Behavioral Neuroscience
1993, Vol. 107, No. 2, 306-316


0735-7044/93/$3.00

# Cholinergic Mechanisms in Startle and Prepulse Inhibition: Effects of the False Cholinergic Precursor N-aminodeanol

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We examined the effects of cholinergic deficiency on prepulse inhibition (PPI) of the acoustic startle. Rats treated with a choline-free diet that contained the false cholinergic precursor N-aminodeanol showed great deficit in PPI. This deficit does not appear to be secondary to an increase of stereotyped behaviors. Startle threshold was also greatly reduced, as these rats startled to the 70-dB prepulse and the baseline startle amplitude was increased by 60% over the control rats. Arecoline (4 mg/kg) partially reversed the deficit in PPI. This improvement persisted beyond the period of drug treatment. On the other hand, scopolamine (1 mg/kg) reduced PPI in the control rats. These results suggest that cholinergic systems play a major role in both the elicitation and prepulse inhibition of startle.



The role cholinergic systems play in modulating the startle reflex is unclear. Previous studies with cholinergic agonists or antagonists have reported only small yet conflicting results (for a review, see Davis, 1980). Recent studies in rats treated chronically with a choline-free diet that contained an unnatural choline analog N-amino-N,N-dimethylaminoethanol (N-aminodeanol; NADe), on the other hand, suggest that cholinergic systems play a major role in the modulation of startle (Newton, Crosland, & Jenden, 1986; Russell et al., 1990). It is not known whether one form of startle modulation, the suppression of startle by a preceding stimulus (*prepulse inhibition*), is also affected by this procedure. It has been hypothesized that the dorsolateral pons, which contains one major source of cholinergic neurons in the brainstem, may mediate prepulse inhibition (Wu, Siegel, Shouse, & Schenkel, 1990). To test this hypothesis we examined prepulse inhibition in choline-deficient, NADe-treated rats by using the dietary protocol developed by Jenden and his colleagues (e.g., Newton et al., 1986). This procedure reduces brain choline level by about 70% and acetylcholine (ACh) level by about 60% (Knusel et al., 1990; Newton, Crosland, & Jenden, 1985). It has been shown that acetylated NADe replaces ACh and is released on stimulation as a false transmitter (Newton & Jenden, 1985). This false transmitter has only about 4% of the potency of ACh on muscarinic receptors (Newton, Ringdahl, & Jenden, 1985).

Prepulse inhibition is the suppression of the startle response produced by a stimulus presented immediately before the eliciting stimulus (Hoffman & Ison, 1980; Ison & Hoffman, 1983). The neurotransmitter system that mediates prepulse inhibition has not been conclusively identified. Prepulse inhibition is reduced by dopaminergic agonists apomorphine and d-amphetamine (Mansbach, Geyer, & Braff, 1988; Swerdlow, Geyer, Braff, & Koob, 1986), an effect possibly mediated through mesolimbic and striatal dopaminergic systems (Swerdlow et al., 1986). Recently, the NMDA antagonists ketamine, phencyclidine, and dizocilpine were found to eliminate prepulse inhibition (Mansbach & Geyer, 1989, 1991), and at least the effects of phencyclidine and dizocilpine were not blocked by the dopaminergic antagonist haloperidol (Keith, Mansbach, & Geyer, 1991). The mechanism by which glutamatergic systems affect the prepulse inhibition mechanism is still unclear. It is possible that multiple neurotransmitter systems may be involved in the mediation of prepulse inhibition (Davis, 1988). A possible involvement of the cholinergic system in prepulse inhibition of the startle reflex has not been studied. In this study we demonstrate for the first time that cholinergic mechanisms are strongly involved in prepulse inhibition of startle.

## General Method

### *Subjects*

The subjects were 13 male Sprague-Dawley rats between 100 and 120 days of age. They were the offspring of pregnant females purchased from Bantin and Kingman (Pleasanton, CA). They were housed 2 to a semibarrier cage inside a room with 12:12-hr light–dark cycle. The rats were cared for as described in Russell et al. (1990).

### *Apparatus*

The apparatus and the testing environment for the startle response was similar to that described in Wu, Mallick, and Siegel (1989). Briefly, animals were tested individually during the light phases of the

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This research was supported by Public Health Service Grants MH17691 and MH43811 and by the Medical Research Service of the Department of Veterans Affairs.

We are grateful to R. S. Szymuziak and D. J. McGinty for their helpful suggestions and for providing facilities for animal care.

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light–dark cycle, inside a chamber with an ambient noise level of 55 ± 2 dB (SPL/A, re 20 μPa). The startle platform was constructed of Plexiglas with an accelerometer (Kistler Instrument Corp., Amherst, NY) mounted underneath. The startle response, as expressed by vertical movement of the platform, was measured by the accelerometer. The signal was amplified and band-passed between 3 and 100 Hz with a Grass 7P511 amplifier. Four hundred milliseconds of the signal, starting 100 ms before the prepulse, was full-wave rectified and digitized at 400 Hz with an IBM PC/XT equipped with Model 1401 interface and signal averaging package (Cambridge Electronic Design, Cambridge, United Kingdom). The startle amplitude was expressed as the integral of the response envelope during the 200 ms after the startle-eliciting stimulus. The initial and peak latencies of the startle response were also measured with the digitized record. The initial latency was defined as the first point after the startle stimulus where the amplitude exceeds the mean amplitude of the prestimulus period by two standard deviations. The peak latency was defined as the point with the largest amplitude during the 200 ms after the delivery of the startle stimulus.

Animals were allowed 10 min to adapt to the test environment inside a plastic test cage (26 × 16 × 10 cm inside dimensions) that was placed on top of the startle platform. The top of the test cage had a removable metal screen. Startle stimuli were presented through two midrange loudspeakers located in diagonally opposite ends of the top of the recording chamber, about 50 cm from the animal's head. Prepulses were delivered through a loudspeaker situated between the other two speakers.

