Latent Inhibition of Conditioned Odor Potentiation of Startle: A Developmental Analysis

ABSTRACT: We conducted a two-part study of age and latent inhibition in the rat. In the first part of the study, rats given odor–shock pairings at 23 or 75 days of age exhibited a potentiated startle response in the presence of the odor the following day. This effect did not occur in rats trained at 16 or 20 days of age. Odor pre-exposure on the day prior to conditioning markedly reduced the odor potentiation of startle effect in 23- and 75-day-old rats but had no effect in 16 and 20-day-olds. In the second part of the study, rats were pre-exposed to the odor at 16 or 20 days of age and then conditioned at 23 days of age. When tested the day after conditioning, these pre-exposed rats exhibited a disruption in the odor potentiation of startle effect. We compare our results with other studies of latent inhibition, and with recent studies on whether conditioned responses are appropriate to the animal’s age at training or their age at test.

Keywords: latent inhibition; development; potentiated startle; olfactory conditioning; rats

Pairings of an initially neutral conditioned stimulus (CS) with a biologically relevant unconditioned stimulus (US) leads to acquisition of an association between these two stimuli. Because of this association, the animal’s response to subsequent presentations of the CS is altered. However, the learning, or at least the expression, of this association is impaired if rats receive non-reinforced exposures to the CS prior to conditioning, an effect known as latent inhibition (Lubow & Moore, 1959). The latent inhibition effect is well established in adult rats (e.g., Aguado, Symonds, & Hall, 1994; Escobar, Arecediano, & Miller, 2002; Killcross, Kiernan, Dwyer, & Westbrook, 1998), but has been considerably less attention paid to this effect in the developing rat.

A developmental analysis of basic associative phenomena, such as latent inhibition, will contribute to our understanding of the differences and similarities in how information is encoded at different points of development. Such research will also potentially provide converging evidence for the neural analysis of these processes in adult animals with experimentally induced brain lesions (e.g., Fanselow & Rudy, 1998). The limited literature on latent inhibition (LI) during development is not consistent. That is, some investigators have reported that LI does not occur in preweanling rats (e.g., Nicolle, Barry, Veronesi, & Stanton, 1989; Wilson, Phinney, & Brennan, 1974), while others have reported that it does (e.g., Kraemer, Hoffman, & Spear, 1988; Rudy & Cheatle, 1979). Nicolle et al. used a conditioned taste aversion procedure and found LI in 32-day-old rats, but not in 18- or 25-day-olds, while Rudy and Cheatle used an odor-avoidance procedure and found LI in rats as young as 8 days of age. Clearly, more research is needed in this area.

In a recent review, Stanton (2000) reported some very interesting results concerning LI in the developing rat. Stanton used the eyeblink conditioning procedure in this work. Earlier research from his laboratory had shown that conditioned eyeblink responses to an auditory CS emerge...
of age. Stanton, Freeman, & Skelton, 1992). In a study described in the review (see Figure 8 in Stanton, 2000), CS pre-exposure impaired eyelink conditioning at 24 days of age (i.e., LI was observed) but facilitated conditioning at 20 days of age (non-pre-exposed rats at this age did not exhibit conditioning). If conditioned freezing was measured, however, then CS pre-exposure produced LI at both ages. Stanton concluded: “This suggests that the cognitive system responsible for latent inhibition interacts differently with the associative systems governing these two conditioned responses, depending on their relative stage of development. CS pre-exposure appears to increase attention to the CS in poorly developed associative systems and decrease attention in more mature associative systems” (p. 34). We have further explored this intriguing idea.

