Pharmacology Biochemistry & Behavior, Vol 20, pp 935–942, 1984 © Ankho International Inc Printed in the U S A 0091-3057/84 $3 00 + 00

The Effects of Clonidine, Prazosin, and Propranolol on Short-term and Long-term Habituation of the Acoustic Startle Response in Rats[1]

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Received 3 October 1983

LEATON, R. N AND J V. CASSELLA. *The effects of clonidine, prazosin and propranolol on short-term and long-term habituation of the acoustic startle response in rats* PHARMACOL BIOCHEM BEHAV **20**(6) 935–942, 1984 —The unique effect of clonidine in facilitating habituation of the acoustic startle response [10] was replicated However, clonidine had no effect on between-session habituation, showing a pharmacological dissociation between short- and long-term habituation Systematic manipulation of ISI showed clonidine's habituation-facilitating effect to be most striking with longer within-session ISIs where habituation was relatively weak in controls Comparing clonidine's effect to that of two other hypotensive agents, prazosin and propranolol, showed that the habituation-facilitating effect was not due to blood pressure effects Prazosin, an alpha$_1$-adrenergic blocker, facilitated short-term habituation, but significantly less so than did clonidine, an alpha$_2$-agonist Propranolol, a beta-adrenergic blocker, had no effect on short-term habituation Both prazosin and propranolol impaired long-term habituation, but propranolol did so without suppressing initial response levels. The data suggest that a synapse with both alpha-$_1$- and alpha$_2$-adrenoceptors may be critically involved in habituation of the acoustic startle response A beta-adrenergic involvement in long-term habituation is tentatively suggested.

Clonidine Habituation Startle response Prazosin Propranolol Alpha-adrenergic receptors Beta-adrenergic receptors Hypotension

THE acoustic startle response in the rat is one of the most commonly used response systems in theoretical and parametric studies of habituation. The pharmacology of the response has received considerable recent attention (for a review see [8]). A variety of drugs modulate startle responsiveness, but there is no concensus concerning the critical underlying transmitter system or systems. Habituation in this response system has been particularly resistant to pharmacological manipulation. In a series of studies Overstreet [24] reported only negative effects on within-session habituation following manipulations of cholinergic, noradrenergic, dopaminergic, or serotonergic systems. Two papers are frequently cited as indicating that depletion of serotonin by injections of parachlorophenylalanine (PCPA) impairs within-session habituation [4,5]. However, neither of these studies showed unambiguous effects on habituation, and the results overall suggest that PCPA produced a hyperresponsiveness or sensitization-like effect rather than an effect on habituation per se. The cholinergic blocking agent scopolamine may impair the transfer of habituation from the drugged to the non-drugged state [38,39] but it does not directly affect habituation of the startle response [24].

Remarkable within this sea of negative and ambiguous results is the report by Davis *et al.* [10]. They found that the presumed adrenergic agonist and hypotensive agent, clonidine, significantly accelerated the rate of within-session habituation of the acoustic startle response, while having little or no effect on the initial responsiveness of the animals. This unique drug effect may be of critical importance for understanding the pharmacology of habituation, and the primary purpose of the present experiments was to study further the effects of clonidine on habituation of the acoustic startle response. It was intended to replicate over a range of doses the effect of clonidine on within-session habituation and to explore the effect of the drug following a systematic manipulation of the interstimulus interval (ISI). In addition, the effect of clonidine on between-session or long-term habituation was investigated. Habituation of the acoustic startle response has been shown to have a significant long-term component [7,22] making it an appropriate response system for the study of the pharmacology of both short- and long-term processes. Finally, the effects of clonidine were compared with the effects of two other hypotensive drugs, prazosin and propranolol.

[1]The research was supported in part by a Faculty Research Grant from Dartmouth College.

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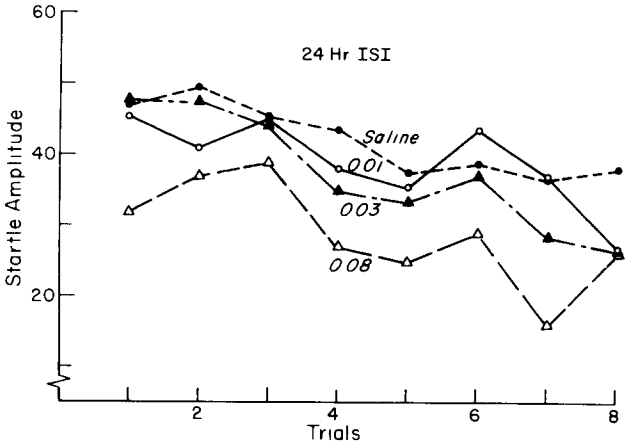

FIG. 1 Mean startle amplitude for the three clonidine-dosage groups (in mg/kg) and the saline groups on a 24-hr ISI