### *Procedures*

The pups were weaned to their respective diets at the age of 29 days. They were assigned randomly to either experimental (NADe) or control (choline) diets. They were fed ad libitum a choline-free basic diet in pellet form with either NADe chloride (experimental, $n = 7$) or choline chloride (control, $n = 6$) at a concentration of 35.8 mmol/kg added to the diet. NADe was synthesized in the laboratory by a method described in Newton, Ringdahl, and Jenden (1983).

Tests for baseline startle response and prepulse inhibition began at the age of 100–120 days. The startle-eliciting stimulus was a 20-ms noise pulse, with a 1-ms rise–fall time, of either 115 or 100 dB SPL/A. The prepulse was a 20-ms, 70-dB noise pulse, with a 3-ms rise–fall time, presented 100 ms before the eliciting stimulus. The standard test procedure consisted of a total of 30 trials presented in counterbalanced order. Half of the trials were presented with the eliciting stimulus alone (S-alone trials), and the other half with the prepulse preceding the eliciting stimulus (P trials). The S-alone trials served as the baseline startle for the prepulse trials. The amount of prepulse inhibition was calculated as the mean difference in startle amplitude between S-alone and P trials and expressed as the percentage of the mean S-alone startle level:

$$100 \times [(\text{Mean Startle Amplitude in S-Alone Trials} - \text{Mean Startle Amplitude in P Trials}) \div \text{Mean Startle Amplitude in S-Alone Trials}].$$

Intertrial interval was between 30 and 40 s. In order to reduce within-subject variability, and because startle amplitude tends to stabilize after the initial few trials, four warm-up S-alone trials were given at the beginning of the test. Except for the study of startle habituation, the data from the warm-up trials were not included in the analyses.

### *Measurement of Choline and N-aminodeanol Levels*

All animals were sacrificed at the end of the experiments. The animals were decapitated, blood was collected, and the brain and the liver were quickly removed. Free and phospholipid-bound choline and NADe levels in the plasma, liver, and cortex were analyzed with high-pressure liquid chromatography (HPLC), as in the procedures described in Knusel et al. (1990).

### *Data Analysis*

For the analysis of startle responses, absolute startle amplitude and percentage of prepulse inhibition were used except when noted. Repeated measures analysis of variance (ANOVA), post hoc comparisons, $t$ and chi-square tests were performed with the SYSTAT package (Wilkinson, 1987).

## Experiment 1

This experiment was designed to examine the effects of the cholinergic false precursor NADe on the elicitation and prepulse inhibition of the acoustic startle response. NADe treatment has been shown to greatly enhance startle response (Newton et al., 1986; Russell et al., 1990). To avoid a possible interference with prepulse inhibition by a ceiling effect due to the heightened startle response, two intensity levels of the eliciting stimulus were used.

NADe-treated rats generally display more spontaneous activity in an open-field test environment than do the choline-treated control subjects (Jenden et al., 1989). Our preliminary observation also indicates that NADe-treated rats are very active in the enclosed test cage in the testing environment. These rats spent more time in grooming and stereotyped behaviors (e.g., intense grooming, sniffing, rearing, and locomoting). Because grooming and stereotyped activities may affect the elicitation and the modification of the startle response (Wecker & Ison, 1986), the behavior of the rat during startle elicitation was also recorded.

### *Method*

The subjects were the 13 rats described in the General Method. Four control rats and 3 experimental rats were first tested with a 115-dB eliciting stimulus, and the others were tested with a 100-dB eliciting stimulus first. The prepulse was a constant 70-dB noise pulse. The test conditions were reversed 4 days later for the two groups.

The rat's behavior was observed through a one-way mirror on the chamber door for 10 s before the trial and was categorized into quiet, face washing, grooming, sniffing, and other activities. The last category consists of such miscellaneous behaviors as locomoting, rearing, and head turning.

### *Results and Discussion*

Figure 1 shows the effect of a 70-dB prepulse on the startle response elicited with a 115-dB or a 100-dB stimulus. Experimental rats showed greater startle response at both intensity levels than did the control rats. The increase in startle amplitude of the experimental rats over the control rats was 60% with the 115-dB stimulus, $F(1, 11) = 4.86$, $p < .05$, and 97% with the 100-dB stimulus, $F(1, 11) = 50.64$, $p < .001$.

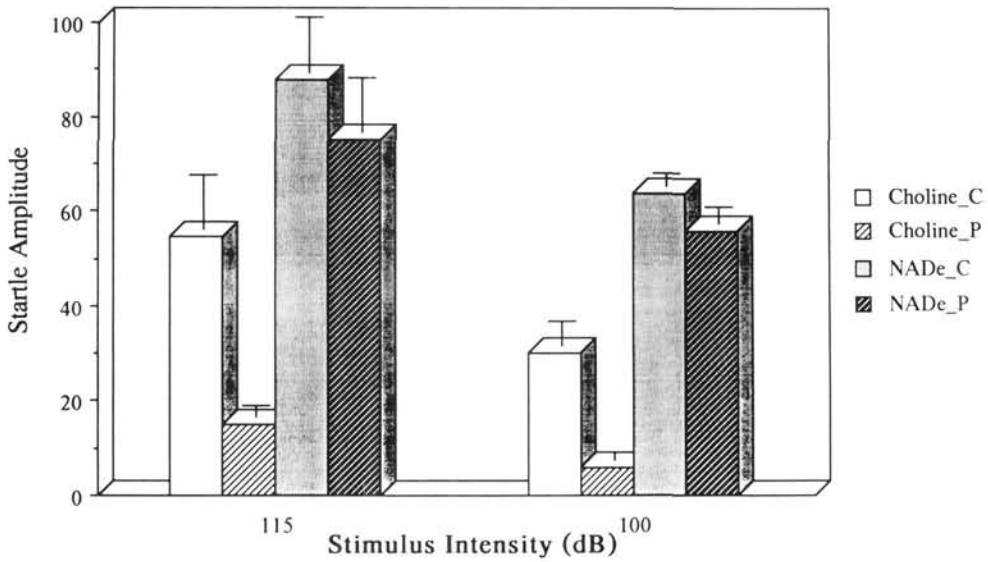


*Figure 1.* Startle response and prepulse inhibition as a function of elicitation intensity in choline- (control) and N-aminodeanol-treated (experimental) rats. (N-aminodeanol-treated rats showed enhanced startle response [60% and 97% increase over the control rats at 115-dB and 100-dB startle intensities, respectively] and a deficit in prepulse inhibition [11.9% and 12.5% inhibition with 115-dB and 100-dB startle intensities, respectively] as compared with the control rats [70.7% and 76.2%]. Startle amplitudes in this and other figures represent absolute response size in arbitrary units. C = trials with startle-eliciting stimulus alone; and P = trials with prepulse.)

