The Ontogeny of Conditioned Odor Potentiation of Startle

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During development, conditioned responses usually occur first to olfactory, then to auditory, and finally to visual cues. The authors of the present study report that fear potentiation of startle to an olfactory conditioned stimulus emerges relatively late in development (i.e., at 23 days of age; Experiments 1 and 2). The failure to observe conditioned odor potentiation of startle (OPS) in younger rats was not due to a failure to either acquire or remember the odor-shock association (Experiment 3). Surprisingly, the authors also found that rats trained at 16 but tested at 23 days of age failed to exhibit the OPS effect even though they did exhibit pronounced odor avoidance (Experiment 4). The results are discussed in terms of (a) sensory-specific sequential emergence of learned fear, (b) the neural circuit involved in fear potentiation of startle, and (c) the concept that conditioned responding is appropriate to the animal’s age at the time of training rather than its age at testing.

Although there has been a long-standing interest in learning and memory during development, there has been a recent upsurge of interest in this area. Developmental studies are being used to provide converging evidence for theoretical models based on lesion studies in adult animals (e.g., Fanselow & Rudy, 1998; Stanton, Fox, & Carter, 1998). Recent developmental studies have also shown that associative learning emerges in a response- and sensory-specific sequence (e.g., for a review, see Hunt & Campbell, 1997).

The various sensory systems develop in a sequential manner. For example, across a wide range of species the functional onset of the auditory system precedes that of the visual system (Gottlieb, 1971, as cited in Alberts, 1984). Further, the functional onset of the tactile and chemical senses usually precedes that of the auditory system (for review of sensory system development in the rat, see Alberts, 1984). Interestingly, associative learning to conditioned stimuli (CSs) in various sensory modalities follows this same sequence, albeit with a delay of a few days. That is, young rats express a learned response to an auditory CS before they express the same learned response to a visual CS, and in both cases the learned reaction usually occurs a few days after that sensory system is functional (for review, see Hunt & Campbell, 1997).

In addition to emerging in a sensory-specific sequence, associative learning emerges in a response-specific sequence. For example, three common measures of learned fear in the rat are freezing, changes in heart rate, and potentiation of the acoustic startle response, and interestingly, these forms of learned fear emerge at different ages. More specifically, if subjects are tested shortly (e.g., 15 min) after training, an auditory CS previously paired with an aversive unconditioned stimulus elicits freezing at a younger age than at which it alters heart rate, and it alters heart rate at a younger age than at which it potentiates the startle response (for review, see Hunt & Campbell, 1997).

The response-specific emergence of learned fear provides converging support for the view that different neural structures mediate the various forms of learned fear. That is, current models of the neural bases of learned fear maintain that various structures downstream from the amygdala mediate the various forms of learned fear. Specifically, freezing is thought to be mediated by the periaqueductal gray, changes in heart rate by the lateral hypothalamus, and startle potentiation by the nucleus reticularis pontis caudalis (PnC; for reviews, see Davis, 1992; LeDoux, 1994). The finding that these forms of learned fear emerge at different ages is usually interpreted as a result of either the delayed development of the specific structure mediating each behavior or the delayed development of the projections from the amygdala to the relevant downstream structure.

There is a substantial literature on both conditioned freezing (or suppression of ongoing behavior) and conditioned changes in heart rate during development (e.g., Campbell & Ampeuro, 1985; Hunt, 1997; Hunt, Richardson, Hess, & Campbell, 1997; Richardson, Wang, & Campbell, 1995; Sananes, Gaddy, & Campbell, 1988). In contrast, there is very little empirical evidence on the development of fear potentiation of startle (FPS). The evidence that has been reported on the ontogeny of conditioned FPS, however, has shown that this form of learned fear emerges relatively late. Hunt, Richardson, and Campbell (1994), for example, reported that an auditory CS elicited freezing in rats as young as 18 days of age but did not potentiate the startle response until the rats were at least 23 days of age. Similarly, Hunt et al. (1994) reported that a visual CS elicited freezing in rats 23 days of age but did not potentiate the startle response until the rats were 30 days of age. Further, Hunt (1999) has recently shown that conditioned FPS to a visual CS emerged later in development than did conditioned changes in heart rate to that stimulus.