Conditioned odor potentiation of startle (OPS) develops relatively late (at about 23 days of age; Richardson, Paxinos, & Lee, 2000) compared to other conditioned responses to an odor CS (e.g., conditioned freezing and heart rate responses are observed as early as 16 days of age; Richardson, Tronson, Bailey, & Parnas, 2002; Sananes, Gaddy, & Campbell, 1988). In the OPS procedure, rats receive either paired or explicitly unpaired presentations of an odor with shock. During a subsequent test, the startle response to a loud, unexpected noise is measured. The odor is not present for the first part of the test but is introduced for the second part of the test. Rats in the paired condition exhibit a pronounced increase in startle response amplitude in the presence of the odor but those in the unpaired condition do not (also see Richardson, Vishney, & Lee, 1999). Importantly, the failure to observe OPS in rats younger than 23 days of age is not due to a failure to acquire the odor–shock association because they exhibit a marked odor avoidance (Richardson & Fan, 2002; Richardson et al., 2000). The failure to observe OPS in rats younger than 23 days of age is also not due to some methodological problem with the startle procedure; rats as young as 16 days of age exhibit a potentiated startle response following direct pharmacological stimulation of the primary startle pathway (Weber & Richardson, 2001). Taken together, these findings would lead one to conclude that the associative system mediating conditioned OPS is “poorly developed” in rats younger than 23 days of age. Therefore, in the present study we examined the effects of CS pre-exposure on the OPS effect in rats either younger or older than 23 days of age. Based on the interesting results reported by Stanton (2000) the prediction was that CS pre-exposure would impair acquisition of conditioned OPS in rats older than 23 days of age but would facilitate acquisition of OPS in rats younger than 23 days of age.

EXPERIMENT 1

The first part of the study examined the effects of odor pre-exposure on conditioned odor potentiation of startle (OPS) in rats at four different ages (16, 20, 23, or 75 days of age). Previous research has demonstrated that 23- and 75-day-old rats should exhibit the OPS effect, while 16 and 20-day- olds should not (Richardson et al., 2000). Based on the findings of Stanton (2000), odor pre-exposure would be expected to impair conditioning in the two older age groups but facilitate conditioning in the two younger age groups.

Methods

Subjects. One hundred twenty experimentally naïve, male, Sprague-Dawley rats, obtained from the breeding colony maintained by the School of Psychology at the University of New South Wales, were used. Rats were 16 (±1), 20 (±1), 23 (±1), or 75 (±15) days of age at training (30 at each age; the data from one adult rat was lost due to experimenter error and the data of a second adult rat was excluded from the analysis because its test performance was more than 5 SD away from the group mean). Rats that were 16–23 days of age were housed in litters of eight, with their mother, in plastic boxes (length × width × height, 37 × 24.5 × 27 cm) that were kept in a room with a 12-hr light/dark cycle (lights on at 06:00 h). No more than one subject from any single litter was included in any experimental group. The 75-day-old rats were housed in groups of eight in plastic boxes (length × width × height, 65 × 40 × 22 cm) in a colony room with a natural light–dark cycle. The adult rats were handled for at least 3 days, 5 min a day, before being used in the experiment. Food and water were continuously available for all rats. All animals were treated according to the principles of animal use outlined in the Australian code of practice for the care and use of animals for scientific purposes (6th Edition, 1997), and the Animal Care and Ethics Committee at the University of New South Wales approved all procedures.

Apparatus. To assess startle, rats were tested in rectangular chambers constructed of Plexiglas and stainless steel bars. The front wall, rear wall, and ceiling of each startle chamber were constructed of clear Plexiglas. The floor and two sidewalls were constructed of 3-mm stainless steel rods (the wall rods were vertically positioned relative to the floor rods). Electric shock (0.6 mA for 1 s duration) could be delivered to the floor of each chamber via a custom-built, constant-current shock generator. The chamber was attached to a piece of Plexiglas onto which a sheet of piezoelectric film had been
laminated. Movement within the chamber caused the piece of Plexiglas to flex, which produced a voltage in the piezoelectric film. The voltage produced by the piezoelectric film was proportional to the intensity of the movement in the chamber; that is, larger movements produced larger voltages. These voltages were amplified and digitized (1-kHz rate) in order to measure startle amplitude. The peak voltage (converted into arbitrary units that ranged from 0 to 32,000; data rounded to 0–320 prior to analysis) in the 250 ms period after stimulus onset was taken as the index of the startle response.

The acoustic startle stimulus was delivered through two high-frequency speakers mounted 8 cm from either side of the startle chamber. The startle stimulus was a 100-ms, 100-dB burst of white noise, with a 1-ms rise–fall time. The intensity of the startle stimulus and of the background noise was measured with a Bruel and Kjaer precision sound level meter (type 2235) placed in the center of the startle cage. Stimulus presentations and the recording of data were both controlled by computer. The software and hardware were custom-developed at the University of New South Wales.