There was no difference in initial startle latency between the two groups (*M*s ± *SE*s = 12.7 ± 0.3 and 13.2 ± 0.4 ms, for experimental and control rats, respectively), $t(11) = 1.02, p > .5$. However, experimental rats tended to show a late peak latency, as compared with the control rats: 67.9 ± 4.2 vs. 50.8 ± 7.6 ms, $t(11) = 2.05, p = .07$, and 65.7 ± 5.8 vs. 48.3 ± 7.5 ms, $t(11) = 1.86, p = .09$, for S-alone and P trials, respectively. It was noticed during testing that experimental rats tended to show two startle responses, an initial, but smaller response, followed by a larger, late response. It is possible that the late peak latency represents a secondary startle response, which was either a late response to the eliciting stimulus or a second startle reaction to the initial one.

In addition, and unlike control rats that showed no or little response to the prepulse, experimental rats frequently responded to the 70-dB prepulse (Figure 2), which suggests a reduced startle threshold. Further analysis of the data showed that there was a significant difference in response amplitude to the prepulse as a function of the intensity of the eliciting stimulus: 1.96 with the 115-dB stimulus vs. 0.97 with the 100-dB stimulus (as compared with 0.18 and 0.23 for the control animals), $F(1, 11) = 9.73, p < .01$. This result suggests that sensitization may be responsible for this differential effect. The response to the prepulse did not facilitate startle, because there was no difference in either initial or peak latency between S-alone and P trials in either experimental or control rats (all *p*s > .5).

Control rats showed an average of 70.7% and 76.2% startle reduction by the 70-dB prepulse with the 115-dB and 100-dB eliciting stimulus, respectively, which is comparable with that in normal rats (e.g., Ison, O'Connor, Bowen, & Bocirnea, 1991). In contrast, the experimental rats showed very little prepulse inhibition under either 115-dB (11.9%) or 100-dB (12.5%) startle conditions. The repeated measures ANOVA revealed a significant difference between experimental and control rats in the effect of prepulse; there was a significant interaction of diet treatment and prepulse effect, $F(1, 11) = 11.07, p < .01$. This difference in prepulse effect between experimental and control rats was true regardless of stimulus intensity; there was no significant interaction of stimulus intensity, diet treatment, and prepulse effect, $F(1, 11) = 0.93, p > .05$. Reducing the intensity of the eliciting stimulus to 100 dB, which reduced the basal startle amplitude of the experimental rats to about the level of the control rats with 110 dB (63.4 ± 7.6 vs. 54.7 ± 25.7), $t(11) = 0.87, p > .05$, did not change the deficit in prepulse inhibition. This result suggests that the diminished prepulse effect in the experimental rats was not secondary to a ceiling effect due to the heightened startle response.

Consistent with prior observations, experimental rats were much more active than the control rats during the test. As shown in Figure 3 (top panel), experimental rats were engaged in face washing, grooming, sniffing, or locomotor activities during 72.6% of the trials, as opposed to the 24% of trials for control rats, $\chi^2(1, N = 60) = 20.3, p < .001$. Face washing with the forepaws was usually followed by intense grooming of the hind flank and genitalia area with the snout. The small trial numbers of this experiment did not allow a comparison of prepulse effects under each behavior category. Experiment 2 was designed to address this question.

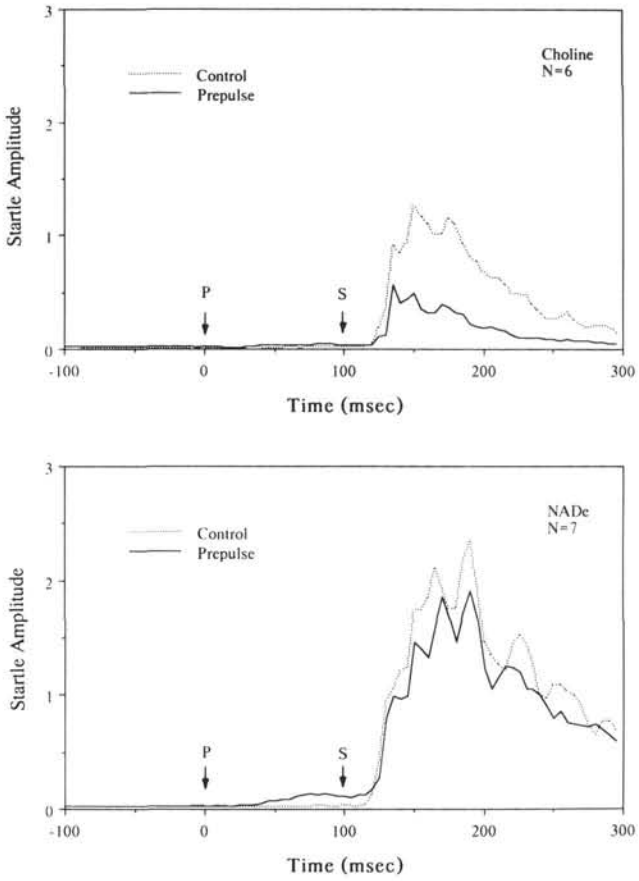


*Figure 2.* Computer averages of startle response (to 115–dB startle-eliciting stimulus) across animals with and without the prepulse in choline- (upper panel) and N-aminodeanol-treated (bottom panel) rats. (N-aminodeanol-treated rats regularly responded to the 70–dB prepulse, in contrast to the control rats, which rarely responded to the prepulse. Time of stimulus presentation is indicated by the arrows. P = prepulse; S = startle-eliciting stimulus.)

