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Startle Modulation Studies in Autism[1]

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We studied acoustic startle response and its modulation by prestimulation and by short-term and long-term habituation in 54 autistic patients and 72 normal age-matched controls. The startle response was measured as the amplitude and onset latency of the integrated orbicularis oculi EMG. There were no consistent significant differences between the autistic and control subjects in startle modulation by inhibitory or facilitatory prestimulation, short-term habituation of startle amplitude, long-term habituation of either startle amplitude or latency, or unmodulated startle amplitude. Differences between autistic and control subjects were limited to prolongation of unmodulated startle onset latencies in the autistics in all of the experimental paradigms (significant $p = .005$ *only in the context of short-term habituation) and a statistically significant* ($p < .05$) *slower rate of short-term habituation of startle onset latency in the autistic patients, relative to the controls. Results provide only limited support*

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for hypotheses of brainstem pathophysiology and no support for hypotheses of cerebellar pathophysiology in autism.

INTRODUCTION

For over three decades, an obtunded response to stimuli that should evoke startle has been observed in patients with autism (Anthony, 1958). However, the startle response in autism has not been studied under controlled laboratory conditions that utilize current understanding of startle modulation and its underlying functional neuroanatomy.

Autism has been attributed to a disorder of sensory processing and directed attention mediated by dysfunction of brainstem and related diencephalic systems and the cascading impact of such dysfunction on higher neural structures which elaborate, refine, and modulate the activities of the lower centers (Ornitz, 1988, 1989a). Following the neurophysiologic model for the mediation of directed attention proposed by Mesulam (1981, 1983), it has been proposed that dysfunction of a distributed system including ascending brainstem, limbic, and polymodal associative cortical projections to the inferior parietal lobule, with efferents to frontal eye fields, striatum, and other structures mediating the motor components of directed attention, is involved in autism (Ornitz, 1989a). The central role of the brainstem was emphasized. Alternatively, cerebellar dysfunction has been implicated in the autistic attentional disorder in view of imaging studies, some (Courchesne, 1991) but not all (Garber & Ritvo, 1992; Kleiman, Neff, & Rosman, 1992; Kleiman, Neff, Rosman, & Boston, 1990; Piven et al., 1992; review in Tsai, 1992) of which have demonstrated reduced size of cerebellar vermal lobules VI and VII.

Because of parallel experimental animal studies, the study of startle modulation by prestimulation or short-term habituation and by long-term habituation in man permits partial assessments of brainstem and cerebellar vermal function, respectively. Startle inhibition by prestimulation is a brainstem function involving the early low-level processing of sensory input. This type of inhibition is mediated by an inhibitory pathway in the mesopontine lateral tegmental area as demonstrated by lesion (Leitner & Cohen, 1985; Leitner, Powers, Stitt, & Hoffman, 1981) and stimulation (Saitoh, Tilson, Shaw, & Dyer, 1987) studies in the rat. This pathway, which parallels the primary startle pathway (Davis, Gendelman, Tischler, & Gendelman, 1982) impinges on the latter at, or prior to, the medial pontomedullary reticular formation (Wu, Suzuki, & Siegel, 1988).

Short-term habituation of the startle response is mediated by mechanisms intrinsic to the sensory side of the primary startle pathway (Davis,

Parisi, Gendelman, Tischler, & Kehne, 1982), and depends on the integrity of the inferior colliculus (Jordan & Leaton, 1983), particularly its dorsal cortex and lateral nucleus (Parham & Willott, 1990). In contrast, there is evidence that long-term habituation of startle is mediated by activity at sites rostral to the direct startle pathway (Leaton, Cassella, & Borszcz, 1985). These sites include dorsal hippocampus, frontal and auditory cortex (Groves, Wilson, & Boyle, 1974), mesencephalic reticular formation (Jordan, 1989; Jordan & Leaton, 1983), and cerebellar vermis and fastigial nuclei (Leaton & Supple, 1986, 1991).

