Maturation of startle reflex habituation in rats

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Measurement of acoustic startle reflex (150 trials) in rats of ages 15, 18, 21, 25, and 100 days indicated that adult-like rates of habituation were first obtained at 25 days. Previous estimates of earlier development of this inhibitory ability may have been assessing a different aspect of the reaction to startle-eliciting stimuli. A specific and testable theoretical framework is offered.

During the past few years, evidence has accumulated to support the notion of substantial postnatal development of inhibitory capacities in the rat. Campbell, Lytle, and Fibiger (1969) used locomotor activity measures as an index of behavioral inhibition and excitation. Rats of various ages were tested following the administration of amphetamine (which increases activity by direct excitation) or scopolamine (which increases activity indirectly through disinhibition). The results of these studies were consistent with the notion that the cholinergic brain systems that controlled this type of behavior did not reach functional maturity until 18-25 days of age.

These basic conclusions have been replicated and extended in a variety of experiments conducted in this and other laboratories (cf. Feigley, 1974; Feigley & Hamilton, 1971; Feigley, Parsons, Hamilton, & Spear, 1972). In particular, these data in combination with those of Campbell and his associates support the notion that certain cholinergic brain systems mature between the ages of 18 and 25 days and that these systems are important in the behavioral inhibition that is reflected in locomotor activity, passive avoidance responding, and exploratory habituation.

Although the results outlined above formed a parsimonious view of the development of inhibitory capabilities, certain inconsistencies began to appear that required modification of these early formulations. As early as 1969, Warburton and Groves had noted that scopolamine injections did not retard the rate of habituation of the startle reflex. Later, Mabry and Campbell (1974) essentially copied the methodology of the Campbell et al. (1969) experiment and concluded that certain inhibitory behaviors may be under the control of a serotonergic system that develops early, perhaps between 10 and 15 days of age. At about the same time, Carlton

and Advokat (1974) demonstrated that parachlophenyl-alanine injections retarded the habituation of startle reflexes in adult rats, and Williams, Hamilton, and Carlton (1975) showed that 15- to 16-day-old rats exhibit faster habituation of an exploratory response than of the startle reflex. Thus, the data suggested that the inhibition reflected by the habituation of the startle reflex might be based on serotonergic brain systems that develop before the cholinergic systems, which appear to control more complicated forms of inhibition. Although other experimenters have shown that startle responses can be modulated by interfering with other systems (most notably, dopaminergic systems; e.g., Davis & Aghajanian, 1976), these effects seem less closely related to the specific phenomenon of habituation.

The present experiments were undertaken as part of a broader program of research investigating the ontogeny of various forms of behavioral inhibition. The designs were based on the notion that the response decrement that occurs with repeated presentation of a startle-eliciting stimulus reflects a form of behavioral inhibition that has reached functional maturity by the age of 15 days. Contrary to these expectations, the results we report here indicate that the response decrement may be biphasic and that the long-term, gradual response decrement that occurs over many trials may, in fact, reach functional maturity only after 21-25 days of age.

METHOD

Subjects

The subjects were first-order descendants of Sprague-Dawley breeding stock obtained from Blue Spruce Farms in Altamont, New York. The pups were reared in litters culled to four males and four females at birth and weaned at 21 days of age. The colony was maintained with constant temperature and humidity with a 14 h light/10 h dark cycle of illumination. Food pellets and water were available ad lib at all times. All behavioral testing was conducted during the light period.

Apparatus

The measurement of the startle response required the development of special apparatus that could detect and record the rapid ballistic movement produced by the rat's muscular response to an auditory stimulus. The core of the apparatus consisted of a 60-cm-square piece of single-thickness glass supported on the hard rubber tips of three heavy doorstops

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that were screwed into a heavy wooden base. The slight flexibility and excellent elastic properties of this large piece of glass provided a base that would essentially amplify the forces exerted by the rat during the startle reflex.

Two types of test cages were placed on this glass base. For adults, a 20 x 20 x 25 cm high Plexiglas chamber with a grid floor was equipped with hard plastic tripod "feet," allowing it to make firm contact with the glass plate. For pups, a 15 x 17 cm high metal cylinder (with a thin layer of wood shavings on the bottom) was mounted on tripod compression springs that were sensitive to the movements of pups at the earliest ages tested.

The startle-eliciting stimulus was the click of a heavy-duty 24-V dc solenoid located approximately 50 cm from the test cage. The entire apparatus was surrounded by thick foam rubber to attenuate external environmental sounds.