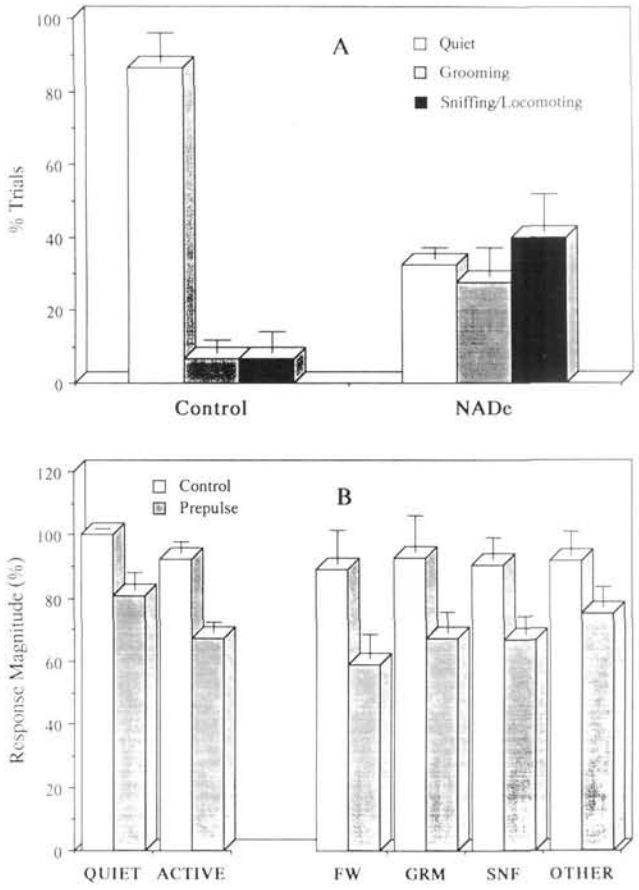


*Figure 3.* N-aminodeanol-treated (NADe) rats displayed more grooming and stereotyped behaviors than the choline controls in Experiment 1 (Panel A), but the amount of prepulse inhibition was comparable in quiet and active states in Experiment 2 (Panel B). (Data in Panel B are expressed as percentage of the basal startle level during quiet state. Scores in active state represent the averages of all active behavioral states. FW = face washing; GRM = grooming; and SNF = sniffing.)

## Experiment 2

One possible explanation for the diminished prepulse inhibition in experimental rats is that prepulse inhibition was disrupted by grooming and stereotyped behaviors these NADe-treated rats displayed during the trial. Stereotyped and locomotor behaviors tend to reduce prepulse inhibition in normal animals (Wecker & Ison, 1986). In order to determine the contribution of each of these activities to the diminished prepulse inhibition in NADe-treated rats, we observed the animals' behavior across an extended series of trials.

### *Method*

The subjects were 4 of the NADe-treated rats used in Experiment 1. The rats were allowed at least 1 week of recovery between Experiments 1 and 2. A total of 150 trials were run, with half of the trials presented with startle stimulus alone and half with a prepulse before the startle stimulus. Trials were presented in a counterbalanced manner. The startle-eliciting stimulus was 110 dB and the prepulse was 70 dB.

### *Results and Discussion*

As shown in Figure 3 (bottom panel), there was no difference in prepulse inhibition during the quiet and active states. An ANOVA revealed no significant difference in prepulse inhibition among different behaviors, $F(4, 8) = 0.17, p > .05$. The baseline startle amplitude in active states was also not significantly different from that during the quiet state, $F(4, 8) = 0.65, p > .05$. Thus, the diminished prepulse inhibition of the NADe-treated rats was not due to the increased grooming and stereotyped behaviors that these rats displayed during the tests.

Although Experiment 2 was not designed specifically to study habituation, there was an indication that habituation of the startle response in the NADe-treated rats was impaired (Figure 4). On the contrary, startle responses appeared to be sensitized over repetitive stimulation. No comparable data on habituation was available for the control rats; however, normal rats do show habituation of response under a similar experimental condition (Wu & Siegel, 1991).

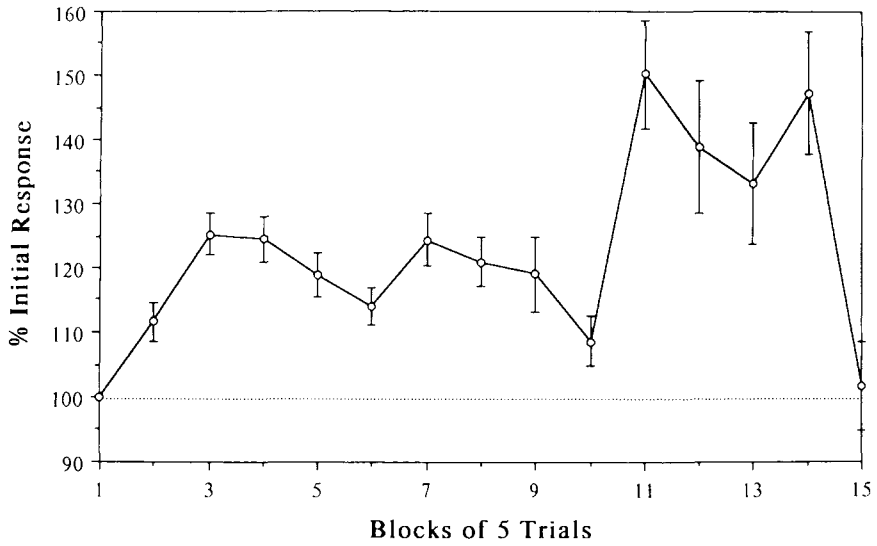


*Figure 4.* Changes ($M \pm SE$) in startle response over trials in N-aminodeanol-treated rats ($n = 4$). (Each data point is the average of five trials with the stimulus-eliciting stimulus alone and expressed as the percentage of initial startle level. Note that prepulse trials were presented intermixed with trials with startle-eliciting stimulus alone in a total of 150-trial series.)



## Experiment 3

In this experiment we used a cholinergic agonist (arecoline) and an antagonist (scopolamine) to test the hypothesis that the deficit in prepulse inhibition of the NADe-treated rats was directly produced by a deficiency in cholinergic mechanisms.