Each chamber was located in a wood cabinet in order to attenuate external noise and visual stimulation. A ventilation fan in the cabinet provided a low-level background noise (60 dB) at all times, and illumination was provided by a 15-W red light on the front door of the wood cabinet. Following each training or test session, the startle chambers were cleaned with tap water and a tray of animal bedding just below each chamber was changed.

There were two pairs of startle chambers: one pair for the 75-day-olds and one for the 16-, 20-, and 23-day-olds (hereafter referred to as “preweanling”). Each chamber within a pair was identical, but the two pairs differed in the following ways. The chambers used for a 75-day-old rat was slightly larger than the chambers used for a preweanling rat (20 × 12 × 12 cm vs. 13 × 9 × 9 cm, respectively). In addition, the stainless steel grids comprising the floor were farther apart, center-to-center, in the chambers used for the 75-day-old rat than in the chambers used for the preweanling rat (1.3 cm vs. 1.0 cm, respectively). Finally, the chambers used for the 75-day-old rat were attached to a slightly thicker piece of Plexiglas than were the chambers used to test the preweanling rat (4.2 mm vs. 3.0 mm, respectively).

**Odor.** The odor used in this study was 0.1 ml of grape flavor (Grape No. 182380019; Wild Flavours, Heidelberg, Germany); in the control condition, 0.1 ml of tap water was used in place of the grape odor. In both cases, the fluid (either water or grape) was squirted onto a piece of paper towel inside a plastic specimen jar. During odor presentations, the jar was placed approximately 10 cm below the startle chamber floor.

**Procedure**

**Pre-Exposure.** On the day prior to conditioning, all rats were placed into a startle chamber, with the door of the wood cabinet slightly ajar, for approximately 35 min. After an initial 5-min adaptation period, a specimen jar was placed under the startle cage for 7–10 s. There were 15 separate presentations of the specimen jar, with an inter-stimulus interval of 1.5, 2.0, or 2.5 min (average = 2 min). For rats in condition Pre-Paired, the specimen jar contained grape (n = 10 at each age, except for the 75 day olds where n = 9). For rats in condition NoPre-Paired the specimen jar contained water (n = 10 at each age). For half of the rats in the Unpaired condition the jar contained grape and for the other half it contained water (subsequent analysis showed that these two unpaired groups did not differ so they were combined into a single group; n = 10 at each age, except for the 75-day-olds where n = 9).

**Training.** A two-stage training procedure was used. The doors of the wood cabinets in which the startle cages were located were kept slightly ajar throughout both stages of training. In Stage 1 of training, rats were placed in the startle chamber and given a 5-min adaptation period, at the end of which the first of five shocks was given. Seven to 10 seconds prior to each shock, a jar was placed beneath the startle chamber; this jar contained grape for rats in the paired condition and water for rats in the unpaired condition. Immediately after the shock, the jar was removed, covered with a lid, and placed on a bench approximately 2 m from the startle chamber. The interval between shock presentations was 1.5, 2.0, or 2.5 min (average = 2 min) and varied pseudo-randomly. After the last shock, rats were removed from the startle chambers and placed in their home cages. This training procedure produces an association between grape and shock in the rats in the paired condition but not in the rats in the unpaired condition. Rats in both conditions, however, would acquire an association between the context (i.e., the startle cage) and shock. Therefore, in order to extinguish this context–shock association, at least partially, and to equate the exposure to the grape odor for rats in the paired and unpaired conditions, a second stage of training was given.

In Stage 2 of training, rats were returned to the startle chamber that they had been in during Stage 1 and given a 2-min adaptation period. A jar, containing water for rats in the paired condition and grape for rats in the unpaired condition, was then presented for 7–10 s. No shocks were administered in stage 2 of training. The jar was presented five times in Stage 2 (i.e., the same number of times as in Stage 1), and the interval between presentations was 1.5, 2.0, or 2.5 min (average = 2 min) and varied pseudo-randomly (i.e., the same as in Stage 1). Both stages of training lasted approximately 15 min, and the interval...
between the two stages was 50–60 min. At the end of each stage of training, the doors and windows were opened for at least 10 min to allow adequate ventilation of the experimental room.