In the present study, we examined whether conditioned FPS would emerge earlier in development if an olfactory, instead of an auditory or visual, stimulus was used as the CS. Based on the
findings that learned fear typically emerges to olfactory CSs at younger ages than to auditory or visual CSs, we predicted that conditioned odor potentiation of startle (OPS) would be observed in rats as young as 16 days of age (an age at which pronounced heart rate conditioning is seen to an olfactory CS; Sananes et al., 1988). The procedures used in the present study are based on a recent demonstration of conditioned OPS in adult rats (Richardson, Vishney, & Lee, 1999).

General Method

Subjects

Experimentally naïve, Sprague-Dawley rats were used. All rats were obtained from the breeding colony maintained by the School of Psychology at the University of New South Wales. Rats were 16 (± 1), 20 (± 1), 23 (± 1), or 75 (± 15) days old at training. Only males were used at 75 days of age, but both sexes were used at the younger ages. The 75-day-olds were housed in groups of 8 in plastic boxes (65 cm long x 40 cm wide x 22 cm high) and kept in a colony room with natural lighting. The 75-day-olds were handled for 3 min a day on each of 3 days before training. The 16-, 20-, and 23-day-olds were housed in litters of 8, with their mother, in plastic boxes (37.0 cm long x 24.5 cm wide x 27.0 cm high) and kept in a breeding room with a 12-hr light-dark cycle (lights on at 6 a.m.). No more than 2 subjects from any single litter were included in a group. The 16-, 20-, and 23-day-old rats were not handled before training. Food and water were continuously available to all rats. Training and testing occurred between 9 a.m. and 5 p.m. All subjects were treated in accord with the principles of animal use maintained by the American Psychological Association, and all procedures were approved by the Animal Care and Ethics Committee at the University of New South Wales.

Apparatus

Two types of chambers were used in this study: startle chambers and an avoidance chamber.

Startle chambers. To assess startle, we tested rats in one of two 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 side walls were constructed of 3-mm stainless steel rods (the wall rods were vertically positioned relative to the floor rods). Electric shock (0.06 mA, 1 s in duration) could be delivered to the floor of each chamber through 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 amplitudes produced larger voltages. These voltages were amplified and digitized (at a 1-kHz rate) so that startle amplitude could be measured. The peak voltage (converted into arbitrary units) in the 250-ms period after stimulus onset was taken as the index of the startle response.

There were two pairs of startle chambers: one pair for the 75-day-olds and one for the 16-, 20-, and 23-day-olds (hereinafter referred to as preweanlings). Each chamber within a pair was identical, but the two pairs differed in the following ways. The chamber used for a 75-day-old rat was slightly larger than the chamber used for the preweanlings (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 preweanling chambers (1.3 cm vs. 1.0 cm, respectively). Finally, the chamber used for the 75-day-old rats was attached to a slightly thicker piece of Plexiglas than was the chamber used to test the preweanlings (4.2 mm vs. 3.0 mm, respectively).

We placed each set of chambers in one of two wooden cabinets to attenuate external noises and sights. 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, a tray of animal bedding just below each startle chamber was changed and the startle chambers were cleaned with tap water.

The 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-full 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; Naerum, Denmark) placed in the center of the startle cage. All stimulus presentations and data recording were controlled by computer. The software and hardware used were custom developed at the University of New South Wales.