### *Method*

The subjects were the same 13 (6 control and 7 experimental) rats used in the previous experiments.

All the rats were treated with saline and arecoline hydrobromide (4 mg/kg, ip). Ten (5 control and 5 experimental) rats were involved in a later treatment with scopolamine hydrochloride (1 mg/kg, ip). The order of the treatment for saline and arecoline was randomly selected for each rat; 2 control and 3 experimental rats were treated with saline first, and 4 control and 4 experimental rats were treated with arecoline first. One week after the saline–arecoline treatments, an additional test for prepulse inhibition was done on experimental rats under saline. Tests for the effects of scopolamine were done next. At least 4 days of recovery were allowed between tests. Two rats were also treated with 8 mg/kg of arecoline, but the tests were stopped because of aversive side effects to the animals.

The intensity for the startle-eliciting stimulus was 110 dB, and that for the prepulse was 70 dB. The animals were sacrificed 2 weeks after the experiment to measure their choline and NADe levels in the brain, liver, and blood, as described in the General Method.

### *Results and Discussion*

The results are shown in Figure 5 (top panel). Arecoline significantly increased prepulse inhibition in the experimental rats without changing that of the control rats. The ANOVA revealed a significant effect of diet on prepulse inhibition, $F(1, 11) = 53.40, p < .001$, and a significant interaction of diet and arecoline treatment, $F(1, 11) = 5.41, p < .05$. Experimental rats showed a significant increase of prepulse inhibition, to 47.2% from 26.7% with saline, $t(6) = 2.54, p < .05$. The basal startle amplitude of the experimental rats was not changed by arecoline, $t(6) = 1.40, p > .05$. At this dose, arecoline did not change either the basal startle amplitude, $t(5) = 0.16, p > .05$, or the amount of prepulse inhibition, $t(5) = 0.51, p > .05$, of the control rats. Arecoline also did not change the pattern of spontaneous activities in either the control or the experimental rats (Figure 6, top panel; compare with Figure 3, top panel).

The amount of prepulse inhibition under saline for the experimental rats was greater in this experiment than that in earlier experiments (26.7% vs. 12.0%). This difference arose because some of the rats have been treated with arecoline first and the improvement on prepulse inhibition by arecoline persisted days after the treatment. As depicted in Figure 5 (bottom panel), experimental rats tested with arecoline first ($n = 4$) showed greater prepulse inhibition in the later test with saline than did those that had no prior experience with arecoline ($n = 3$; 38.7% ± 6.0% vs. 12.9% ± 5.1%), $t(5) = 3.62, p < .02$. These two groups of experimental rats did not differ in prepulse inhibition under arecoline (49.2% ± 2.7% vs. 45.6% ± 11.6%), $t(5) = 1.10, p > .05$. There was also no difference in prepulse inhibition between these two groups when tested 1 week later with saline, $t(5) = 1.14, p > .05$.

These results suggest that ACh is responsible for the NADe-induced deficit in prepulse inhibition and that ACh is essential for the initial expression of prepulse inhibition. However, the remaining cholinergic function in the NADe-treated rats may be sufficient for the expression of the partially restored prepulse inhibition once it is acquired, because prepulse inhibition was retained in a later test with saline given 1-2 weeks after arecoline treatment.

Scopolamine at the dose of 1.0 mg/kg, which did not

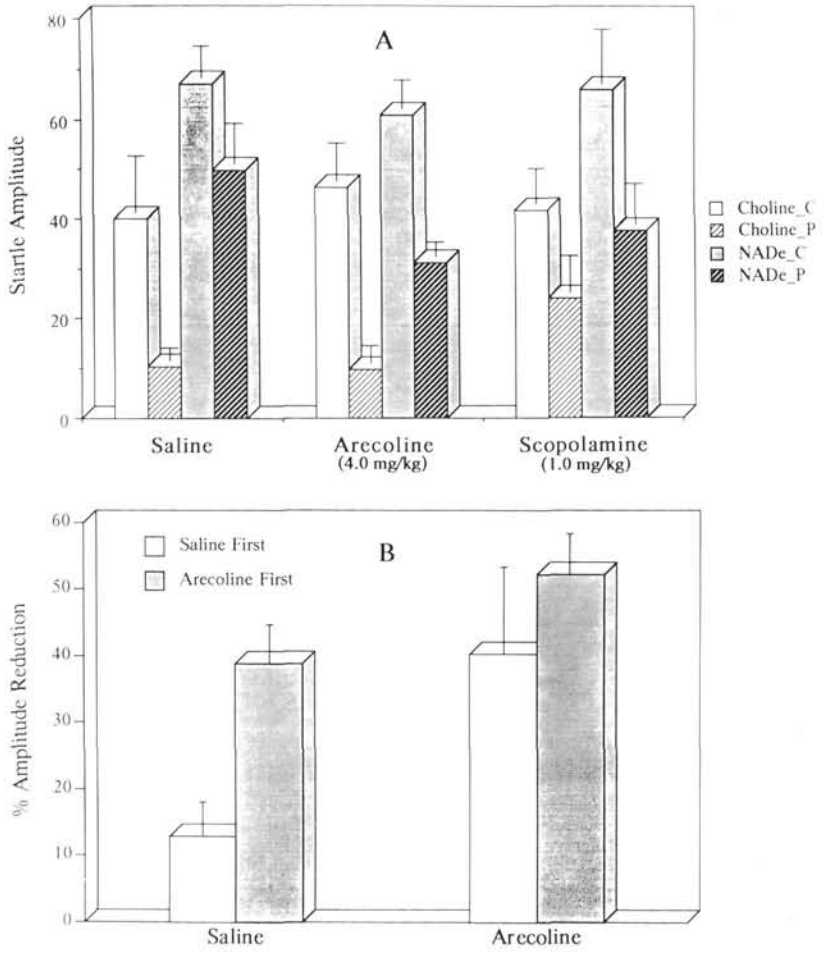


*Figure 5.* Effects of arecoline and scopolamine on the elicitation and prepulse inhibition of the startle response in the choline- (control) and N-aminodeanol-treated rats (top panel), and effects of arecoline on prepulse inhibition of startle in N-aminodeanol-treated rats as a function of order of treatment (bottom panel). (Data represent the percentage reduction from basal startle level. C = trials with startle-eliciting stimulus alone; and P = trials with prepulse.)