Hence, this investigation of startle modulation by prestimulation and habituation addresses the proposals that brainstem and/or cerebellar pathoneurophysiology may be involved in autism. Startle responses from patients with autism and normal controls were compared in four experiments, two of which evaluated prestimulation modulation of startle at different prestimulation intervals and two of which evaluated habituation. Experiments 1 and 2 evaluated prestimulation modulation of startle. Experiment 1 used 60 msec and 120 msec prestimulation intervals since these intervals are known to induce startle latency facilitation and startle amplitude inhibition, respectively. Experiment 2 used 120 msec and 2000 msec prestimulation intervals, providing replication for Experiment 1 and testing for startle facilitation. The effect of age was evaluated in the prestimulation modulation experiments because of the established influence of maturation during childhood on prestimulation modulation of startle (Ornitz, Guthrie, Kaplan, Lane, & Norman, 1986). Experiment 3 evaluated short-term habituation (STH) of startle and Experiment 4 evaluated both short-term and long-term habituation of startle (LTH) and their interaction.

METHODS

Subjects

A total of 54 patients with autism were successfully entered into 74 experimental sessions distributed among Experiments 1, 2, and 3 (10 patients participated in two of these experiments and 5 participated in all three experiments). A total of 72 normal age-matched controls were also entered into 74 experimental sessions distributed among Experiments 1, 2, and 3 (2 subjects participated in two of these experiments). Data from an additional 12 autistic patients and 37 normal subjects could not be obtained or used for the reasons listed in Table I.

All autistic patients met DSM-III-R criteria (American Psychiatric Association, 1987) for Autistic Disorder by both history taken from the

Table I. Numbers of Subjects Whose Data Could Not Be Obtained or Used

Reason for rejecting subject	Rejection frequencies	
	Autistics	Normals
Audiometric deficit[a]	0	9
Excessive facial movements, excessively restless, unable to cooperate with experimental conditions, or upset during recording session	11	9
Drowsiness	0	8
Computer problem, electrode contact, or other technical problem	0	3
Subject would not accept electrode placement	1	3
Background orbicularis EMG too high	0	1
Subject showed very small or absent responses to startling stimuli	0	3
Abnormal EEG observed during recording session	0	1
Totals	12	37

[a]Of those autistic subjects who cooperated with audiometric testing.

Table II. Number of Autistic Subjects in Each IQ Range

IQ range	Verbal IQ Wechsler scales (WS)[a]	Performance IQ		Full-scale IQ	
		WS[a]	MP[b] or L[c]	WS[a]	SB[d]
130–145	1	2		1	
115–129	2	2		1	
100–114	4	4	3	4	1
85–99	6	12	2	8	1
79–84	8	7	4	11	2
55–69	9	3	2	4	5
40–54			1	1	2

[a]Wechsler scales (WAIS-R, WISC-R, or WPPSI).
[b]Merrill-Palmer Preschool Performance Test.
[c]Leiter International Performance Test.
[d]Stanford-Binet.

parents and by direct examination. Additionally, the early childhood development of these patients was characterized by disturbances of motility (e.g.,

hand flapping) and sensory modulation (e.g., under- and overreactivity to sounds) (Ornitz, 1974, 1987b, 1988, 1989b). At the time of this study, 11 patients with autism were nonverbal, the speech of 16 was limited to echolalia, and the other 27 used speech primarily to communicate their immediate needs and ideosyncratic interests. Psychometric test results could be obtained from 44 autistic patients (Table II).

Of the 54 patients with autism, 37 had normal prenatal and perinatal histories and had no history or current evidence of chronic illness, psychiatric disorder, developmental disorder other than autism, or neurological disorder. Of the remaining 17 patients, 12 had a history of perinatal disorder and 10 had a diagnosed medical or neurological disorder (Table III). Chromosomal analyses, particularly testing for fragile X, were not systematically available for these patients with autism.

Normal controls recruited from the general population through newspaper ads and word-of-mouth were screened for good health, normal development, and absence of any symptoms suggesting middle or inner ear, ocular, or neurological pathology. Additional screening excluded potential subjects with chronic illness, psychosis, severe mood or anxiety disorder, tic disorders, eating disorder, somatization disorder, or specific developmental disorders, and history of head injury, seizures, meningitis, encephalitis, or significant educational impairment or delay. Otherwise, normal subjects were selected only to match the autistic subjects for chronological age and sex (Table IV). These normal subjects came from similar socioeconomic environments to those of the autistic patients. Psychometric data were not available. All control subjects old enough to be or to have been in school had unremarkable school histories.