**Testing.** Rats were returned to the startle chamber in which they had been trained, and after a 5-min adaptation period, 30 startle-eliciting noise bursts, separated by 30 s, were presented. No odor was present during these initial 30 bursts. The average startle response on these initial 30 trials was taken as an estimate of the rat’s baseline startle response. During a 60-s period following the 30th noise burst, a jar containing grape was placed beneath the startle chamber floor and remained there for the rest of the test session (~15 min). After the introduction of the grape odor, an additional 30 noise bursts, each separated by 30 s, were presented. At the end of the testing session, subjects were removed from the startle chamber and returned to their home cage. Testing occurred approximately 24 hr after training.

Odor potentiation of startle (OPS) was examined by comparing the average startle amplitude when the odor was present to the average startle amplitude during baseline and was calculated using the following equation: \[ \frac{(T - B)/B \times 100 = \text{OPS}} \] where \( T \) = mean startle amplitude over the 30 test trials, \( B \) = mean startle amplitude over the 30 baseline trials, and OPS = percentage increase in startle.

**RESULTS AND DISCUSSION**

**Baseline Startle Amplitude**

Analysis of baseline startle amplitudes, with a 4 (age) \( \times 3 \) (condition) ANOVA, yielded a significant effect of age \( [F(3, 106) = 7.59, p < .001] \). The main effect of condition and the interaction of age and condition were both non-significant [largest \( F(2, 106) = 1.15, p = .31 \)]. The effect of age was due to the 16-day-olds having lower baseline startle amplitudes than did the rats at the other three ages (Table 1). However, this significant main effect does not necessarily reflect a true age difference. That is, we vary the gain setting of the startle chambers for rats of different ages in an attempt to produce comparable baselines (mean of approximately 50). Obviously, we were unsuccessful in this effort in this particular experiment. In any case, given that we convert each animal’s test score into a percentage change from baseline score, the effects of the age differences in baseline startle amplitude is minimized. Furthermore, the low baselines in the 16-day-olds maximize the likelihood of observing the OPS effect in rats this age (which is opposite to our predictions).

<table>
<thead>
<tr>
<th>Group</th>
<th>Startle Response</th>
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<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
</tr>
<tr>
<td>16-day-olds</td>
<td>23.13 (4.6)</td>
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<tr>
<td>20-day-olds</td>
<td>45.30 (4.6)</td>
</tr>
<tr>
<td>23-day-olds</td>
<td>47.17 (4.6)</td>
</tr>
<tr>
<td>75-day-olds</td>
<td>52.10 (4.8)</td>
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<tr>
<td>Experiment 2</td>
<td></td>
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<tr>
<td>Pre16–Paired23</td>
<td>53.68 (10.3)</td>
</tr>
<tr>
<td>Pre20–Paired23</td>
<td>53.63 (10.3)</td>
</tr>
<tr>
<td>NoPre–Paired23</td>
<td>65.7 (14.3)</td>
</tr>
<tr>
<td>Unpaired</td>
<td>46.3 (9.2)</td>
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Note: Values are expressed as mean (\( \pm \text{SEM} \)).

**Odor potentiation of startle**

Analysis of the test data yielded a significant main effect of age \( [F(3, 106) = 5.53, p = .04] \), condition \( [F(2, 106) = 14.05, p < .001] \), and interaction \( [F(6, 106) = 5.22, p < .001] \). Post hoc comparisons, with Tukey’s HSD test, showed that the interaction was due to the rats in condition NoPre-Paired at the two older ages exhibiting a substantial increase in startle response amplitude in the presence of the odor compared to rats in the Unpaired and Pre-Paired conditions (smallest \( p = .004 \)), while there were no group differences at the two youngest ages. These data are shown in Figure 1.