significantly change the basal startle amplitude of either the control, $t(4) = 0.16, p > .05$, or the experimental, $t(4) = 1.53$, $p > .05$, rats, significantly decreased prepulse inhibition of the control rats (47.4% vs. 78.4% with saline), $t(4) = 4.03, p < .02$, without affecting that of the experimental rats (41.8% vs. 48.1% with saline, tested 1 week after arecoline), $t(4) = 1.63$, $p > .05$. Scopolamine, however, increased stereotyped behaviors, primarily sniffing, in the control rats (an increase from 8.9% of trials with sniffing under saline to 48.7% after scopolamine), $t(4) = 2.84$, $p < .05$ (Figure 6). Although scopolamine did not change the overall percentage of time the experimental rats engaged in grooming and stereotyped behaviors (65.2% vs. 62.0%), sniffing activities also tended to be increased (48.0% vs. 26.2% with saline), $t(4) = 2.40, p = .08$, similar to the effect on the control rats.

Neither arecoline nor scopolamine significantly changed the response amplitude to the prepulse in either experimental or control rats, when compared with their respective responses with saline (all $ps > .5$). Experimental rats still showed greater responses to the prepulse than did the control rats after arecoline or scopolamine treatment, $t(11) = 2.23, p < .05$, and $t(9) = 3.06, p < .02$, respectively. However, in the experimental rats, the responses to the prepulse in this experiment were significantly smaller than their responses in Experiment 1 with 115-dB stimulus intensity, $t(6) = 3.01, p < .03$, and $t(4) = 7.96$, $ps < .001$, respectively, for baseline–saline and baseline–scopolamine comparisons.

Cholinergic deficiency in the experimental rats was confirmed by chromatographic analyses of the choline and NADe levels in the cortex, liver, and blood. Figure 7 shows the results of these analyses from the control and experimental rats. Phospholipid-bound choline in the cortex, liver, plasma, and erythrocytes (not shown in Figure 7) of the experimental rats was reduced to 32%, 23%, 12%, and 33%, respectively, of the control rats. Free choline in plasma and erythrocytes (not shown in Figure 7) fell to 37% and 15%, respectively, of the choline controls. These results are similar to those reported previously (Russell et al., 1990).

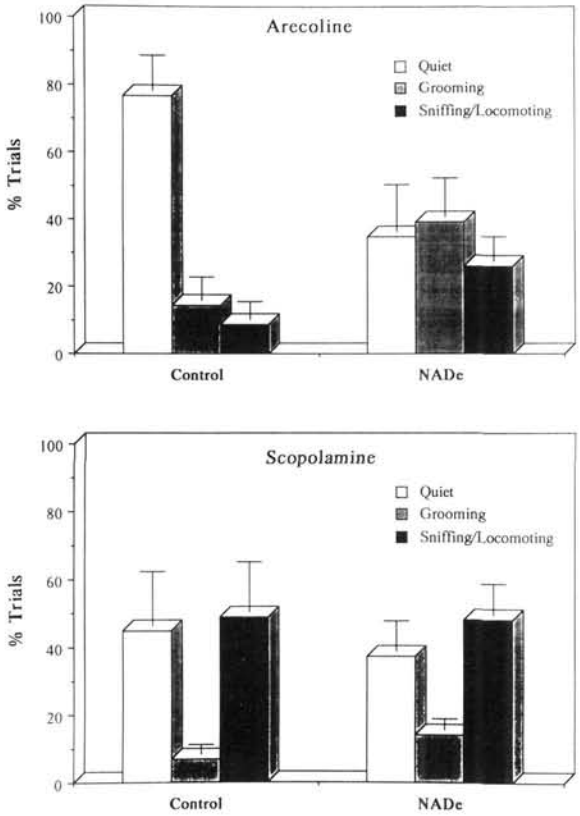


*Figure 6.* Effects of arecoline and scopolamine on spontaneous activity in the choline- and N-aminodeanol-treated rats.

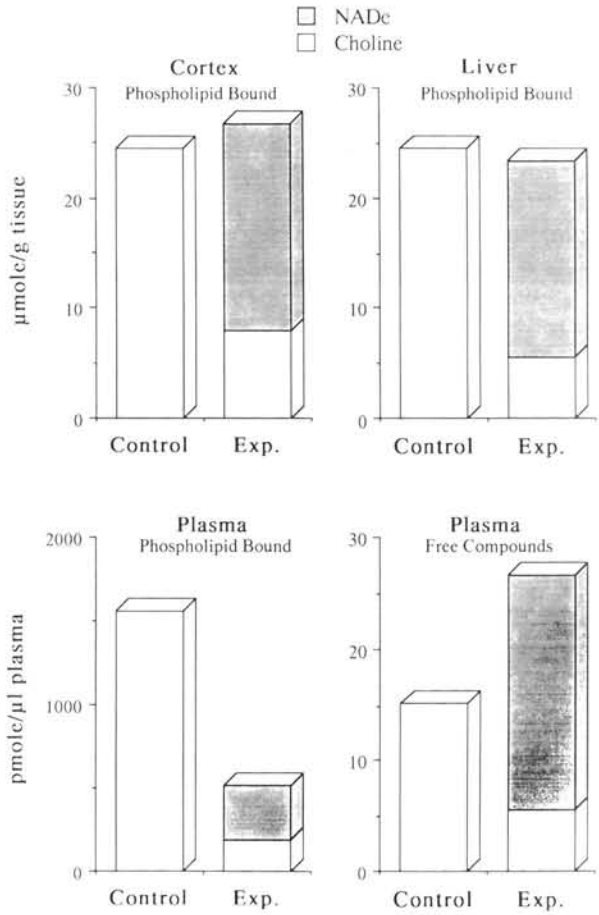


*Figure 7.* Chromatographic analysis of choline and N-aminodeanol (NADe) levels in the cortex, liver, and blood. (Control = choline-treated rats; and Exp. = NADe-treated rats.)