Stimulation

During Experiments 1 and 2, prestimulation [75 dB Sound Pressure Level (SPL) 1,000-Hz tones, 4-msec rise and fall times] and startle stimuli (SS, 104 dB SPL, zero rise time, 50-msec white noise bursts) were presented binaurally through TDH-49 circumaural earphones. SS were presented alone and preceded by 25-msec tones 60 and 120 msec before the SS in Experiment 1. In Experiment 2, SS were presented alone and preceded by the 25-msec tones 120 msec before and by continuous tones for 2,000 msec before the SS. In each experiment, the order of trials was balanced across subjects. During Experiments 3 and 4, only SS were presented. Forty SS were presented at 23- to 26-sec intervals.

Table III. Perinatal and Medical or Neurological Conditions Associated with Autism in 17 of the 54 Autistic Subjects[a]

Case	Perinatal condition	Medical or neurological condition
1	Postpartum respiratory distress	Petit mal and grand mal seizures
2	Cyanosis, low Apgar scores	Seizure disorder, abnormal EEG with right temporal-central focus
3		Seizure disorder, nonspecific abnormal EEG
4.	Neonatal jaundice	Head injury with loss of consciousness at 2 years, abnormal EEG at 6 years
5		Aseptic viral meningitis at 5 months
6	Congenital cytomegalovirus	Microcephaly
7		Kartagener's syndrome
8		Head injury without loss of consciousness at 18 months
9	Fetal heart rate deceleration neonatal jaundice	Pyloric stenosis
10		Congenital heart disease
11	Dystocia, low Apgar scores	
12	Fetal distress	
13	Toxemia, respiratory distress, neonatal jaundice	
14	Meconium staining	
15	Neonatal jaundice	
16	Neonatal jaundice	
17	Neonatal jaundice	

[a]All clinical data ascertained from clinic or hospital records.

Dependent Variables

Orbicularis oculi EMG was recorded bipolarly from disc electrodes taped to the skin about 12 mm apart, and about 10 mm below the left lower lid margin, with the lateral electrode about 7 mm medial to the outer canthus. The raw EMG was AC-amplified with filters set at half-amplitude 100–1000 Hz, then rectified and smoothed through a Coulbourn contour-following integrator (time constant 3.5 msec), the output of which was digitized on a DEC 11/23 laboratory computer at a 500-Hz sampling rate. The computer was programmed to measure the *onset latency* (the first increment greater than 2 standard deviations above average baseline which was not followed by return to this EMG level within a window 20–80 msec following

Table IV. Numbers of Autistic Patients and Normal Controls, Age (Years), Sex, and Medication Status, Successfully Enrolled in Each Experiment

	Autistic patients			Normal controls	
Experiment	*n* (female)	Mean age (range)	No. on medication[a]	*n* (female)	Mean age (range)
1	20(1)	9.2(2.8–18.2)	3	20(1)	9.3(3.5–18.2)
2	30(2)	8.6(3.2–15.1)	0	31(2)	8.7(3.4–15.7)
3	24(1)	13.7(3.6–33)	1	23(1)	13.5(3.7–32)
4[b]	9(0)	19.6(6.0–33)	0	8(0)	20.6(6.0–32)

[a]In all cases, medication was fenfluramine. Preliminary data analyses with and without these few patients on medication revealed no effect of medication on the parameters under investigation.

[b]These 9 autistic patients and 8 normal controls were part of the Experiment 3 (short-term habituation) groups; they returned for four additional days to complete Experiment 4 (long-term habituation).

SS onset) and the *peak amplitude* (the highest point within a window from response onset to 105 msec following SS onset). These variables were used as the measures of the startle response.

Variables Used to Control Stimulus Delivery

A vertical electrooculogram (EOG) to monitor lid position, spontaneous blinking, and vertical saccades, a single channel of EEG subjected to a broadband spectral analysis for delta, theta, alpha, and beta activity to monitor change in state, heart rate, and movement (detected by increased EMG activity of the upper extremities and trunk) and the tonic level of the mean integrated orbicularis oculi EMG were recorded as described in Ornitz et al. (1986).

Procedures

Prior to the experimental session each subject was familiarized with the laboratory and exposed to one sample of prestimulation (in Experiments 1 and 2) and one SS, electrodes were applied, and an audiometric test (1,000-, 2,000-, and 4,000-Hz tones at 20, 30, 40, and 60 dB SPL) was performed. All normal subjects entered into the four experiments passed the audiometric test. Forty-five autistic subjects passed the audiometric

test. Four autistic patients could not cooperate at all; however, 3 of these patients had previously tested normal. Five autistic patients did not respond to the 20-dB tones at some but not all frequencies; problems in cooperation may have been a contributing factor. These 5 autistic patients did respond to all frequencies at 30 dB. In our experience, this 10-dB difference does not appear to affect the response to either the startle stimulus or the prestimulus. The responses of all 9 patients were not different from those of the 45 patients who responded at 20 dB to all frequencies tested.