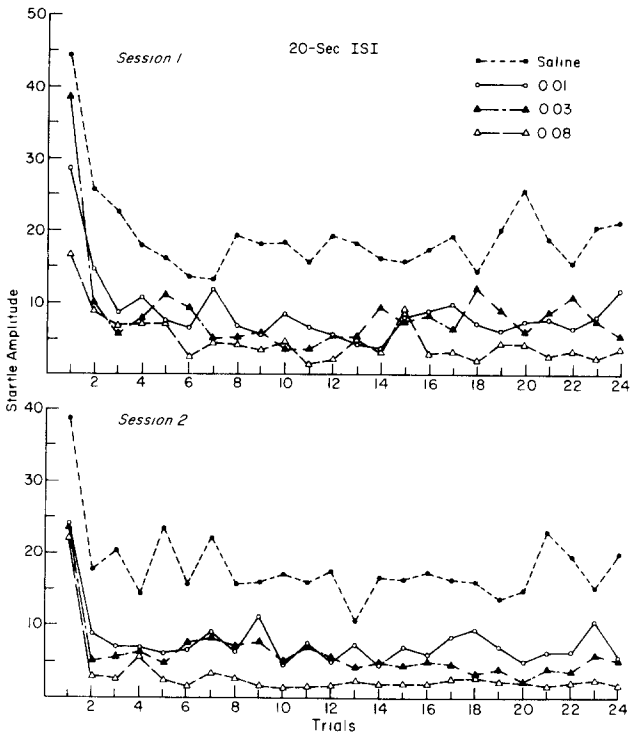

FIG 2 Mean startle amplitude for the three clonidine-dosage groups and the saline group for the two sessions on a 20-sec ISI

EXPERIMENT 1

Method

Subjects. Subjects were 48 experimentally naive male albino rats (Holtzman). They were approximately 110 days old and weighed between 420 and 480 g when testing began. They were individually housed, maintained on a 14:10 light-dark cycle, and allowed free access to food and water throughout the experiment.

Apparatus. The startle apparatus has been described previously [22]. Briefly, animals were tested in one of two 20×12×14 cm startle chambers enclosed within separate dimly illuminated, sound-attenuating boxes Startle amplitude was measured as the difference between the digitized and integrated output of the transducer for the 200-msec epochs before and after stimulus onset.

A 9-cm piezo-electric tweeter (Herald Electronics) centered 12 cm from the long wall of each startle chamber delivered the test stimuli which were 4-kHz pure tones, 118 dB SPL intensity, 100 msec in duration with a 5-msec rise/fall time Continuous white noise (61 dB SPL) from a 9-cm speaker located directly above the tweeter helped mask extraneous auditory stimuli. Intensity of the auditory stimuli was measured with a General Radio sound-level meter (Model 1551-C, 20 kHz setting) with the microphone centered inside the startle chamber

Procedure. Animals were divided randomly into four groups of 12 rats each, one group to receive 0.01 mg/kg, one 0 03 mg/kg, and one 0.08 mg/kg of clonidine hydrochloride, based on the weight of the salt The fourth group received injections of normal saline All injections were given intraperitoneally in a volume of 1 cc/kg. (The clonidine was generously supplied by Boehringer Ingleheim, Ltd.)

Each rat was given a 3-min adaptation session in the test chamber on each of 3 consecutive days and was injected with normal saline just prior to each session. Following these sessions drug testing began. Each animal was given one test session on each of 8 consecutive days. The appropriate drug was injected just prior to each session which consisted of one presentation of the test stimulus presented 20 min after the rat was placed in the startle chamber The rat was removed from the chamber 1 min after stimulus presentation (This injection procedure and the timing of the first tone presentation of a session remained constant throughout Experiment 1) Following these eight one-trial test sessions animals were given two sessions on consecutive days, each consisting of 24 tone presentations on a 20-sec ISI. On the following day a third session was given consisting of 16 tones on a 40-sec ISI

Following these sessions animals were tested on two consecutive days to evaluate responsiveness over a range of ISIs Each animal received 14 tone presentations during each session, providing a total of five tests at each of the following ISIs 5, 10, 30, 60 and 120 sec. Order of presentation was balanced so that each ISI followed itself and every other ISI an approximately equal number of times. The first stimulus presentation on each of these days, along with the first presentation on each of the three preceding test days, was used to assess an animal's long-term response asymptote (The response to the second tone in the second session was discarded from data analysis because it replicated the last ISI of the previous session)

Results

Figure 1 shows the startle response for the four groups over the initial 24-hr ISI. Analysis of variance showed that the decrease over trials was significant, $F(7,308)=10\ 00$, $p<0.0001$. Neither the difference among the groups, $F(3,44)=1\ 67$, $p>0.10$ nor the Groups × Trials interaction ($F<1$) was significant

Figure 2 shows the startle response for the two sessions on the 20-sec ISI. Analysis of the first session yielded a significant Groups effect, $F(3,44)=6.59$, $p<0\ 001$, Trials effect, $F(23,1012)=17.9$, $p<0.0001$, and Groups × Trials in-

TABLE 1

MEDIAN STARTLE RESPONSIVENESS ON TRIAL 1 AND AT ASYMPTOTE AND % DECREASE FROM TRIAL 1 TO ASYMPTOTE FOR THE TWO 20-SEC ISI SESSIONS AND THE 40-SEC ISI SESSION

Group	Trial 1	Asymptote	% Decrease
		20-sec ISI	
Na	45 2	15.4	64.0
0 01	26.5	6.4	74.0
0 03	25 5	3.9	84.4
0 08	13 8	1 8	84.5
	H=9 92*	H=17.01†	H=8 59*
		40-sec ISI	
Na	33 0	18.4	52 8
0 01	36.5	8 6	79.4
0 03	20 0	1 6	88.0
0 08	17 0	0 3	98.7
	H=5 40	H=19 42†	H=12.89†