## General Discussion

This study demonstrated for the first time that cholinergic systems play a major role in prepulse inhibition of startle. Cholinergic hypofunction, induced by chronic choline-free, NADe diet, produced a deficit in prepulse inhibition (Experiment 1). A single treatment with the cholinergic agonist arecoline partially restored prepulse inhibition in NADe-treated rats (Experiment 3), which suggests that cholinergic deficiency is directly responsible for the diminished prepulse inhibition. Most interestingly, however, NADe-treated rats retained the restored prepulse inhibition weeks after the arecoline treatment. Administration of the cholinergic antagonist scopolamine did not reverse the restored prepulse inhibition in the NADe-treated rats but attenuated prepulse inhibition in the control rats (Experiment 3).

Cholinergic deficiency also induced an increase of startle amplitude, consistent with previous reports (Newton et al., 1986; Russell et al., 1990). The startle threshold may be reduced by cholinergic hypofunction, because NADe-treated rats startled even to the 70-dB prepulse. These rats also appeared to have an impaired habituation of startle.

The NADe-treated animals showed an increase of grooming and stereotyped behaviors (Experiments 2 and 3), which may be responsible for the impaired prepulse inhibition (Wecker & Ison, 1986). However, prepulse inhibition was equally impaired in quiet and active states in the NADe-treated rats (Experiment 2). Furthermore, arecoline treatment restored prepulse inhibition but did not change grooming and stereotyped behaviors (Experiment 3). These results suggest that the deficit was not secondary to the increase of grooming and stereotyped behaviors.

The reduced prepulse inhibition was also unlikely to be due to an overestimation of the startle response, as a result of a possible overlap of responses to the prepulse and the startle pulse, because the response to the prepulse during the 100-ms sampling period was less than 3% of that to the startle stimulus, too small to account for the 60% difference in inhibition. The response to the prepulse per se is also unlikely to be directly responsible for the deficit in prepulse inhibition, because in normal animals the amount of prepulse inhibition is positively correlated with the intensity of the prepulse. In other words, although responses to the prepulse can be elicited at high intensities, the inhibition is in fact increased rather than decreased with the increase of prepulse intensity (Hoffman & Searle, 1968). Thus, an impairment of the prepulse inhibition mechanism must be involved to account for the deficit.

Two aspects of prepulse inhibition, which may not be mutually exclusive, may account for the deficit induced by the choline-free, NADe diet treatment. First, the deficit may

represent an immature, underlying prepulse-inhibition mechanism. In the rat, prepulse inhibition begins to mature during the 2nd and 3rd postnatal weeks (Parisi & Ison, 1979). The maturation process may continue throughout the early months of life, during which period normal cholinergic development may be crucial. Therefore, although the choline-free, NADe diet was not administered until 4 weeks after birth in this study, it is possible that cholinergic hypofunction may hinder the prepulse inhibition mechanism from further maturation. It will be interesting to see if prepulse inhibition is affected when cholinergic deficiency is made later in development, after prepulse inhibition mechanism is fully developed. On the other hand, and secondly, the deficit in prepulse inhibition may represent an impairment in the acquisition process. Prepulse inhibition is generally considered an unconditioned phenomenon that can be demonstrated the first time a prestimulus is administered (Hoffman & Ison, 1980). However, improvement of inhibition with a visual or a weak auditory prepulse through repetitive testing has been reported (Ison, Hammond, & Krauter, 1973). Similar findings were also found in later studies with a cutaneous prepulse (Wu, Ison, Wecker, & Lapham, 1985) or a gap in noise (Crofton, Dean, Sheets, & Peele, 1990; Dean, Sheets, Crofton, & Reiter, 1990). Although classical conditioning has been ruled out as an explanation for the experiential effect (Ison et al., 1973; Wecker, 1985), for gap inhibition, pairing of the gap and the eliciting stimulus was required to demonstrate the experiential effect (Crofton et al., 1990). Thus, a learning process, either associative or nonassociative, may be involved for optimal expression of prepulse and gap inhibition. Cholinergic systems have been hypothesized to be involved in, among other things, learning and memory processes (Carlton, 1964, 1969; Deutch & Rogers, 1979). NADe-treated rats have been shown to have deficits in a variety of learning and memory tests (Russell et al., 1990). The fact that prepulse inhibition is eliminated in the NADe-treated rats and can be restored, possibly permanently, by a single treatment of the cholinergic agonist arecoline, suggests that an acquisition process is involved and that cholinergic activation is required for this process. Some preliminary data have shown that scopolamine blocked the improvement of gap inhibition over repeated tests (Crofton, Sheets, Dean, & Peele, 1988). Our results also suggest that the remaining cholinergic function in the NADe-treated animals may be sufficient for the expression of the partially restored prepulse inhibition once it is acquired. Prepulse inhibition in the control animals operated under a normal cholinergic system and thus may be more susceptible to cholinergic challenge. This may explain why scopolamine only attenuated prepulse inhibition in the control rats but not in the experimental animals.

Strong dopaminergic and cholinergic interactions are known to exist in certain brain regions (e.g., the nigrostriatal system; Roth & Bunney, 1976). Dopaminergic activations in both humans and animals have been shown to induce locomotor and stereotyped behaviors (e.g., Randrup & Munkvad, 1967, 1974). The behavioral sequence of face washing and the subsequent intense grooming of the hind flank seen in NADe-treated rats is characteristic of D1 dopamine receptor activation (Murray & Waddington, 1989). Furthermore, and similar to cholinergic hypofunction, dopaminergic activation enhances startle response (Davis, 1980). It is possible that cholinergic hypofunction may modulate mesolimbic and nigrostriatal dopaminergic systems, the result of which in turn causes the increase in locomotor and grooming and stereotyped behaviors. Dopaminergic systems may also interact with glutamatergic systems in the mediation of stereotyped behaviors (Tiedtke, Bischoff, & Schmidt, 1990). It has been suggested that the increase in stereotyped behaviors after dopaminergic activation of the mesolimbic systems may be caused by a deficit in sensory gating (Swerdlow, Braff, Masten, & Geyer, 1990). However, a deficit in prepulse inhibition does not necessarily accompany an increase in locomotor or stereotyped behaviors. Neurotoxic lesions of the dorsolateral tegmental area, which has been implicated in prepulse inhibition (Leitner, Powers, Stitt, & Hoffman, 1981; Saitoh, Tilson, Shaw, & Dyer, 1987), attenuate prepulse inhibition but do not induce an increase in locomotor or stereotyped behaviors in the rat (Wu & Siegel, 1991). This suggests that a deficit in prepulse inhibition or sensory gating and an increase in locomotor and stereotyped behaviors are not causally related, but whatever mechanism that induces locomotor or stereotyped behaviors may be able to modulate prepulse inhibition as well.