For experiments 1 and 2, during a 2-min period of adaptation during which the subject watched silent cartoons or movies, the range of resting levels of orbicularis oculi, scalp and upper body EMG, delta, theta, alpha, and beta EEG, heart rate, and lid position were noted and entered into the computer which then attempted to present trials according to a bounded random schedule of minimal intertrial intervals (ITI) every 25–45 sec. A trial consisted of blanking the television screen for 2.5–3.0 sec (to avoid a conflicting visual stimulus) and then presenting the auditory stimuli. Following each minimal ITI, the computer withheld the stimuli until the heart rate, EEG parameters, lid position level, and EMG levels were within the initially selected ranges according to the procedure described and evaluated in Ornitz et al. (1986). Consequently, the *actual* ITI was the sum of the *minimal* ITI and the additional waiting period.

For Experiments 3 and 4, the 40 startle stimuli (SS) were presented at almost constant intervals (every 23 to 26 sec) and the silent television was not blanked. Experiment 3 consisted of one session during which 40 SS were presented. During Experiment 4, 9 of the autistic and 8 of the control subjects returned for four additional sessions (a total of five sessions at daily intervals). All sessions were during the morning hours.

In these experiments, drowsiness, which can be a difficult confounding variable, was identified by continuous observation on a closed-circuit television monitor focused on the subject's face, persistent lid closure and/or rolling eye movements (indicated by changes in the EOG recording) and/or persistent slowing and increased amplitude of the EEG.

Data Analyses

For Experiments 1 and 2, during 101 sessions, all subjects completed 9 trials and data analyses were based on these 909 trials. The 9 trials were divided into three successive blocks of 3 trials each. All trials without prestimulation were usable. Of the 606 trials with prestimulation, 20 (3.3%) had to be discarded (because lid or eye movements occurred during the

20 msec just after SS onset). Amplitude and latency values for each discarded trial were replaced with the average of the values of the remaining trials of the same trial-type. There were also 6 blink responses on trials without prestimulation and 11 on trials with prestimulation with amplitudes too small to be measured reliably. Amplitude and latency values for these trials were replaced according to imputation procedures described in Ornitz et al. (1986).

For Experiments 1 and 2, both autistic and normal subjects were each divided into two age groups, those younger than 90 months and those older than 90 months. An analysis of variance included the two age groups and diagnoses as intersubject factors, and the three stimulus conditions and the three blocks as intrasubject factors. Planned contrasts were used to compare the effect of each prestimulation interval on the startle response across age and diagnostic groups. Reflex blink amplitudes were log-transformed to diminish the effect of skewness of their distribution.

Preliminary analyses revealed no statistically significant multivariate main effects of block, or block by age or diagnosis interaction on amplitude or latency modulation across the 3 blocks of 3 trials each used in these data analyses. Therefore blocks were treated as replications in Experiments 1 and 2.

For Experiments 3 and 4, successive blocks of 4 trials were averaged on each day. Trials were rejected if onset latency was earlier than 20 msec following stimulus onset or if there was movement artifact or increased EMG during the 200 msec prior to stimulus onset. When a trial was rejected, the response amplitudes were averaged over the remaining trials within a block. For Experiments 3 and 4, an average of 4.1 and 3.9 trials per subject per day for normals and 7.1 and 7.7 trials per subject per day for autistic subjects were rejected. Trials with zero amplitudes were included. There were 92 such trials in the 4600 trials presented in Experiment 3 and Experiment 4. These trials were imputed as described for Experiments 1 and 2. Since the habituation curves neared asymptotes after 20 trials, the first five blocks of trials from each day in Experiment 3 and Experiment 4 were analyzed with a repeated measures analysis of variance (BMDP2V). Blocks and days were used as crossed intrasubject factors and diagnosis as a grouping factor in Experiment 4. Greenhouse-Geisser epsilon corrections were used for significance tests. Significance tests were obtained for block and day main effects, for interaction of block and day, and for orthogonal linear, quadratic, cubic, and quartic components of blocks, days, and interactions. Similar analyses on block effects were done for the single day in Experiment 3, and age was entered as a continuous covariate.