These results replicate our previous findings that conditioned OPS emerges relatively late in development (i.e., in 23-day-old rats, but not in 20-day-olds; Richardson et al., 2000). Furthermore, they show that CS pre-exposure markedly reduces the magnitude of the OPS effect during presentation of grape odor. Rats were 16, 20, 23, or 75 days of age at the time of training. At each age, some rats received the odor CS paired with the shock US, while others received the two stimuli in an explicitly unpaired fashion. Furthermore, some of the rats in the paired condition had been pre-exposed to the odor CS on the day prior to training. Rats in all conditions were tested on the day after training.
effect at both 23 and 75 days of age. There was no detec-
table effect of stimulus pre-exposure in the 16- and
20-day-old rats, however. This latter finding does not
support the intriguing finding reported by Stanton (2000)
with the eyeblink preparation. That is, in the present study,
CS pre-exposure did not facilitate conditioning in rats
trained at an age where the associative system mediating
the conditioned response was “poorly developed.” How-
ever, it must be acknowledged that such an effect might be
seen with different parameters. Changing the number of
stimulus pre-exposures, the interval between stimulus
presentations, or the interval between pre-exposure and
training (or some other factor) might lead to a facilitatory
effect being observed. All that can be concluded from
the present study is that such an effect was not observed with
the parameters used.

EXPERIMENT 2

We did not detect any effects of odor pre-exposure in the
16- and 20-day-old rats in Experiment 1. Because rats
at these ages do not exhibit conditioned OPS, it is obvious
that LI could not have been observed. However, based on
the findings of Stanton (2000), we predicted that CS pre-
exposure would facilitate conditioning of OPS at these
two ages (i.e., ages at which the associative system me-
diating fear potentiation of startle is poorly developed).
Clearly, this was not observed. Therefore, a slightly dif-
ferent approach was taken in the present experiment in
order to see whether odor pre-exposure at these ages
affects conditioned OPS. Specifically, the present experi-
ment was designed to examine whether odor pre-exposure
at 16 or 20 days of age would produce latent inhibition if
conditioning took place at an age when the rat does exhibit
conditioned odor potentiation of startle (i.e., 23 days of
age).

Method

Subjects. Forty-eight experimentally naïve, male, Spra-
gue-Dawley rats, obtained from the same source as those
in Experiment 1, were used (the data from two rats were
excluded from the analysis, one because of experimenter
error and one because its test performance was more than
5 SD away from the group mean). All rats were 23 (±1)
days of age at the time of training. All housing conditions
were as described in Experiment 1.

Apparatus. The startle cages used for the preweanling
rats in Experiment 1 were used in this experiment. All
stimulus parameters were as described in Experiment 1.

Procedure. All procedures were the same as in Experi-
ment 1, with the following exceptions. This experiment
consisted of a four-group, non-factorial design. Rats in
one condition (Pre16–Paired23; n = 12) were given 15
odor pre-exposures at 16 days of age and then 5 odor–
shock pairings at 23 days of age. Rats in a second
condition (Pre20–Paired23; n = 12) were given 15 odor
pre-exposures at 20 days of age and five odor–shock
pairings at 23 days of age. Half of the rats in a third
condition (NoPre–Paired23; n = 11) received 15 expo-
sures to water at 16 days of age and five odor–shock
pairings at 23 days of age, while the other half received 15
water exposures at 20 days of age and five odor–shock
pairings at 23 days of age (statistical analysis showed that
these two conditions did not differ so their data were
collapsed into a single group). Finally, rats in the fourth
condition (Unpaired; n = 11) received no treatment at 16
or 20 days of age and five explicitly unpaired presentations
of the odor and shock at 23 days of age. All rats were tested
for OPS on the day after conditioning.

Results and Discussion

Baseline Startle Amplitude. Analysis of baseline startle
amplitudes showed that the four groups did not differ
[F < 1.0, Table 1].

Odor Potentiation Of Startle. Inspection of the test data
(Figure 2) indicated that the non-pre-exposed rats given

![FIGURE 2](#)
odor–shock pairings at 23 days of age exhibited a potentiated startle response in the presence of the odor compared to the rats in the unpaired condition. Furthermore, rats given odor–shock pairings at 23 days of age, but pre-exposed to the odor at either 16 or 20 days of age, exhibited a marked reduction of the OPS effect (i.e., latent inhibition). Statistical analysis confirmed these interpretations of the data. Specifically, an overall ANOVA yielded a significant effect of group \( F(3, 42) = 3.79, p = .017 \). Subsequent pairwise comparisons, with Tukey’s HSD test, showed that rats in group NoPre–Paired23 exhibited a potentiated startle response in the presence of the odor compared to rats in the Unpaired group \( (p = .044) \) and to the rats in either of the pre-exposed groups \( (p = .03 \) and \( .047 \) for the comparisons with groups Pre16–Paired23 and Pre20–Paired23, respectively). The rats in the two pre-exposed groups did not differ, nor did they differ from the rats in the Unpaired group \( (all ps > .60) \).