Avoidance chamber. A rectangular chamber (29 cm long × 15 cm wide) made of Plexiglas was placed on a grid floor consisting of stainless steel grids (3 mm in diameter, 0.7 cm apart, center to center). The chamber was divided into three sections. The middle 4.5 cm of the chamber was termed the “neutral” zone, one end was termed the “odor” zone, and the other end was termed the “no-odor” zone (each end was 12.25 cm long). A petri dish containing the grape odorant (see below) was placed underneath the odor zone. The time spent in both the odor and the no-odor zones during a 3-min test was recorded. A rat was considered to be out of the neutral zone whenever its head left that area of the chamber.

Odor. The odor used in this study was 0.15 mL of grape flavor (Grape No. 182380019; Wild Flavors, Heidelberg, Germany). In the control condition, 0.15 mL of tap water was used in place of the grape odor. Odor stimuli were prepared by placing a small amount of fluid (either water or grape flavor) on a piece of paper. The odorant was contained in a petri dish (a specimen jar was used in Experiment 2). During odor presentation, the petri dish was placed approximately 10 cm below the startle or avoidance chamber floor.

Procedure

Training. A two-stage training procedure was used in all experiments, and training always occurred in a startle chamber. 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 15 shocks was given. Seven to 10 seconds before each of these 15 shocks, a petri dish containing grape for rats in the paired group (water for rats in the unpaired group) was placed beneath the startle chamber. Immediately after the shock, the petri dish was removed, covered with a lid, and placed on a bench approximately 2 m from the startle chamber. The interval between shock presentations was either 1.5 min or 2.5 min and varied pseudorandomly. 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 exposure to the grape odor, a second stage of training was given. In Stage 2 of training, all rats were returned to the same startle chamber that they had been in during Stage 1 and were given a 2-min adaptation period. After this, a petri dish, containing water for rats in the paired condition and grape for rats in the unpaired condition, was presented for 7–10 s. No shocks were administered in Stage 2 of training. The petri dish was presented 15 times in Stage 2 (i.e., the same number of times as in Stage 1), and the interval between presentations was either 1.5 min or 2.5 min and varied pseudorandomly (i.e., the same as in Stage 1). Both stages of training took approximately 35 min to complete, and the interval between the two stages was 50–55 min. At the end of each stage of training, the doors and
Method

comparisons within an age group because we were interested in

Results and Discussion

and shock (see General Method). All rats were given the startle test 1 day

effective at potentiating the startle response in paired rats at all

each age, there were two training conditions: Rats in the

procedures were identical for the two conditions (see General

in startle.

In Experiments 3 and 4, some rats were tested for odor avoidance. In this
test, the rats were placed in the neutral zone of the avoidance chamber
described above for a single 3-min test session. The time spent in the odor
and the no-odor zones was recorded with stopwatches.

Experiment 1

Although it has been shown that an odor previously paired with
shock is effective at potentiating the startle response (e.g., Rich-

ardson et al., 1999; Vishney & Richardson, in press), this effect
has been documented only in adult rats. In the present experiment,
we examined the ontogeny of conditioned OPS. Specifically, we
tested whether an odor previously paired with shock potentiates
the acoustic startle response in 16-, 23-, and 75-day-old rats. At
each age, there were two training conditions: Rats in the paired
condition received 15 pairings of grape with shock, and rats in the
unpaired condition received the same number of odor presenta-
tions and shock but in an explicitly unpaired manner. The test
procedures were identical for the two conditions (see General
Method). Based on the general finding that conditioned fear
emerges in a sensory-specific sequence (for review, see Hunt &
Campbell, 1997), we predicted that the olfactory CS would be
effective at potentiating the startle response in paired rats at all
three ages.

Method

Twenty rats were trained at 16, 23, and 75 days of age (total of 60 rats).
Half of the subjects at each age received grape-shock pairings at training,
and the other half received explicitly unpaired presentations of the grape
and shock (see General Method). All rats were given the startle test 1 day
after training.