RESULTS

Experiments 1 and 2, Prestimulation Modulation of Startle

Effects of Age and Diagnosis on Unmodulated Startle Amplitude and Onset Latency (Table V). Although amplitudes were smaller and latencies were longer in the autistic than in the normal subjects in both Experiments 1 and 2, there were no significant age effects, diagnostic effects, or age by diagnosis interactions for either startle amplitude or startle latency during trials without prestimulation during either experiment.

Effect of Age and Diagnosis on Prestimulation Modulation of Startle Amplitude (Table VI). For the difference between the startle response on trials with and without prestimulation (Experiments 1 and 2), there were no significant diagnostic effects and no diagnosis by age interactions with the exception of one significant diagnosis by age interaction at the 120-msec prestimulation interval in Experiment 1 (not replicated in Experiment 2). There were significant age effects for the 60-msec and 120-msec prestimulation intervals (with subjects under 7.5 years of age showing deficient inhibitory startle amplitude modulation) but not for the 2000-msec prestimulation interval.

Effect of Age and Diagnosis on Prestimulation Modulation of Startle Onset Latency (Table VI). For the difference between the startle response on trials with and without prestimulation (Experiments 1 and 2), there were no significant diagnostic effects and no diagnosis by age interactions with the exception of one significant diagnosis by age interaction at the 60-msec prestimulation interval in Experiment 1. Significant age effects were limited to the 120-msec prestimulation interval (Experiment 1 and combined data

Table V. Effects of Age and Diagnosis on Unmodulated Startle Amplitude and Latency **(Experiments 1 and 2)**

	Age < 7.5 vs. > 7.5 years			Diagnosis autistic vs. controls		Interaction Age × Diagnosis	
	df	*F*	*p*	*F*	*p*	*F*	*p*
			Amplitudes				
Experiment 1	1/36	0.78	ns	1.43	ns	0.01	ns
Experiment 2	1/57	0.05	ns	3.11	ns	1.60	ns
			Latencies				
Experiment 1	1/36	0.04	ns	2.38	ns	0.23	ns
Experiment 2	1/57	0.34	ns	0.88	ns	0.44	ns

Table VI. Effects of Age and Diagnosis on Prestimulation Modulation of Startle Amplitude and Latency (Experiments 1 and 2)

	Age < 7.5 vs. > 7.5 years			Diagnosis autistic vs. controls		Interaction Age × Diagnosis	
	df	*F*	*p*	*F*	*p*	*F*	*p*
Amplitude							
60-msec Prestimulation interval							
Experiment 1	1/36	6.51	.015	0.61	ns	3.46	.071
120 msec Prestimulation interval							
Experiment 1	1/36	6.92	.013	2.85	ns	4.92	.033
Experiment 2	1/57	16.7	.0001	0.64	ns	0.29	ns
Combined Exps. 1 and 2	1/93	20.0	.0001	2.41	ns	0.79	ns
2000 msec Prestimulation interval							
Experiment 2	1/57	0.01	ns	1.89	ns	1.27	ns
Latency							
60-msec Prestimulation interval							
Experiment 1	1/36	0.16	ns	0.01	ns	4.43	.042
120-msec Prestimulation interval							
Experiment 1	1/36	9.63	.004	0.75	ns	2.92	ns
Experiment 2	1/57	2.94	ns	1.10	ns	1.01	ns
Combined Exps. 1 and 2	1/93	9.53	.003	1.61	ns	0.09	ns
2000-msec Prestimulation interval							
Experiment 2	1/57	0.81	ns	3.59	.063	0.49	ns