*$p<0$ 05
†$p<0.01$

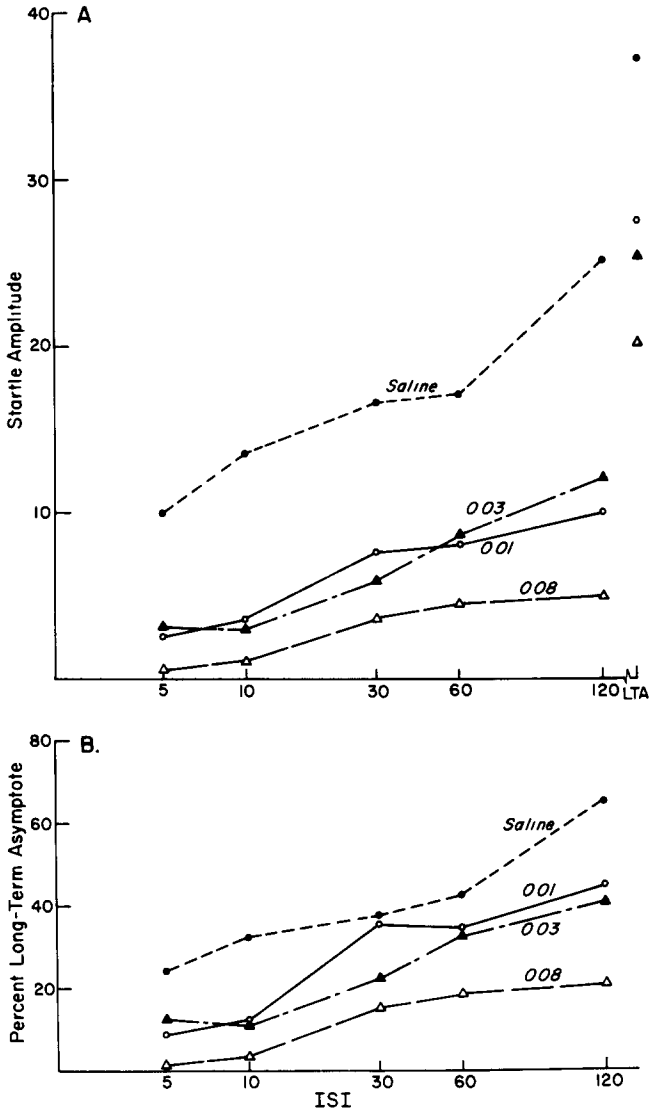

FIG. 3. Startle amplitude as a function of ISI (in sec). A. Mean startle amplitude. LTA indicates the long-term asymptote for each group estimated from the means of the first trial of five sessions B Startle responsiveness expressed as the mean percentage of each animal's long-term asymptote

teraction, $F(69,1012)=1.5$, $p<0.005$. Analysis of the second session revealed significant Groups, $F(3,44)=8.2$, $p<0.001$, and Trials, $F(23,1012)=14.1$, $p<0.0001$, effects, but the interaction was no longer significant, $F<1$. The differences in first-trial responsiveness were significant in Session 1, $H(3)=15.07$, $p<0.01$, but not in Session 2, $H(3)=4.70$, $p>0.15$. (The non-parametric Kruskal-Wallis one-way analysis of variance [29] was used whenever possible because of variance differences.) Because of these first-trial differences a clearer view of the effects of the drug was obtained by comparing the percentage of decrease in responsiveness from Trial 1 to asymptote. Asymptote was defined as the mean responsiveness over the last 10 trials of a session. These comparisons are shown in Table 1 for the two 20-sec ISI sessions combined. The groups differed significantly on Trial 1 responsiveness, asymptotic responsiveness and, importantly, on the percentage of decrease from Trial 1 to the asymptote

The data for the 40-sec ISI session were similar to that for the previous sessions except that the drug-control differences were more pronounced because the controls showed, as expected, a reduced percentage decrease over the longer ISI. These data are summarized in Table 1. The first-trial responsiveness differences were not significant in this case, but the asymptotic response levels and the percentages of decrease were highly significant.

Figure 3 shows the results of the systematic manipulation of ISI. Analysis of startle responsiveness (Fig. 3A) yielded significant Groups, $F(3,44)=6.53$, $p<0.01$, and ISI, $F(4,176)=33.51$, $p<0.0001$, effects and a significant interaction, $F(12,176)=2.14$, $p<0.05$. The long-term asymptote of responsiveness, based upon the mean of the first trial of five sessions (the two variable ISI sessions, the two 20-sec ISI sessions, and the 40-sec ISI session) is shown at the right of Fig. 3A. These differences did not reach significance, $H(3)=6.38$, $p<0.10$.

Figure 3B shows the ISI data as a percentage of each animal's long-term asymptote in order to reduce the effects of initial responsiveness differences. Analysis of these percentage data revealed a significant Groups, $F(3,44)=4$ 91, $p<0.01$, and ISI effect, $F(4,176)=38.2$, $p<0.0001$, but the interaction was not significant, $F(12,176)=1.58$, $p>0.10$. Note that at the 120-sec ISI the control group had only shown a decrease from its long-term asymptote of 35.5% while the drug groups had decreased, respectively, 57.9%, 59.5% and 79.6%. These differences at the 120-sec ISI were significant, $H(3)=16.13$, $p<0.002$. Group comparisons by the Mann-Whitney U test showed that the control group differed significantly from each drug group (all ps<0.05), the 0.01 and 0.03 drug-dosage groups did not differ, but both of these dosage groups differed from the 0.08 dosage group (ps<0.05).

Discussion

The present results on short-term habituation replicate and extend the results of Davis *et al.* [10]. Clonidine facilitated short-term habituation of the acoustic startle response, an effect unique in the pharmacology of habituation. Clonidine did have some suppressive effect on initial response levels, an effect which was apparently dose related. However, the initial responsiveness differences were statistically inconsistent and were small when compared with the consistently significant differential suppressions that followed stimulus presentations. Clonidine produced robust and dose-related increases in the percentage of decrement in responsiveness.