The motion transducer was a phonographic cartridge that rested on the glass surface. Rapid, although slight, motor movements resulted in the generation of an electrical voltage that was amplified via a solid state operational amplifier chip to a voltage ranging from -6 to +6 V. The amplified voltage was full-wave rectified before being sent to the recording system.

The voltage produced by the startle response was recorded by interfacing the apparatus with a Nova-2 computer equipped with an analogue-to-digital converter. The computer was programmed to wait 50 msec after a signal was presented (to avoid the stimulus artifact) and then to cumulate the voltages that occurred during the next successive 450 msec. In order to control for the level of motor activity in the absence of the startle-eliciting stimulus a "control signal" was introduced 1 sec before each "startle signal." For purposes of data analysis, the voltages recorded following the control signals were subtracted from that following the startle signals, yielding an index of the startle reflex that was corrected for general activity.

Procedure

The pair of signals were programmed to occur every 30 sec for a period of 90 min. During the first 15 min, the solenoid was not operated, thereby providing a period of time for the rat to adapt to the test environment. During the next 75 min (150 trials), the second signal was always accompanied by the operation of the solenoid. For purposes of data analysis, the 150 test trials were divided into 30 five-trial blocks, with a median score being used as the index of the startle reflex during that period.

RESULTS

During the first 15 min after being placed in the apparatus, 30 pseudostartle trials were programmed. As expected, the difference scores during this portion of the session resulted in very small voltages that were distributed above and below zero. The first of the 150 test trials were presented during the 16th minute and typically resulted in a very large, positive difference score. The scores generated during these 150 test trials (reduced to 30 scores based on 5-trial medians) form the bases for the following analyses.

The first step in the data analysis was a comparison of the absolute magnitude of the startle reflex as a function of repeated exposures. As suggested by the data summarized in Table 1, the magnitude of the startle reflex varied as a function of age [$F(4,45) = 8.08$, $p < .01$]. This difference per se is of little importance in the present context, since it reflects primarily the

Table 1
Median Response Amplitude in Blocks of Five Trials

Trials	Age (in Days)				
	100	25	21	18	15
1	82	30	72	31	34
2	64	17*	62	23	43
3	76	18*	33	19	39
4	64	19*	60	15	20
5	64	14*	30	13	14
6	60	16*	58	17	21
7	66	26	59	15	17
8	59	16*	56	29	15
9	57	14*	43	20	22
10	44*	15*	50	34	23
11	68	16*	57	21	24
12	57	10†	58	28	14
13	52	10†	61	16	14
14	58	11†	35	27	20
15	32*	14*	32	18	15
16	45*	10†	50	27	21
17	42*	6†	40	24	24
18	41*	6†	41	32	13
19	36*	8†	44	26	19
20	37*	9†	36	20	16
21	37*	10†	43	15	24
22	32*	11*	26	22	16
23	28*	11*	44	25	26
24	29*	10†	36	17	12
25	30*	5†	35	9	11
26	26*	9†	37	16	12
27	26*	7†	52	22	17
28	27*	6†	52	27	14
29	21*	6†	56	21	10
30	23*	10†	43	25	10

Note—Critical value ($p = .05$): 100 days = 36, 25 days = 9, 21 days = 40, 18 days = 20, 15 days = 18. *1 CV below initial block. †2 CVs below initial block.

differences in body weights of the different age groups (hence, the progressive increase in magnitude for the three youngest age groups that were tested in the small apparatus, and an even greater disparity between the 25-day and 100-day groups that were tested in the large chamber). Of greater importance is the significant Age by Trials interaction [$F(116,1305) = 1.30$, $p < .05$], which indicates that the response decrement of the various groups proceeded at different rates irrespective of the magnitude of the response. To extend this analysis of the raw data, separate analyses of variance were performed on each age group to allow the calculation of a critical value (CV; see Winer, 1971) for the trials effect. The CVs (listed in Table 1) revealed that the response decrement over trials can be observed regardless of the baseline (cf. the 25- and 100-day age groups).

The large differences in the size of the CVs (an index of variability) cautioned against too heavy a reliance on the overall analysis of the raw data. Instead, the scores were transformed into standard scores that could be used as an index of the rate and characteristics of the habituation process. The standard scores were similar

to z scores in that they were based on the variance of the particular group. Specifically, each individual score was expressed as a multiple of the critical value (shown in Table 1) for the trials effect of that group. This standardized transformation resulted in much more comparable ranges of scores, allowing a more meaningful comparison of the response decrement of the various age groups.