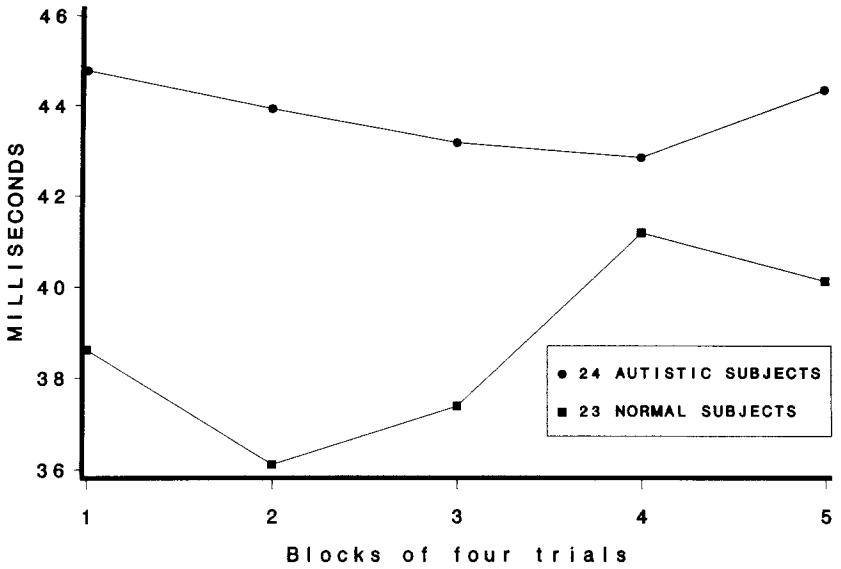

Fig. 1. Short-term habituation of startle onset latency from 1 day (data from Experiment 3). On the horizontal ordinate, each of the five successive blocks represents the mean of four trials (two or three trials in the case of missing data within a block) for each subject, averaged across all subjects.

from Experiments 1 and 2), with subjects under 7.5 years of age showing relative latency facilitation.

Experiment 3, Short-Term Habituation of Startle

Analysis of response amplitudes (Table VII) showed a significant block main effect, reflecting short-term habituation (STH). There was no effect of diagnosis and no block (STH) by diagnosis interaction. The age covariate was not significant.

Analysis of onset latencies (Table VII) showed a block main effect, reflecting increasing latency (STH) of the startle response over successive trial blocks, a diagnosis main effect which reflected an overall prolongation of onset latencies in the autistic (43.9 msec) compared to the normal subjects (38.7 msec) (Fig. 1), and a block (STH) by diagnosis interaction reflecting a different pattern of habituation in the autistic and normal subjects. The block effect was both quadratic and cubic and the block by diagnosis interaction was cubic with a linear trend (Fig. 1). As with short-term habituation of amplitude, the age covariate was not significant.

Table VII. Effects of Diagnosis on Short-Term Habituation (STH) and Long-Term Habituation (LTH) of Startle Amplitudes and Latencies (Experiments 3 and 4)

	Block (STH) or Day (LTH) effects			Diagnosis autistic vs. controls			Interactions (Block or Day × Diagnosis)		
	df	*F*	*p*	*df*	*F*	p	*df*	*F*	*p*
Experiment 3									
Amplitudes (STH)	4/180	8.59	.002[a]	1/44	0.58	ns	4/180	1.35	ns
Latencies (STH)	4/180	2.78	.046[a]	1/44	8.64	.005	4/180	2.80	.045[a]
Linear	1/45	1.30	ns				1/45	2.82	ns
Quadratic	1/45	6.00	.018				1/45	0.03	ns
Cubic	1/45	4.10	.049				1/45	7.22	.010
Experiment 4									
Amplitudes				1/15	0.03	ns			
STH (block effects within days)	4/60	27.3	.0001[a]				4/60	1.28	ns
Linear	1/15	43.5	.0001				1/15	1.03	ns
Quadratic	1/15	10.1	.006				1/15	0.44	ns
LTH (between-day effects)	4/60	16.2	.0001[a]				4/60	1.69	ns
Linear	1/15	45.9	.0001				1/15	0.15	ns
Quadratic	1/15	7.7	.014				1/15	0.81	ns
Latencies				1/15	0.30	ns			
STH (block effects within days)	4/60	4.34	.009[a]				4/60	3.20	.031[a]
Linear	1/15	11.45	.004				1/15	8.74	.010
LTH (between-day effects)	4/60	4.42	.006[a]				4/60	2.23	ns
Linear	1/15	10.1	.006				1/15	2.92	ns

[a]Greenhouse-Geisser corrected *p* values.