Clonidine's facilitating effect on short-term habituation was seen most clearly with the longer ISIs which produced relatively less decrement in the controls. This was apparent when comparing the 20-sec and 40-sec fixed-ISI sessions (see Table 1) and was most strikingly seen at the 120-sec ISI in the ISI series. At this relatively long ISI responsiveness was depressed by only 35% in the controls whereas responsiveness was depressed approximately 60% in the 0.01 and 0.03 clonidine groups and by almost 80% in the 0.08 clonidine group. Indeed, if a drug had its response-suppressive effect by facilitating short-term habituation one would expect to find the most striking drug-control comparisons at the longer ISIs where habituating effects should be relatively weak in controls. These results emphasize the importance of studying a range of ISIs in any investigation of habituation.

Analysis of the ISI function yielded a significant Groups by ISI interaction, but the interaction disappeared when responsiveness was expressed as a percentage of the long-term asymptote. This percentage analysis suggests that the interaction resulted from depressions from the control groups higher starting point rather than from any fundamental change in the ISI function over the 5- to 120-sec range. The most significant effect of clonidine had already occurred at the 120-sec ISI.

In contrast to its effect on short-term habituation clonidine had no detectable effect on long-term habituation. All groups habituated over the 24-hr ISI and no group differences or interactions were significant (see Fig 1). The habituation-facilitating effect of the drug which was so strongly present 120 sec following stimulus exposure had disappeared within 24 hr. Unreinforced stimulus presentations produce at least two decremental processes. One is a relatively permanent process which has been found to show no recovery for as long as 30 days. The second is a relatively short-term process which shows complete recovery within a few minutes [22,23]. Clonidine deepened and extended the short-term process but had no effect on the long-term process. It has been proposed frequently [2, 34, 37] and shown experimentally [6, 17, 22, 23] that short- and long-term habituation are independent processes that demand different underlying neural mechanisms. The present data suggest that they demand different neurochemical mechanisms as well.

EXPERIMENT 2

While Experiment 1 replicated and elaborated clonidine's unique habituation-facilitating effect, it did not contribute to our understanding of the mechanism underlying the effect. Clonidine is known to be a central agonist of norepinephrine (NE) [27] but as Davis [8] notes it is difficult to account for its effects on habituation in terms of the known actions of NE. Whatever its underlying mode of action, it is well established that clonidine is a potent hypotensive agent [19]. Davis *et al.* [10] discounted the effects of blood pressure on startle amplitude because they found no increase in startle responsiveness during the initial period when clonidine produces an increase in blood pressure. However, the initial hypertensive effect of clonidine is short lived [26], and any effect on habituation may easily have been obscured.

Experiment 2 was designed to determine if clonidine's habituation-facilitating effects could be accounted for by its hypotensive effects. To this end the effects of two clinically effective hypotensive agents, prazosin and propranolol, were compared with the effects of clonidine in habituation paradigms similar to those used in Experiment 1. Each of these drugs derives its hypotensive action by a different mechanism: clonidine as a central alpha-adrenergic agonist [20], prazosin as an alpha-adrenergic blocker [13] and propranolol as a beta-adrenergic blocker [28]. Comparing their effects should clarify the role of a drug's hypotensive action on habituation.

Little is known about the effects of either alpha- or beta-adrenergic blockers on habituation of the startle response, although some effects on response amplitude have been reported. Phenoxybenzamine, an alpha-blocker, decreases the amplitude of the acoustic [9,18] and tactile [15] startle response. The alpha-blocker piperoxane potentiates startle amplitude at low doses [10] and blocks the startle-depressive effect of clonidine [10] and the startle-excitatory effect of d-amphetamine [15]. Propranolol either decreases [15,25] or leaves unchanged [9] startle amplitude. Thus, Experiment 2 will provide data on the effects on habituation of these alpha- and beta-blockers as well as evaluating the effects of hypotensive agents.

Method

Subjects and apparatus Subjects were 44 experimentally naive male albino rats (Holtzman). They were approximately 130 days old and weighed between 450 and 560 g when testing began. Housing and maintenance conditions were the same as in Experiment 1. The startle apparatus and test stimuli were the same as described in Experiment 1

Procedure The general running and injection procedures were the same as described above. Initially all rats were divided randomly into four groups of 11 rats each, one group to receive 0.03 mg/kg clonidine hydrochloride, one 5 mg/kg dl-propranolol hydrochloride, and one 1 mg/kg prazosin hydrochloride. The fourth group served as a water-injected control. All drugs were dissolved in distilled water. (The prazosin was generously supplied by Pfizer Laboratories.) These dosages were selected for initial testing because they were expected to produce comparable effects on blood pressure [14, 26, 32].

All animals were given five 5-min adaptation sessions in the test chamber on consecutive days. Animals received an injection of distilled water prior to each of the first four adaptation sessions and an injection of the appropriate drug prior to the fifth session. Following the adaptation sessions rats were given eight 4-min sessions in the startle chamber on consecutive days to assess between-session habituation. Each session consisted of one presentation of the startle stimulus which occurred approximately 1 min after placement in the chamber. Appropriate drug injections were made 90 min prior to each session.

On the third day following the one-trial-per-day procedure testing began to evaluate within-session habituation.

