time course of the recovery cycle (refractory period) of the acoustic startle response in rats was determined. The refractory effect was shown to be highly dependent upon the interval between two startle stimuli but independent of the intensity of the stimuli at a given interval, provided that the intensity of both stimuli was the same. A model based on summation of refractory effects to repetitive stimulation did not predict habituation to repetitive stimulation. The interaction between refractory effect and habituation was discussed, and it was suggested that short interstimulus intervals reduce the effective intensity of the habituation stimulus so that the effects of frequency and intensity become confounded in habituation studies where the interval between habituation stimuli invades the refractory period of the response being studied.

INTRODUCTION
The acoustic startle reaction is a brief short-latency sequence of generalized muscular contractions to the onset of a loud noise (Dodge & Louttit, 1926; Fleshler, 1965; Landis & Hunt, 1939). It occurs in a wide variety of species and shows pronounced decrement in response magnitude to a repetitive stimulus delivered at interstimulus intervals (ISIs) of from seconds to minutes (Cohen, 1929; Prosser & Hunter, 1936). For this reason, it has proven to be a useful response system for studying elementary forms of behavioral plasticity such as habituation and sensitization (Davis & Wagner, 1968; Groves & Thompson, 1970; Moyer, 1963; Prosser & Hunter, 1936). Continuous auditory background stimulation has been shown to facilitate the amplitude of the acoustic startle response (Hoffman & Fleshler, 1963; Ison & Hammond, 1971) whereas pulsed background stimulation produces a pronounced decrease in the amplitude of the reaction (Hoffman & Fleshler, 1963). The explanation of the latter phenomenon has been that the acoustic startle response is refractory for some period of time following its occurrence (Cohen, 1929; Dodge & Louttit, 1926). Thus, if one startle stimulus precedes another within a restricted time period, the second stimulus will produce a relatively smaller response (Dodge & Louttit, 1926; Hoffman & Searle, 1965; Ison & Hammond, 1971). This effect, which has been called variously "prepulse inhibition" or "refractory period," is dependent upon the time between the two startle stimuli, with shorter intervals producing more marked refractoriness. Although it has been shown that the degree of refractory effect is dependent upon the intensity of the first stimulus (Hoffman & Searle, 1965; Ison & Hammond, 1971), a higher intensity producing greater decrement in the second response, whether the refractory period is dependent upon intensity when the intensity of both stimuli is held constant, has not been reported. Another question of recent concern has been the role of the refractory period of the acoustic startle response, as well as other response systems, in the progressive decrement in response amplitude (habituation) which results from repetition of the acoustic stimulus. The hypothesis that response refractory period might account for habituation was first suggested by Cohen (1932) for habituation of the eye blink to acoustic stimulation in humans and, later, as part of a general theory of habituation by Ratner (1970). The present experiments were designed to determine (a) the effects of absolute stimulus intensity and ISI on the refractory period of the acoustic startle response in the rat and (b) the possible contribution of refractory period to habituation of the acoustic startle.
response. We report that the refractory period is independent of stimulus intensity when both stimuli are held constant and is a function of the interval between the two stimuli. Further, refractory period cannot account for habituation of the acoustic startle response in rats.

EXPERIMENT 1 In this experiment, the time course of the refractory effect of the acoustic startle response was determined over an ISI of 1-32 sec. These are ISIs commonly used in studies of habituation of the acoustic startle response. Stimulus intensity was held constant while interval was varied.

Method
Ten albino male rats supplied by Horton Laboratories (Oakland, California) served as subjects and weighed 179-250 gm. at the time of testing. Each animal received 60 trials, each trial consisting of two equally intense stimuli, either 105 db or 112 db., with an ISI of 1, 2, 4, 8, 16, or 32 sec. Every animal received each of these 12 stimulus configurations five times, arranged randomly except that all of the 12 possible configurations had occurred before any was repeated. The intertrial interval (ITI) was varied randomly from 50 to 70 sec., with a mean of 60 sec. All animals were handled, weighed, and given a 15-min adaptation period in the startle response apparatus on the day preceding their testing.

The startle response apparatus has been described in detail elsewhere (Wilson & Groves, 1972). Briefly, it consists of a Plexiglas animal chamber mounted on a spring-suspended lever in an acoustically insulated box. Movements of the chamber are translated directly by a force transducer (Gross Strain Gauge, Model FT 03C) placed under the suspended lever. The amplitude of the startle response is then written out on a Grass Model 5 polygraph as a vertical pen deflection, the amplitude of which is directly proportional to force. The startle stimuli consisted of 20-msec. bursts of white noise generated by a Grason-Stadler random noise generator (Model 901B), amplified by an audio amplifier and switched with Hunter interval timers. Nominal stimulus intensities were measured inside the animal chamber with a General Radio Model 1551-C sound-level meter (re .0002 dynes/cm2).