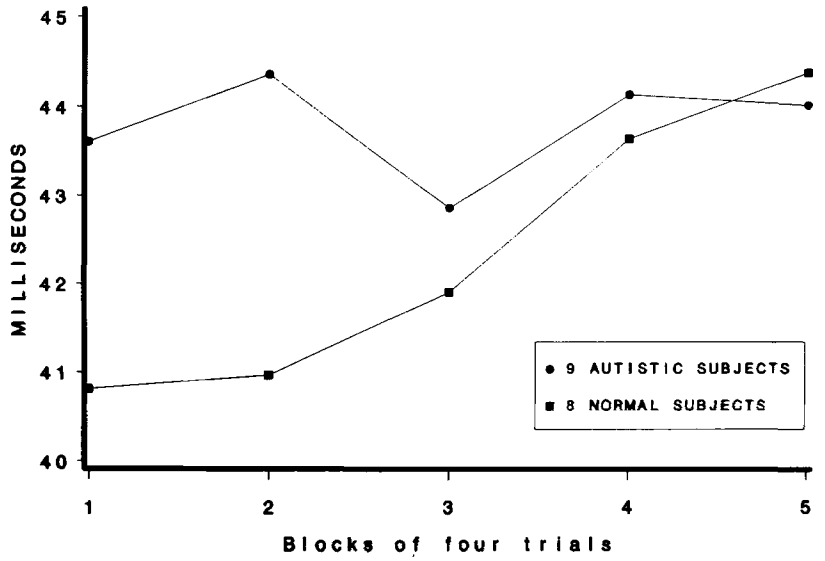

Fig. 2. Short-term habituation of startle onset latency (data from Experiment 4). As in Fig. 1, except that responses for each block are also averaged across the five successive days of Experiment 4).

Experiment 4, Long-Term Habituation of Startle

Analysis of response amplitudes (Table VII) showed significant block and day main effects, reflecting both short- and long-term habituation (LTH). There was no effect of diagnosis.

There was a statistically significant linear decrement of response amplitude (and a quadratic effect) within days (STH), and a significant linear response decrement (and quadratic effect) across days (LTH). There was no interaction between blocks and days, that is, between STH and LTH, $F(16, 240) = 0.51$, ns, confirming the independence of STH and LTH previously reported by Ornitz and Guthrie (1989). There were no interactions between diagnosis and blocks or days, hence no differences between autistic and normal subjects in either STH or LTH.

Analysis of onset latencies (Table VII) showed significant block and day main effects. There was no effect of diagnosis.

There was a statistically significant linear increment of onset latencies (block effect) within days. This result suggests short-term habituation and is congruent with the within-days decrement of response amplitude. Across days, there was a statistically significant linear effect. This result

suggests long-term habituation and is congruent with the long-term habituation of startle response amplitude. As with response amplitude, there was no interaction, $F(16, 240) = 1.19$, ns, between days and blocks of trials for the latency data. There was a significant linear interaction between diagnosis and blocks. Perusal of the group means across blocks showed that this interaction reflected a slower rate for short-term latency habituation in the subjects with autism than in the normal subjects (Fig. 2). There was a nonsignificant interaction between diagnosis and days.

DISCUSSION

Motivated by clinical observations of hyporeactivity to intense stimuli, particularly startling stimuli (Anthony, 1958), and theoretical considerations (Ornitz, 1989a) and imaging studies (Courchesne, 1991) that suggested brainstem and cerebellar vermal involvement, respectively, in autism, we compared startle and its modulation by prestimulation and habituation in autistic and normal subjects.

Studies of unmodulated startle, startle modulation by prestimulation, and startle modulation by short-term and long-term habituation provide functional anatomical specificity for potential involvement of particular brainstem and cerebellar regions. An additional advantage of these startle modulation protocols for studies of autism is that no tasks are involved, that is, they require neither motivation nor significant cooperation.

The absence of significant amplitude differences in the unmodulated startle response and in inhibitory prestimulation modulation of startle argues against functional pathophysiology involving the primary startle pathway leading to the nucleus reticularis pontis caudalis (Davis et al., 1982) or the mesopontine tegmental regions (Leitner & Cohen, 1985; Leitner et al., 1981; Saitoh et al., 1987) in the brainstem. On the other hand, the *prolongation of the onset latencies of the unmodulated startle response* by 5.2 msec ($p = .005$) in Experiment 3 and the *deficient short-term latency habituation* in the autistic subjects observed in both Experiments 3 and 4 ($p < .05$) provide some suggestion of pathoneurophysiology involving transmission through brainstem structures, for example, the inferior colliculus (Jordan & Leaton, 1983; Parham & Willott, 1990). The interpretation of these findings should be qualified by two considerations. First, perusal of Figs. 1 and 2 suggests that the differences in short-term habituation could be explained by the longer latencies in the autistic subjects, that is, there could be a ceiling effect for the autistic subjects, preventing increased latency habituation across successive blocks of trials. Second, the longer latencies in the autistic subjects could be explained either by the mental