The results of this experiment show that pre-exposing rats to an odor CS at either 16 or 20 days of age impairs acquisition of an odor–shock association at 23 days of age. Furthermore, it appears that 16-day-old rats are capable of remembering the odor-only experience quite well over at least a 1-week interval given that the amount of LI in these rats is complete (compare the LI in group Pre16–23Paired in this experiment to that of the 23-day-old pre-exposed rats in Experiment 1).

**GENERAL DISCUSSION**

The results of this study replicate our earlier findings that conditioned OPS develops relatively late; that is, it occurs in rats trained at 23 days of age but not in those trained at 20 days of age or younger (Richardson et al., 2000). The lack of OPS in rats trained prior to 23 days of age is not due to a failure to acquire the odor–shock association: younger rats exhibit pronounced odor-elicited freezing (Richardson et al., 2002) and odor-elicited avoidance (Richardson & Fan, 2002; Richardson et al., 2000). This developmental dissociation of how learned fear is expressed provides unique opportunities for exploring fundamental issues that concern both memory processes and the neural bases of learned fear.

The results of this study clearly demonstrate latent inhibition of conditioned odor potentiation of startle, at least in rats trained at 23 or 75 days of age. This finding supports the recent work of Schauz and Koch (1998, 2000) who reported latent inhibition to a visual CS in adult rats tested in a fear potentiation of startle procedure. As Schauz and Koch noted, given the vast amount of information known about the neural circuitry mediating conditioned fear potentiation of startle, it would seem a particularly promising procedure to use in the analysis of the neural bases of various basic associative phenomena (such as latent inhibition). Our findings of latent inhibition in the OPS procedure also support the work of Otto, Cousins, and Rajewski (1997) who demonstrated latent inhibition of olfactory conditioning in adult rats, using odor-elicited freezing as the measure of learned fear. Otto et al. (1997) reported that LI of olfactory conditioning, at least in adults, was context specific and persistent (for at least 31 days, which is the longest interval tested). In other conditioning preparations, the introduction of an interval between conditioning and test often leads to a reduction in LI (e.g., Aguado et al., 1994). It will be interesting to determine if LI of olfactory conditioning is affected differently by a long conditioning-test interval than is LI of CSs in other sensory modalities.

In Experiment 2, we found that pre-exposing rats to an odor at 16 or 20 days of age impaired their acquisition, or their expression, of an odor–shock association at 23 days of age. In other words, although odor pre-exposure at these ages failed to affect conditioned OPS the following day (Experiment 1), it did affect conditioned OPS if training occurred at 23 days of age. These findings are relevant to several recent studies from our laboratory on the effects of early odor experience on later performance of conditioned OPS. More specifically, recent results from our laboratory have shown that rats given odor–shock pairings at 16 days of age retain that association over a 1-week interval but (a) fail to express it via conditioned OPS (Richardson & Fan, 2002; Richardson et al., 2000; Richardson et al., 2002); (b) do not acquire OPS at a faster rate (i.e., with fewer odor–shock pairings) than do naïve rats (Richardson & Fan, 2002); and (c) do not exhibit increased resistance to extinction of OPS compared to previously naïve rats following training at 23 days of age (Richardson et al., 2002). In other words, we have failed to see any effects of odor–shock pairings at 16 days of age on later performance of OPS at 23 days of age. One might be tempted to suggest that the OPS procedure is merely insensitive to any early experiences. However, our findings clearly do not support such a conclusion. That is, this study shows equivalent latent inhibition of OPS in 23-day-old rats that had been pre-exposed to the odor CS either the day before training or at 16 days of age.

How can one explain the consistent finding that odor-only exposure at 16 days of age affects conditioned OPS at 23 days of age, whereas odor–shock pairings do not? At present, we can only offer a speculative explanation of this pattern of results. The amygdala is generally considered to be a key structure in learned fear. That is, pairings of a CS with an aversive US lead to neural changes occurring in the amygdala, particularly the basolateral nucleus. This neural plasticity is thought to represent a central state of fear (e.g., Davis, 1992). Subsequent presentations of the CS elicits this central state, which can be expressed via
any of a number of responses (e.g., freezing, changes in heart rate, or a potentiated startle response). However, this basic assumption is challenged by some of our recent work on the development of learned fear.