Results and Discussion

In this study, we primarily focused the data analysis on pairwise
comparisons within an age group because we were interested in
whether rats at a particular age exhibit the OPS effect, not in age
comparisons of the magnitude of the effect (i.e., adult rats exhibit
a larger effect than do 23-day-olds). Furthermore, the gain settings
of the piezoelectric outputs of the startle cages were adjusted at
each age in an effort to have roughly comparable baseline startle
amplitudes at each age. That is, the piezoelectric output could vary
between 0 and 320 arbitrary units, and we adjusted the gain
settings at each age in an effort to have mean startle baselines of
approximately 50 units. This practice is important for ensuring that
a failure to observe potentiation of startle at test is not due to rats
at that age having baselines at the upper end of the range, but it
means that it is inappropriate to make direct age comparisons.

As can be seen in Figure 1, presentation of the grape odor
produced a marked potentiation in startle response amplitude in
23- and 75-day-old rats in the paired condition. This effect was not
observed in the 16-day-olds. Statistical analysis with t tests sup-
ported these interpretations of the results. That is, rats in the paired
condition differed from unpaired controls at 23 and 75 days of age,
t(18) = 3.06 and 3.05, respectively, both ps = .007, but not at 16
days of age, t(18) = 0.38, p = .74. Importantly, rats in the paired
and unpaired groups did not differ in baseline startle amplitude
at any age, largest t(18) = 1.66, p = .11 (see Table 1).

In earlier studies on conditioned OPS in adult rats, it has been
found that the OPS effect is relatively constant across the test
session (Richardson et al., 1999; Vishney & Richardson, in press).
That is, the OPS effect does not appear to extinguish over a
25-30-min test session. However, younger rats may respond dif-
frently. Specifically, younger rats may exhibit the OPS effect at
the start of the test, but then this effect may extinguish over the
25-30-min test session. Therefore, collapsing the test data across
the entire test session into a single score could mask the occurrence
of the OPS effect at the start of the test in the 16-day-old rats. To
explore this possibility, we further analyzed the test data by divid-
ing the test session into five blocks of 10 trials each (each block
of 10 trials represents 5 min of the test session).

The analysis of these data yielded essentially the same pattern of
results as described above: The 16-day-old rats did not exhibit the
OPS effect, but the 23- and 75-day-old rats did (see Figure 2).

Figure 1. Mean (+ SEM) percentage of change in startle amplitude
during presentation of grape odor. Rats were 16, 23, or 75 days of age
at the time of training, and all were tested 1 day after training. Rats in
the paired condition (open bars) received 15 pairings of grape and shock
at training, whereas rats in the unpaired condition (filled bars) received
the same number of grape and shock presentations but in an explicitly
unpaired fashion. Each group contained 10 subjects.
Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>16-day-olds</th>
<th>23-day-olds</th>
<th>75-day-olds</th>
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<tbody>
<tr>
<td>Paired</td>
<td>37.3 (3.1)</td>
<td>47.4 (6.2)</td>
<td>36.5 (3.7)</td>
</tr>
<tr>
<td>Unpaired</td>
<td>38.7 (1.6)</td>
<td>54.4 (5.1)</td>
<td>48.7 (6.4)</td>
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More specifically, a 2 (condition) × 3 (age) × 5 (blocks) mixed-design analysis of variance failed to yield any significant effect of block or any significant interaction of block with any other factor, largest $F(8, 216) = 1.18, p = .54$. As can be seen in Figure 2, the 16-day-old rats failed to exhibit conditioned OPS on any block. Further, the lack of extinction across the 25–30-min test session that has been observed in our earlier studies was replicated in the 75-day-olds and was observed in the 23-day-old rats as well.