For the purposes of statistical analyses, the standard scores were cumulated across trials during the first half and second half of the session. The difference between the cumulated scores (15 scores in each half) would indicate a progressive decrement in the response magnitude (i.e., habituation). As shown in Figure 1, the 100-day-old rats and the 25-day-old rats exhibited a substantial and progressive decrement in responding over the course of the session, whereas the 18- and 21-day-old pups did not. Although the 15-day-old pups showed some differences, these were primarily due to an initial high level of responding followed by a virtually unchanging low level of responding. These different patterns of declining response magnitude were reflected in a significant Age by Trials interaction [$F(56,630) = 2.78, p < .01$] in the analysis of the differences in the cumulated scores.

DISCUSSION

The results of these experiments indicate that previous estimates of the timetable for the development of startle reflex habituation may have been in error—or, at least, incomplete. In the experiments of Williams et al. (1975), we suggested that the habituation of the startle reflex was functionally mature by about 15 days of age, consistent with the observations that certain serotonergic inhibitory systems develop at this time (Mabry & Campbell, 1974) and that depletion of serotonin retards the habituation of the startle reflex (Carlton & Advokat, 1974). Furthermore, it was suggested that this form of inhibition essentially complemented a more complex form of inhibition that develops later (18-25 days) and appears to be under cholinergic control (cf. Campbell et al., 1969; Feigley, 1974; Feigley et al., 1972; Williams et al., 1975). The present data require a modification of this conceptualization because the rats in the 18- and 21-day-old groups showed little or no habituation relative to the 25- and 100-day-old rats. And, while the 15-day-old rats did, in fact, show a decrement in response as a function of trials, this effect was not characteristic of the type of function that is usually termed habituation. Examination of the data in Table 1 shows that these pups tended to exhibit the greatest response during the second block of trials, followed by a very rapid decline to a baseline that exhibits little further change. Although this could be viewed as rapid and complete habituation, the absence of habituation in the 18- and 21-day-old rats suggests that this is not the case. Rather, it seems more likely that this early pattern of responding may represent the rapid waning of an excitatory reaction already developed at an early age. This notion would still be consistent with the previous findings of Williams et al. (1975), which were based on approximately half as many trials, and with the Carlton and Advokat (1974) data, which showed that serotonin depletion impaired the response decrement that occurred over only 30 trials.

A further observation may be useful in conceptualizing the behavioral implications of the repeated presentation of a startle-inducing stimulus over a long period of time. In Pavlovian (1927/1960) terminology, this would represent internal inhibition, and one might expect considerable waxing and waning of

CUMULATED STANDARD SCORES

Figure 1. Standard scores (see text) cumulated across 5-trial blocks of stimulus presentations during the first half (75 trials) and second half (75 trials) of the session. For graphical purposes, the cumulated scores of the first half of the session are repeated on the right side of the figure and a shaded insert indicates the disparity in response amplitude between the first and second halves of the session.

the response as the organism went through successive periods of dishabituation and continued habituation. To the extent that the organism may begin, after many trials, to suppress the normally disinhibitory effects of environmental stimuli, it might be expected that the neural substrates involved in this more complicated form of behavioral inhibition might be those that mature at a later stage of ontogenetic development.

Although the present data do not include pharmacological manipulations, the pattern of these results in conjunction with previous experiments suggests a specific and testable set of hypotheses concerning the ontogeny of neural substrates that mediate habituation of the startle reflex. These hypotheses are based on the intersection of behavioral, developmental, and pharmacological aspects of the phenomenon. Specifically, habituation of the startle reflex appears to show two behavioral components. (1) An early, rapid decline in response magnitude: (a) This aspect of the behavior may be more closely related to a decline in excitation than to specific inhibitory capacities. (b) Manipulations that interfere with central serotonergic systems should interfere with this aspect of the behavior. (c) The capacity to exhibit this behavior is present by 15 days of age in the rat, and perhaps as early as 10 days. (2) A prolonged, gradual decline in response magnitude: (a) This aspect of the behavior is proposed to be more akin to Pavlovian (1927/1960) internal inhibition, specifically as it relates to the control of external disinhibitory effects. (b) Manipulations that interfere with central cholinergic systems should interfere with this aspect of behavior. (c) The capacity to exhibit this behavior should appear specifically between the ages of 18 and 25 days of age in the rat.

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