Each animal was tested every other day until it completed 10 test sessions. Each test session consisted of 20 tone presentations on a 20-sec ISI. The first stimulus of each session was presented 1 min after the session began and the animal was removed from the test chamber 1 min following the last tone presentation. Each animal received a different one of the following 10 injection treatments 90 min prior to a session: distilled water; 0.01, 0.03, and 0.08 mg/kg clonidine; 0.3, 1, and 3 mg/kg prazosin; 1, 5, and 10 mg/kg propranolol. Each animal received each drug treatment once and only once. The order of the treatments was balanced across the 10 test sessions and each treatment followed every other treatment an approximately equal number of times. To allow for the statistical comparison of the same animals under the 10 different drug conditions a rate of habituation score was calculated for each animal under each drug condition. Rate was taken as the slope of the line from the first trial of a session to the asymptote of responsiveness for that session. Asymptote was defined as the mean response over the last 10 trials of a session. Order effects could be assessed by comparing animals that received a particular treatment early with those that received that same treatment late.

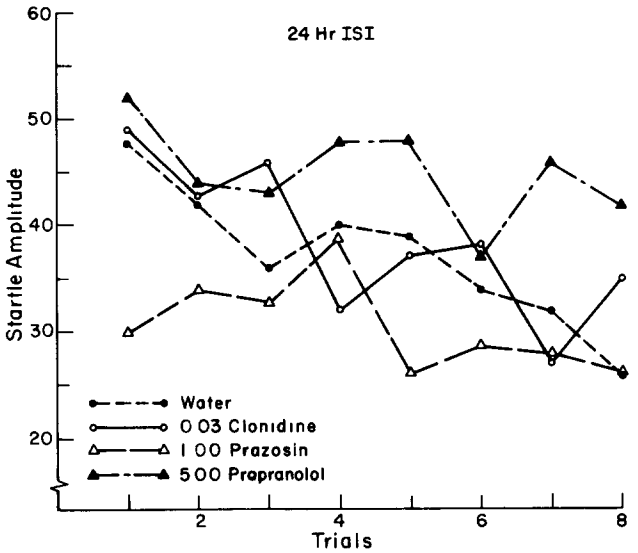

FIG 4 Mean startle amplitude for the four groups on a 24-hr ISI

Results

Figure 4 shows the startle response for all groups in the one-trial-per-day procedure. Analysis of variance yielded significant Groups, $F(3,40)=3.34$, $p<0.05$, and Trials, $F(7,280)=5.63$, $p<0.001$, effects and a significant Groups × Trials interaction, $F(21,280)=1.86$, $p<0.05$. Further analysis of the overall groups difference showed that only the prazosin and propranolol groups differed significantly overall, $F(1,20)=11.4$, $p<0.005$. Further analysis of the Groups × Trials interaction showed that the decreased responsiveness over trials was significant for the water, $F(7,70)=4.34$, $p<0.001$, and clonidine, $F(7,70)=4.63$, $p<0.001$, groups but not for the prazosin, $F(7,70)=1.33$, $p>0.20$, or propranolol, $F(7,70)=1.82$, $p>0.10$, groups.

The results of the within-session habituation tests are shown in Fig. 5 as log-log plots for each of the drug treatment conditions. The rate of response decrement over trials can be visualized directly from these plots. Note that the control curve is repeated in each panel. Clonidine dramatically increased the rate of response decrement in an apparently dose-related manner. Prazosin increased the rate of decrement to a lesser extent and the increased rate was not as clearly dose related. Propranolol had little or no effect on the rate of habituation, reducing it if anything.

These data were converted to rate measures for statistical analysis, and the rates are displayed in Fig. 6. Overall analysis of the habituation rates was significant, $F(9,387)=13.03$, $p<0.001$, and planned comparisons provided a more detailed analysis. Collapsed across dosages both the clonidine treatment and the prazosin treatment produced significantly greater rates of habituation than the water treatment ($ps<0.005$), and the propranolol treatment ($ps<0.0001$). Clonidine produced significantly greater rates of habituation than prazosin ($p<0.001$). Propranolol treatment did not differ from water ($F<1$). Analysis of the three clonidine doses yielded a significant effect of dose ($p<0.001$) as did analysis of the three doses of prazosin ($p<0.05$). Propranolol treatment did not yield a significant dose-response

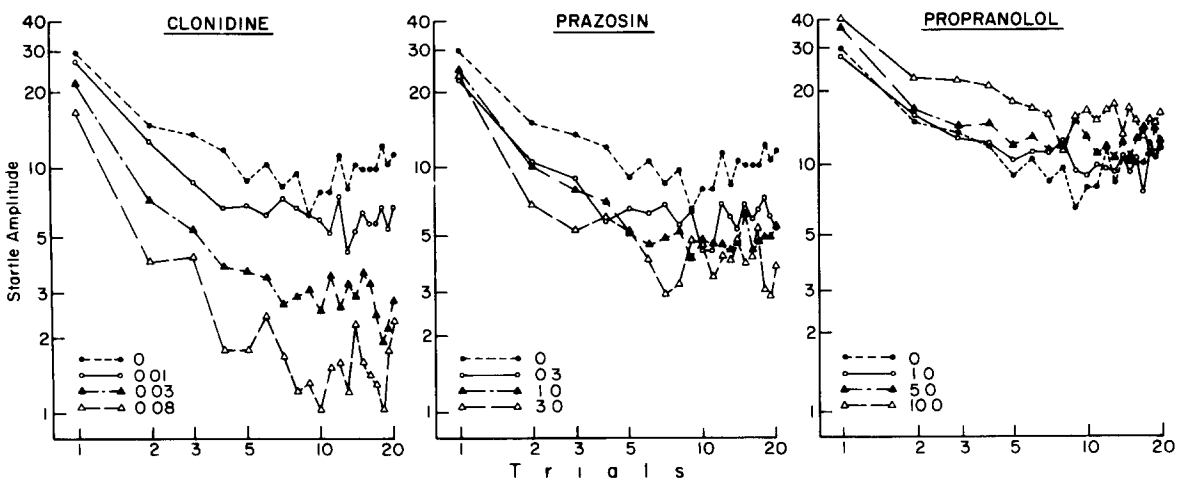

FIG. 5 Log-log plots of mean startle amplitude for each of the 10 drug treatment conditions on a 20-sec ISI Note that the water condition is repeated in each panel.