retardation or by the generally delayed maturation associated with autism. There are no data on the effect of IQ per se on startle or its modulation. However it has been shown that startle latencies become shorter with increasing age across the childhood years (Ornitz et al., 1986, Ornitz, Guthrie, Sadeghpour, & Sugiyama, 1991). However, within the age ranges in this study, there were neither age-related effects on unmodulated startle amplitude and latency nor age by diagnosis interactions. Furthermore, while there were significant age effects such that the children under 7.5 years of age did not show the inhibitory amplitude and latency modulation following the 120-msec prestimulation interval and the amplitude inhibition following the 60-msec prestimulation interval that were characteristic of older subjects, results consistent with our previous maturational studies (Ornitz, Hanna, & de Traversay, 1992; Ornitz et al., 1986, 1991), there were only minimal age by diagnosis effects in prestimulation-induced startle modulation. Hence, these studies of startle modulation provide limited neurophysiologic evidence for functional deficiencies or abnormalities in brainstem structures in autism.

Brainstem functional neuroanatomy can be studied by several complementary methodologies. Imaging studies provide an overall estimate of size and consequently development. Brainstem auditory evoked responses (BAER) measure function in the direct auditory pathway through the brainstem. Vestibular studies provide measures of vestibular nuclear complex functioning (the gain of vestibular nystagmus) and the more diffuse modulating influence of the reticular activating system (the time constant of vestibular nystagmus). Startle modulation by prestimulation and short-term habituation provide measures of mesopontine tegmental and collicular function respectively, as described above. The current study provides limited evidence for collicular dysfunction. There is conflicting evidence for other aspects of brainstem pathophysiology from BAER, vestibular, and imaging studies. Past failures to replicate studies supporting BAER abnormalities (see reviews by Minshew, 1991, and Ornitz, 1987a) suggested normal function through the brainstem auditory pathways. On the other hand, recent studies using a masking technique (Thivierge, Bedard, Cote, & Maziade, 1990) and large autistic populations (Wong & Wong, 1991) have documented BAER abnormalities. Also, prolonged nystagmus time constants (Ornitz, Atwell, Kaplan, & Westlake, 1985) provide some support for more diffuse brainstem pathoneurophysiology involving the reticular activating system. It is possible that the current results from prestimulation-induced modulation of startle reflect function in more circumscribed brainstem pathways than those that are involved in the ocular adaptation to vestibular stimulation as measured by nystagmus time constants (Ornitz et al., 1985). There is conflicting evidence for brainstem involvement from magnetic

resonance imaging (MRI) studies with reports of both reduced (Gaffney, Kuperman, Tsai, & Minchin, 1988; Hashimoto et al., 1991) and normal (Hsu, Yeung-Courchesne, Courchesne, & Press, 1991) size of brainstem structures.

This investigation provides no evidence for functional abnormalities in the cerebellum. In particular, the absence of differences in long-term habituation of startle suggests that the cerebellar vermal structural deficiencies found in earlier (review in Courchesne, 1991) but not more recent (Ekman et al., 1991; Garber & Ritvo, 1992; Kleiman et al. 1992; Piven et al., 1992; review by Tsai, 1992) MRI studies may not be of functional significance. This finding is consistent with the fact that atrophic cerebellar lesions are associated with short time constants of nystagmus whereas children with autism have prolonged nystagmus time constants relative to normal controls (Ornitz et al., 1985). Hence, the consistent loss of Purkinje cells in autistic cerebellar cortex (review by Bauman, 1991; Kemper & Bauman, 1992) may prove to be an important developmental but functionally nonsignificant neuropathologic marker in autism.

In summary, these studies of startle modulation provide limited neurophysiologic evidence (the prolonged onset latencies and the short-term latency habituation findings) for functional deficiencies or abnormalities in brainstem structures in autism; they provide no evidence for functional abnormalities in the cerebellum, particularly the cerebellar vermis. The negative findings, normal prestimulation modulation of startle and normal long-term habituation of startle, do not, of course, exclude the possibility of abnormalities in brainstem tegmental pathways or cerebellar vermal regions, respectively, but they do not provide support for dysfunction in these regions.

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