Results and Discussion
The recovery cycle of the acoustic startle response is shown in Figure 1, in which the amplitude of the second response is plotted as the percentage of the first response. The curves for the two different intensities have been combined because there was no significant difference between the recovery cycle curves for the two intensities (F = 1.37, df = 1/9, p > .25). There was also no significant interaction between intensity of the pulse pairs and recovery cycle interval (F = 1.00, df = 5/45, p > .25). The effect of pulse interval, however, was highly statistically significant (F = 13.71, df = 5/45, p < .01). The recovery curve is of the same general form as that found by other investigators using stimuli of different parameters (Brown, Meryman, & Marzocco, 1956; Buckland, Buckland, Jamie-son, & Ison, 1969; Hoffman & Searle, 1965). The effect of the first stimulus upon responsiveness to the second stimulus is particularly marked. A stimulus following another of equal intensity by 1 sec., as shown in Figure 1, elicits a startle response which is only about 29% of the initial

![Figure 1. Recovery cycle of the acoustic startle response of the rat. (Stimulus pairs of equal intensity were presented at six ISIs. The ordinate represents the mean amplitude of the response to the second stimulus [R2] expressed as percentage of the mean amplitude of the response to the first stimulus)](image_url)
response. With a separation of 32 sec. between the pair, the second response is still not completely recovered. Thus, the startle response shows marked refractoriness, particularly at short ISIs, and the degree of recovery is directly related to the interval between the two stimuli, short intervals producing very marked refractoriness and longer intervals producing relatively less refractoriness.

EXPERIMENT 2
The failure to find a significant effect of intensity in Experiment 1 suggested that the refractory effect at a given interval may be independent of intensity when both stimuli in a given pair are held constant. In view of the finding that the refractory effect is a function of the intensity of the first stimulus in the pair (Hoffman & Searle, 1965) and the results of Experiment 1, it was hypothesized that the refractory effect may be some function of the ratio of the intensities of the first and second stimulus or of the ratio of the response amplitudes characteristic of these stimulus intensities, rather than the absolute intensities per se. To test this possibility, ISI was held constant at 5 sec. while the intensity of the two stimuli was varied over a range of six values from 102 to 112 db. These intensities were chosen because previous work indicated that they produce a range of response amplitudes comparable to those which normally occur during habituation to a 112-db. stimulus series (P. M. Groves & C. Wilson, unpublished observations, 1972). The intensities of S1 and S2 were again constant on any trial, but were covaried during the experiment over the range indicated.

Method
Ten male albino rats weighing 180-240 gm. at the time of testing were each given 48 trials, 8 trials at each of six intensities consisting of 102, 104, 106, 108, 110, or 112 db. Each trial consisted of two equally intense acoustic pulses at an interval of 5 sec., with an ITI of 60 sec. The order of presentation of trials was again block random-

Results
The results of Experiment 2 are shown in Figure 2, in which the absolute amplitude of the first response is plotted against the amplitude of the second response. Although variations in stimulus intensity produced a wide range of response amplitudes, the ratio between R1 and R2 was nearly constant as indicated by the linear relationship shown in Figure 2. While the effect of stimulus intensity on response amplitude was highly significant ($F = 12.70$, df = 5/45, $p < .01$), the effect of intensity on the ratios of the first to the second response was not significant ($F = .05$, df = 5/45, $p > .25$). Thus, the refractory effect for a given interval is independent of stimulus
intensity if the intensity of the two stimuli is held constant.

EXPERIMENT 3
Having established in Experiments 1 and 2 that an ISI of 5 sec. between two consecutive stimuli produces a substantial decrease in the second response which is independent of intensity when both stimuli are held constant, Experiment 3 was designed to test the prediction that the progressive decrement caused by stimuli of constant intensity delivered repetitively at 5-sec. ISIs could be accounted for by summation of the refractory period. If the refractory effect produces approximately a 50% decrease in each subsequent response, the second response would be 50% of the first; the third, 50% of the second, etc.

Method
Ten male albino rats weighing 180-233 gm. at the time of testing were given 60 stimuli, each consisting of one 20-msec. 112-db. burst of white noise with a constant ISI of 5 sec. Handling procedures and apparatus were the same as described for Experiment 1.

Results
Figure 3 illustrates the results of this experiment. The dashed line represents the predicted outcome based upon a 50% refractory effect in which each response is one half of the preceding response; the solid line illustrates the actual experimental outcome. It is very clear from these results that although habituation to the repetitive stimulus was marked (F = 7.38, df = 59/531, p < .01), it did not follow the predicted outcome. The effects of the recovery cycle alone are better seen in Figure 4, in which the first eight trials of Experiment 3 are plotted. From these data it is apparent that at an ISI of 5 sec., the second response is approximately 50% of the first, after which response amplitude quickly stabilizes near that value. Thus, prior to habituation, refractory effects alone produce only a rapid decrement which quickly stabilizes and upon which habituation may then be superimposed.