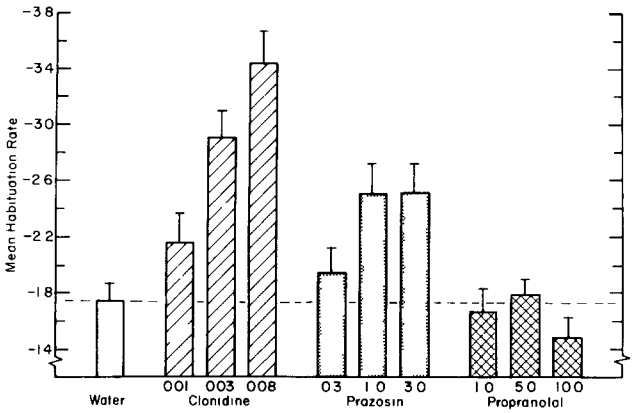

FIG. 6. Habituation rates for the 10 drug treatment conditions calculated as the slope of the line from the first trial of a session to the asymptote of responsiveness for that session Note that the more negative the number the steeper is the slope. A dashed line is extended from the mean of the water condition for comparison Standard error of the mean is shown atop each bar

effect ($p>0.20$). Each clonidine dose differed significantly from every other clonidine dose ($ps<0.05$) whereas for prazosin only the lowest dose differed significantly from the other two doses ($p<0.05$).

The effect of repeated drug testing with the same animals was assessed by comparing the rate of habituation for each treatment for the 22 animals that received that treatment on one of the first five treatment days with the 22 that received that treatment on one of the last five treatment days. The general tendency was for the rate of habituation to be greater during the last five as compared with the first five sessions. This trend reached significance for the water treatment, $t(42)=2.15$, $p<0.05$, the 0.01 mg/kg clonidine treatment, $t(42)=1.99$, $p<0.051$, and the 1 mg/kg propranolol treatment, $t(42)=2.51$, $p<0.05$.

Discussion

Consistent with Experiment 1 and with the previous report of Davis *et al.* [10] clonidine again strikingly facilitated short-term habituation of the acoustic startle response. Prazosin, the alpha-adrenergic blocker, also facilitated short-term habituation, as compared to the control condition, but it had significantly less effect than clonidine. The significant difference between the habituation-facilitating effects of clonidine and prazosin could not have resulted simply from differences in dosage. The effect of prazosin had leveled off at the two highest doses while clonidine had progressively more effect at each higher dose. Propranolol, the beta-adrenergic blocker, had no significant effect on short-term habituation.

The effects of repeatedly testing the same animals under each drug condition had no significant effect on the overall comparisons among water, clonidine and prazosin Animals tested under the water condition during the last five sessions showed significantly higher rates of habituation than those tested during the first five sessions. This trend may have made it somewhat more difficult to reveal the habituation-facilitating effects of clonidine and prazosin over the ten test sessions. Of the six clonidine and prazosin conditions only the 0.01 clonidine condition showed significantly higher habituation rates during the last five sessions. The higher habituation rates in later sessions in the water condition could have made it somewhat easier to reveal habituation-attenuating effects, and any suggestion that propranolol reduced habituation rates would have to be evaluated accordingly.

The data strongly suggest that clonidine's dramatic habituation-facilitating effect is not directly related to its potent hypotensive action. No dose of propranolol produced even a suggestive facilitation of short-term habituation, and the dose ranges for clonidine and propranolol should have produced significant overlaps in blood pressure effects. Also, over the dose ranges used prazosin should have had at least as much effect on blood pressure as clonidine yet it had significantly less effect on short-term habituation. These data are consistent with the suggestion of Davis *et al.* [10] that changes in blood pressure are not related to clonidine's effect on startle amplitude

The question remains as to why clonidine should have such a potent habituation-facilitating effect. Clonidine is an NE agonist, and the weight of the evidence [8] suggests that NE is excitatory to the startle response. Yet clonidine suppresses startle and facilitates habituation. The answer may lie in clonidine's selectivity for a specific subpopulation of alpha-adrenergic receptors. A considerable body of pharmacological evidence suggests that alpha-adrenergic receptors are subdivided into distinct $alpha_1$- and $alpha_2$-subtypes [3, 21, 26, 33, 36], and clonidine has been shown to stimulate selectively the $alpha_2$-adrenoceptor [3, 31, 33]. While stimulation of the $alpha_1$-adrenoceptor has the anticipated NE-excitatory effects, stimulation of the $alpha_2$-receptor reduces the release of NE and thus provides a negative-feedback control of transmitter release [12, 21, 30]. It has been widely assumed that these $alpha_2$-receptors are located on the presynaptic nerve terminal [11, 12, 21, 30, 33], but recent evidence (see [31]) questions this assumption. Whatever the anatomical locus of the receptor clonidine, by selectively stimulating the $alpha_2$-adrenoceptor, acts to reduce the availability of NE at the postsynaptic receptor and thus acts like a special variety of NE antagonist.