In our experiments, arecoline (4 mg/kg) did not restore prepulse inhibition completely, and scopolamine (1 mg/kg) only attenuated, but did not eliminate, prepulse inhibition in the control animals. These doses have been shown to affect startle responses and locomotor activities (Payne & Anderson, 1967; Williams, Hamilton, & Carlton, 1974) or to activate brain cholinoceptive regions (Soncrant & Nurnberger, 1989). Although it is possible that prepulse inhibition may be completely restored or eliminated at other doses, it is also likely that other noncholinergic processes may be involved in the expression of prepulse inhibition. From this study we cannot rule out the possible involvement of dopaminergic and glutamatergic systems in the deficit of prepulse inhibition produced by cholinergic hypofunction. Future studies with dopaminergic antagonists and glutamatergic agonists in NADe-treated animals may be able to answer some of these questions. However, there may be differences in the effects produced by dopaminergic agonists and NMDA antagonists and in the effects produced by choline deficiency and NADe treatment. It has been suggested that dopaminergic agonists interfere with prepulse inhibition by reducing the detectability of the prepulse, because the effects were most evident when prepulse intensity was no more than 10 dB above the background (Davis, 1988; Davis et al., 1990; but see Swerdlow, Keith, Braff, & Geyer, 1991). The fact that in this study the intensity of the prepulse was 15 dB over the background and that NADe-treated rats responded to the 70-dB prepulse suggests that detectability of the prepulse is not a contributing factor for the diminished prepulse inhibition seen in these animals.

Given that cholinergic systems are involved in prepulse inhibition, as strongly suggested by our results, the anatomical loci through which the effect is exerted remains obscure. Although prepulse inhibition is generally believed to be mediated by structures below the midbrain, as the effect is retained in decerebrate rats (Davis & Gendelman, 1977), rostral structures are able to modulate this process (e.g., Caine, Geyer, & Swerdlow, 1991; Swerdlow et al., 1986). Thus,

cholinergic hypofunction may interfere with prepulse inhibition through cortical or forebrain cholinergic systems, or indirectly through the mesolimbic and striatal structures by interacting with the dopaminergic systems. Alternatively, the mesopontine cholinergic systems may be directly responsible for the deficit. The dorsolateral tegmental area at the mesopontine level that encompasses the pedunculopontine tegmental nucleus (PPN) in and around the rostral brachium conjunctivum and the adjacent nucleus cuneiformis (NCF) has been implicated in prepulse inhibition (Leitner et al., 1981; Saitoh et al., 1987). The PPN is one of the two major sources of cholinergic-containing neurons in the pons (Jones & Beaudet, 1987; Reiner & Vincent, 1987; Shiromani, Armstrong, & Berkowitz, 1987). Recent evidence seems to suggest that the PPN, but not the NCF, is the primary structure to mediate prepulse inhibition. Neurotoxic lesions of the NCF, which spared the cholinergic PPN neurons, or electrolytic lesions of a cholinoceptive area just medial to the PPN, produced no effect on prepulse inhibition (Wu & Siegel, 1991; Wu et al., 1990). On the other hand, lesions of PPN alone greatly attenuated prepulse inhibition (Wu & Siegel, 1991). In a preliminary report (Swerdlow, Caine, & Geyer, 1991), electrolytic lesions of the PPN area also attenuated prepulse inhibition. PPN receives indirect projections, through ventral pallidum, from the nucleus accumbens (Jackson & Crossman, 1981; Mogenson, Swanson, & Wu, 1983), an area that has been suggested to mediate dopaminergic effect on prepulse inhibition (Swerdlow et al., 1990; Swerdlow et al., 1986). Thus, PPN may be the final common path for the cholinergic and other neurotransmitter systems to affect prepulse inhibition. It is conceivable that cortical and other forebrain structures may affect prepulse and gap inhibition mechanisms through this pathway (e.g., Caine et al., 1991; Ison, Bowen, & O'Connor, 1991; Ison, O'Connor, Bowen, & Bocirnea, 1991).

The inferior colliculus (IC) also may be involved in the deficit produced by the diet treatment. The IC is involved in prepulse inhibition, given that lesions of the IC abolished auditory prepulse inhibition in the rat (Leitner & Cohen, 1985). NADe-treated rats have heightened sensorimotor responses to sensory stimuli. These animals are hyperalgesic as indicated by a lower flinch-jump threshold to footshock (Russell et al., 1990). In our experiments these rats responded to the modest 70-dB prepulse, which is subthreshold for startle in normal animals. NADe-treated rats are susceptible to tonic-clonic seizure induced by intense sounds (Russell et al., 1990). The IC is important for the generation of audiogenic seizures (Henry, Wallick, & Davis, 1972; Wada, Terao, White, & Jung, 1970; Willott & Lu, 1980). It remains to be determined whether the IC and the dorsolateral pontine area are responsible for the enhanced seizure susceptibility and sensorimotor responses and for the diminished prepulse inhibition of startle in NADe-treated rats.

In conclusion, we demonstrated a strong role that cholinergic systems play in the mediation of prepulse inhibition and the modulation of startle. Along with previous findings, these results suggest that the cholinergic neurons in the dorsolateral tegmental area may mediate prepulse inhibition.



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Received July 1, 1991
Revision received October 14, 1992
Accepted October 14, 1992 ■