EXPERIMENT 4
The relationship between habituation and the recovery cycle, in light of the results above, is apparently not a simple one; and recovery cycle alone, at least based upon a model where each refractory effect sums with subsequent ones, cannot predict the course of habituation. It is possible that habituation to a repetitive stimulus alters the recovery cycle so that the effect becomes less pronounced as habituation proceeds. This would be contrary to the results of Experiment 2 if the effect of habituation were simply one of decreasing response amplitude in the same way that reducing intensity decreases response amplitude. To test this, animals were given pairs of stimuli at 5-sec. ISIs, separated by 35-sec. These values were chosen because a 5-sec. ISI was shown in previous experiments to produce a pronounced refractory effect, whereas an ITI of 35 sec. was one which would produce little refractoriness but one at which habituation would proceed fairly rapidly.

Method
Ten male albino rats weighing 201-228 gm. at the time of testing were given 60 habituation trials at 35-sec. ITIs. Each trial consisted of two equally intense stimuli (112 db.) separated by 5 sec. Habituation was determined by the progressive decrement in response to repeated trials, while response refractory period was noted for each pulse pair constituting a trial. Handling procedures and apparatus were the same as described previously.

Results
Habituation of the acoustic startle response as a function of trials is shown in Figure 5, as is the recovery cycle for each trial. It may be noted first that response amplitude to the first stimulus in each pair varied over approximately the same range as response amplitude in Experiment 2, in which amplitude was varied by varying stimulus intensity rather than by habituation. Habituation across trials was quite marked and highly statistically reliable (F = 3.25, df = 59/1,062, p < .01). Although the refractory effect between trials showed considerable variability, there was no systematic change in ratio of the first to the second response (F = .98, df = 59/531, p > .25). Thus, decreasing response amplitude during habituation produced essentially the same effect as decreasing response amplitude in Experiment 2 by changing stimulus intensity. Namely, the response refractory effect did not change as a function of response amplitude. Therefore, habituation per se does not alter the refractory effect of the acoustic startle response.

GENERAL DISCUSSION
There is substantial indirect evidence to suggest that recovery cycle effects alone cannot account for response habituation. Groves and Thompson (1970), for example, pointed out that responses may show marked habituation at ISIs which exceed their refractory period. Additionally, some responses show sensitization prior to habituation, which is not consistent with a refractory period explanation of habituation to repetitive stimulation. Other evidence can be cited to support the view that refractory period alone cannot account for habituation. The refractory effect of stimulation on the acoustic startle response, for example, has been shown to be, to some extent, nonspecific. Thus, a tactile or visual prepulse will produce a pronounced refractory effect on subsequent acoustic startle (Brown et al., 1956; Buckland et al., 1969). Habituation, on the other hand, is characterized by its specificity. An alteration in stimulus parameters, not to mention stimulus modality, will produce sensitization-
increase in subsequent response, not a decrease (Prosser & Hunter, 1936; Thompson & Spencer, 1966). However, because many studies of habituation include ISIs which invade the refractory period of the response being studied, one must presume that these include some refractory effect on responses subsequent to the first response in the series. In the experiments reported above, we found that a simple summating model based on "prepulse inhibition" at an ISI of 5 sec. could not account for habituation. Further, it was demonstrated that habituation per se did not alter the refractory effect and that refractory period was similarly independent of stimulus intensity. A closer examination of the first few responses in a series of responses to repetitive stimulation, as shown in Figure 4, would suggest that the decrement produced in response amplitude by refractory effects alone would approximate the decrement produced by the first on the second response in any series. The degree of decrement would be a function of the frequency of repetition, shorter intervals producing more pronounced decrement. However, to account for the progressive decrease in response which exceeds that produced in the second response as was the case in Experiment 3, an additional process must be involved. This process is habituation as commonly defined (Thompson & Spencer, 1966).

Because the response amplitude of the acoustic startle is markedly affected by refractory effects, it may be that the properties of habituation are altered when frequencies of repetition are used that invade the refractory period. For example, refractory effects might alter the effective intensity of the stimulus such that, at short intervals, the intensity of the stimulus producing habituation is effectively less. By "effective stimulus intensity" we mean simply that neurons in the startle pathway undergoing habituation would receive less effective input due to refractory effects. These neurons need not be in the specific sensory pathway. Indeed, initial speculation would place them in the brainstem reticular formation because the prepulse inhibition effect is, to some extent, multimodal (Ison & Hammond, 1971), and the reticular formation has been suggested to contain the labile elements responsible for habituation (Groves & Lynch, 1972). If this were the case, then comparisons of rate and degree of habituation at ISIs which invade the refractory period might be confounded by differences in effective stimulus intensity (e.g., Davis, 1970a, 1970b). This possibility was noted by Davis (1970b), for example, although he suggested that it may involve a reduced "perceived" intensity, rather than simply a reduced effective stimulus intensity impinging on habituating neurons. The latter may or may not correspond to a reduction in perceived stimulus intensity.

In conclusion, we suggest that habituation cannot be accounted for on the basis of "prepulse inhibition" alone and that response refractoriness, although it produces some rapid decrement in response amplitude at short ISIs, is not identical with the habituation process. Further, studies of habituation at ISIs which invade the refractory period of the response being studied must take this into account, as well as the fact that refractory period may alter effective stimulus intensity so that the frequency and intensity of the habituating stimulus become confounded, particularly at short ISIs.

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