Handley and Thomas [15] suggested that clonidine's suppression of tactile startle was due to stimulation of presynaptic alpha-adrenergic receptors, and a negative feedback control of transmitter release, whether pre- or postsynaptic, could provide an excellent mechanism for short-term habituation. Clonidine's near unique habituation-facilitating effect, and its selectivity for the $alpha_2$-adrenoceptor, provide suggestive but indirect evidence for the involvement of an $alpha_2$-adrenoceptor in the short-term habituation process The picture is made potentially more complex by recent evidence (see [31]) suggesting that clonidine is a partial $alpha_2$-agonist, stimulating only a subpopulation of $alpha_2$-adrenoceptors. Of course, it may well be that clonidine derives its effects, as Davis suggested [8], by action on an as yet unidentified neurochemical system, and the complexity of the noradrenergic synapse alone provides an abundance of possibilities. As reviewed by Starke [30] noradrenergic nerve terminals may contain, in addition to alpha-adrenergic receptors, nicotinic and muscarinic receptors, beta-adrenergic receptors, and receptors for dopamine, prostaglandins and angiotensin Whatever the neurochemical system involved, any hypothesis about clonidine's mode of action must account for the fact that it depresses responsiveness to repeated stimulation significantly more than it depresses initial startle responsiveness. None of the suggestions solve this problem of mechanism

Although less effective than clonidine, prazosin also facilitated habituation and may have done so because it too reduced alpha-adrenergic activity. Prazosin selectively blocks alpha$_1$-adrenoceptors [1, 3, 35, 36] and has a very low affinity for receptor sites labelled by clonidine [16]. Clonidine reduces NE release by stimulating alpha$_2$-receptors, and prazosin reduces the impact of the released NE by blocking postsynaptic alpha$_1$-receptors. If a specific type of alpha$_2$-adrenoceptor represents a critical feedback mechanism for habituation, one might expect that attacking it directly by clonidine would produce a more pronounced effect than a blockade of the postsynaptic alpha$_1$-adrenoceptor. One would also expect non-specific agonists and antagonist to produce ambiguous effects on habituation, as has usually been the case [8]. Handley and Thomas [15] suggested that a noradrenergic synapse with pre- and postsynaptic alpha-adrenergic receptors is critically involved in the control of the amplitude of the tactile startle response. The present data suggest that an adrenergic synapse with two distinct receptor types may be critically involved in short-term habituation of the acoustic startle response, although the pre- and postsynaptic distinction may no longer be valid.

Experiment 2 again showed the pharmacological independence of short- and long-term habituation. Both clonidine and water treated animals showed significant habituation on a 24-hr ISI. However, neither the prazosin nor the propranolol treated animals showed significant between-session habituation. The effect of propranolol may be more interesting than the effect of prazosin because propranolol did not suppress initial response levels as did prazosin. This is to our knowledge the first report of a pharmacological effect on long-term habituation of the acoustic startle response, and the effects of both of these drugs need further study. The suggestion of a beta-adrenergic involvement in long-term habituation may prove particularly interesting.