In several recent studies, we have exploited the sequential emergence of learned fear responses (for review, see Hunt & Campbell, 1997) to explore a fundamental issue concerning memory development. Specifically, is an animal’s conditioned response appropriate to their age at training or their age at testing? That is, what happens if a rat is trained at an age where it can express learned fear via one response but not via a second response, and is tested at a later age where it can express learned fear via both responses? The common view that an aversive CS elicits a central state makes a very clear prediction: if the rat retains the CS–US association, then it should express the learned fear via both responses. However, in both a between-groups (Richardson et al., 2000) and a within-subjects (Richardson & Fan, 2002; Richardson et al., 2002) design, we have consistently found that rats respond in a manner appropriate to their age of training, not their age of testing. In other words, rats given odor–shock pairings at 16 days of age, and tested the following day, express that fear via odor avoidance (or odor-elicted freezing) but not via OPS. Rats given odor–shock pairings at 23 days of age, and tested the following day, express that learned fear via any of those responses. Most importantly, rats trained at 16 days of age, but not tested until 23 days of age, retain the odor–shock association but express it only via odor avoidance (or odor-elicted freezing).

These findings suggest that our current conceptualization of learned fear needs to be modified. That is, these studies indicate that CS–US pairings produce neural plasticity not only in the amygdala but also in structures downstream to the amygdala. These downstream structures (e.g., periacqueductal gray, nucleus reticularis pontis caudalis) are responsible for mediating specific forms of learned fear. If the pathway between the amygdala and the downstream structure is not functional at the time of training (e.g., because of immaturity), then the necessary neural changes required for expressing that specific form of learned fear will not occur. Therefore, rats will respond in a manner appropriate to their age at the time of training.

In the present study, the early learning did not involve CS–US pairings. Rather, the early experience involved odor-only exposures. There is some evidence that the amygdala is involved in latent inhibition. For example, Schauz and Koch (2000) found that infusions of AP5, an NMDA antagonist, into the basolateral amygdala prior to pre-exposing adult rats to a light CS markedly reduced the amount of LI to the light CS. However, there is other evidence that indicates that the amygdala is not necessarily involved in LI (e.g., Weiner, Tarrasch, & Feldon, 1996). Clearly, much more research is needed in order to determine whether the amygdala is involved in LI or not. However, for the present study, the suggestion is that the LI effect does not involve neural plasticity in structures downstream from the amygdala. Future research will have to determine the validity of this suggestion.

Finally, the results of the present study clearly fail to support the intriguing idea, proposed by Stanton (2000), that CS pre-exposure facilitates conditioning in a poorly developed system (like the OPS system in 16- and 20-day-old rats). Although it is possible that greater or lesser amounts of stimulus pre-exposure might have yielded the predicted outcome, there was absolutely no hint of any facilitation with the current parameters. It is also possible that the conditioned fear potentiation of startle and conditioned eyeblink preparation (which Stanton used) are fundamentally different. That is, although these two model preparations appear to have a number of similarities (i.e., both develop relatively late, both involve a basic reflex, and both involve aversive conditioning procedures), they also differ in a number of important ways. For example, conditioned fear potentiation of startle involves modulation of a reflexive response, while eyeblink conditioning involves a reflexive response being expressed to a stimulus that previously did not elicit it. In addition, Stanton, Fox, and Carter (1998) reported that giving 17-day-old rats CS–US pairings facilitated their acquisition of a conditioned eyeblink response to that CS when later trained at 24 days of age, but we failed to see such a “savings” effect with the conditioned odor potentiation of startle procedure (Richardson & Fan, 2002). These two model preparations both hold considerable promise as tools for exploring the development of learning and memory processes from a behavioral and a neural perspective. However, given these apparent differences, it will be important for future research to more closely compare and contrast these two procedures in the developing rat.

NOTE

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REFERENCES

Aguado, L., Symonds, M., & Hall, G. (1994). Interval between pre-exposure and test determines the magnitude of latent