REFERENCES

1. Atkins, F. L and G L. Nicolosi Alpha-adrenergic blocking activity of prazosin. *Biochem Pharmacol* **28:** 1233–1237, 1979.
2. Buchwald, J S. and G. L. Humphrey. An analysis of habituation in the specific sensory systems In *Progress in Physiological Psychology,* vol 5, edited by E. Stellar and J M Sprague. New York Academic Press, 1973, pp 1–75.
3. Bylund, D. B and D. C. U'Prichard Characterization of alpha$_1$-and alpha$_2$-adrenergic receptors. *Int Rev Neurobiol* **24:** 343–431, 1983.
4. Carlton, P L and C. Advokat Attenuated habituation due to parachlorophenylalanine *Pharmacol Biochem Behav* **1:** 657–663, 1973
5. Conner, R. L., J. M. Stolk, J D. Barchas and S Levine. Parachlorophenlyalanine and habituation to repetitive auditory startle stimuli in rats. *Physiol Behav* **5:** 1215–1219, 1970
6. Davis, M Effects of interstimulus interval length and variability on startle-response habituation in the rat *J Comp Physiol Psychol* **72:** 177–192, 1970.
7. Davis, M. Differential retention of sensitization and habituation of the startle response in the rat. *J Comp Physiol Psychol* **78:** 260–267, 1972.
8. Davis, M. Neurochemical modulation of sensory-motor reactivity: Acoustic and tactile startle reflexes. *Neurosci Biobehav Rev* **4:** 241–263, 1980
9. Davis, M , D. I Astrachan, P. M. Gendelman and D S. Gendelman 5-Methoxy-N, N-dimethyltryptamine Spinal cord and brainstem mediation of excitatory effects on acoustic startle. *Psychopharmacology (Berlin)* **70:** 123–130, 1980.
10. Davis, M., J M Cedarbaum, G K. Aghajanian and D. S. Gendelman Effects of clonidine on habituation and sensitization of acoustic startle in normal, decerebrate, and locus coeruleus lesioned rats *Psychopharmacology (Berlin)* **51:** 243–253, 1977.
11. DeLangen, C. D. J., F. Hogenboom and A H Mulder Presynaptic noradrenergic alpha-receptors and modulation of 3h-noradrenaline release from rat brain synaptosomes *Eur J Pharmacol* **60:** 79–89, 1979
12. Farnebo, L. O. and B Hamberger Drug-induced changes in the release of 3H-monoamines from field stimulated rat brain slices *Acta Physiol Scand* Suppl **371:** 35–44, 1971.
13. Fuller, R. W , H D Snoddy and K W Perry Effect of prazosin on norepinephrine concentration and turnover in rat brain and heart *Arch Int Pharmacodyn* **231:** 30–41, 1978.
14. Graham, R M., H F Oates, L. M. Stoker and G. S. Stokes. Alpha blocking action of the antihypertensive agent, prazosin. *J Pharmacol Exp Ther* **201:** 747–752, 1977.
15. Handley, S. L and K V. Thomas Potentiation of startle response by d- and l-amphetamine The possible involvement of pre- and postsynaptic alpha-adrenoceptors and other transmitter systems in the modulation of a tactile startle response. *Psychopharmacology (Berlin)* **64:** 105–111, 1979.
16. Hornung, R., P. Presek and H Glossman. Alpha adrenoceptors in rat brain· Direct identification with prazosin. *Arch Pharmacol* **308:** 223–230, 1979.
17. Jordan, W. P and R N. Leaton Effects of mesencephalic reticular formation lesions on habituation of startle and lick suppression responses in the rat *J Comp Physiol Psychol* **96:** 170–183, 1982
18. Kehne, J. H and C. A Sorenson The effects of pimozide and phenoxybenzamine pretreatments on amphetamine and apomorphine potentiation of the acoustic startle response in rats *Psychopharmacology (Berlin)* **58:** 137–144, 1978.
19. Kobinger, W. Pharmacologic basis of the cardiovascular actions of clonidine. In. *Hypertension Mechanisms and Management,* edited by G. Onesti, K. E Kim and J. H. Moyer. New York. Grune and Stratton, 1973, pp. 369–380
20. Kobinger, W. and L. Pichler. Evidence for direct alpha-adrenoceptor stimulation of effector neurons in cardiovascular centers by clonidine. *Eur J Pharmacol* **27:** 151–154, 1974.
21. Langer, S. Z Presynaptic receptors and their role in the regulation of transmitter release. *Br J Pharmacol* **60:** 481–497, 1977
22. Leaton, R. N. Long-term retention of the habituation of lick suppression and startle response produced by a single auditory stimulus. *J Exp Psychol (Anim Behav)* **2:** 248–259, 1976.
23. Leaton, R. N and W P Jordan. Habituation of the EEG arousal response in rats short- and long-term effects, frequency specificity, and wake-sleep transfer. *J Comp Physiol Psychol* **92:** 803–814, 1978.
24. Overstreet, D. H Pharmacological approaches to habituation of the acoustic startle response in rats *Physiol Psychol* **5:** 230–238, 1977
25. Pohorecky, L. A., M. Cagan, J. Brick and L. S. Jaffe Startle response in rats: Effects of ethanol. *Pharmacol Biochem Behav* **4:** 311–316, 1976.
26. Rand, M. J. and J. Wilson. Mechanisms of the pressor and depressor actions of St 155 (2-(2,6-dichlorophenylamino)-2-imidazloline hydrochloride, Catapres). *Eur J Pharmacol* **3:** 27–33, 1968.
27. Sharma, J. N , B B. Sandrew and S. C. Wang CNS site of clonidine induced hypotentions: A microiontophoretic study of bulbar cardiovascular neurons *Brain Res* **151:** 127–133, 1978.

28. Sharma, J. N , B. B. Sandrew and S. C. Wang. CNS site of beta-adrenergic blocker-induced hypotension in the cat: a microiontophoretic study of bulbar cardiovascular neurons. *Neuropharmacology* **18:** 1–5, 1979
29. Siegel, S *Nonparametric Statistics* New York. McGraw-Hill, 1956
30. Starke, K. Regulation of noradrenaline release by presynaptic receptor systems. *Rev Physiol Biochem Pharmacol* **77:** 1–124, 1977
31 Starke, K. and K. P. Altman. Inhibition of adrenergic neurotransmission by clonidine. An action on prejunctional alpha-receptors. *Neuropharmacology* **12:** 339–347, 1973
32 Sugawara, K. and M. Ozaki On acute hypotensive effects of pindolol and propranolol in conscious rats *Gen Pharmacol* **11:** 213–219, 1980
33 Tanaka, T. and K. Starke Antagonist/agonist-preferring alpha-adrenoceptors or alpha-1/alpha-2 adrenoceptors? *Eur J Pharmacol* **63:** 191–194, 1980.
34. Thompson, R. F. and W. A Spencer Habituation. A model phenomenon for the study of neuronal substrates of behavior *Psychol Rev* **73:** 16–43, 1966
35 Timmermans, P B. M W M , E Lam and P A VanZwieten The interaction between prazosin and clonidine at alpha-adrenoceptors in rats and cats. *Eur J Pharmacol* **55:** 57–66, 1979
36. U'Prichard, D C , M. E Charness, D. Robertson and S. H Snyder Prazosin: Differential affinities for two populations of alpha-noradrenergic receptor binding sites. *Eur J Pharmacol* **50:** 87–89, 1978
37. Wagner, A R. Priming in STM An information processing mechanism for self-generated or retrieval-generated depression in performance. In: *Habituation Perspectives from Child Development, Animal Behavior and Neurophysiology*, edited by T. J Tighe and R N Leaton. New Jersey Erlbaum, 1976
38 Warburton, D. M and P M Groves The effects of scopolamine on habituation of acoustic startle in rats *Commun Behav Biol* **3:** 289–293, 1969.
39 Williams, J. M., L W. Hamilton and P. L Carlton Pharmacological and anatomical dissociation of two types of habituation. *J Comp Physiol Psychol* **87:** 724–732, 1974