Experiment 2

The results of Experiment 1 clearly show that an odor previously paired with shock was effective in potentiating the startle response in 23- and 75-day-old rats but not in 16-day-olds. The failure to observe OPS in the 16-day-olds was not due to a masking effect of within-session extinction, because these rats failed to exhibit OPS even on the first block of test trials (i.e., in the first 5 min of test). These results did not support our prediction that an olfactory CS would be effective in potentiating the startle response at all three ages. This prediction was based on the finding that conditioning emerges in a sensory-specific sequence, with olfactory CSs effective at younger ages than auditory CSs (for review, see Hunt & Campbell, 1997). Because conditioned FPS occurs to auditory stimuli at 23 days of age (Hunt et al., 1994), an olfactory CS should be effective in producing conditioned FPS at a younger age. Perhaps this prediction was not supported because we tested rats at too early an age. That is, perhaps an olfactory CS would be effective in potentiating the startle response at an age between 16 and 23 days. This idea was tested in Experiment 2.

Method

Twenty rats were trained at 17, 20, and 23 days of age (total of 60 rats). Half of the subjects at each age received grape-shock pairings at training, and the other half received explicitly unpaired presentations of the grape and shock. All procedures were as described in General Method except that the number of trials in each stage of training was reduced from 15 to 5. That is, in Stage 1 of training, rats received 5 shocks instead of 15 (7–10 s before each shock, rats in the paired condition were exposed to grape and rats in the unpaired condition to water). In Stage 2, the grape odor was presented five times to the rats in the unpaired condition, and water was presented to the rats in the paired condition five times. This reduction in the number of trials in each stage of training resulted in each stage being reduced from 35 min to 15 min. The interval between each stage was kept at 50–55 min.

Results and Discussion

The data of one rat in the 23-day-old unpaired condition was excluded from analysis (it was more than 6 SEMs away from the group mean), leaving a sample size of 9 for that condition.

As can be seen in Figure 3, presentation of the grape odor produced a marked potentiation in startle response amplitude in 23-day-old rats in the paired condition. This effect was not observed in the 17- or 20-day-olds. Statistical analysis with *t* tests supported these interpretations of the results. That is, rats in the paired condition differed from unpaired controls at 23 days of age, $t(17) = 2.17, p = .04$, but not at 17 or 20 days of age, largest $t(18) = 0.51, p = .62$. Importantly, rats in the paired and unpaired groups did not differ in baseline startle amplitude at any age, largest $t(18) = 1.31, p = .21$ (see Table 2).

The results of this experiment replicate and extend those of Experiment 1. Once again, 23-day-old rats exhibited a significant OPS effect. Indeed, even though the number of odor–shock pair-
paired condition (open bars) received five pairings of grape and shock at the time of training, and all were tested 1 day after training. Rats in the Figure 3.

Method

conditioned FPS to an auditory CS at 23 days of age (Hunt et al., Richardson, in press). Furthermore, rats younger than 23 days of age was very comparable across the two experiments (mean increase in group, n = 9).

was given 15 pairings of the grape odor with shock, and the others were given the same number of grape and shock presentations but in an explicitly unpaired fashion. Each group contained 10 subjects (except the 23-day-old unpaired group, n = 9).

ings was decreased from 15 to 5, the magnitude of the OPS effect was very comparable across the two experiments (mean increase in startle in the 23-day-old paired subjects was 27% in Experiment 1 and 25% in Experiment 2). This is similar to what has been observed with adult rats (cf. Richardson et al., 1999; Vishney & Richardson, in press). Furthermore, rats younger than 23 days of age failed to exhibit the conditioned OPS effect. In the present experiment, this failure to exhibit OPS was observed in 17- and 20-day-old rats. On the basis of the finding that rats exhibit conditioned FPS to an auditory CS at 23 days of age (Hunt et al., 1994), we had predicted that this effect would emerge earlier in development with an olfactory CS. However, the results of Experiments 1 and 2 do not provide any support for this prediction.

Experiment 3

It is possible that rats younger than 23 days of age failed to exhibit the conditioned OPS effect in Experiments 1 and 2 because they failed to acquire the odor–shock association (i.e., the training parameters were effective for 23-day-olds but not for younger rats). It is also possible that the younger rats acquired the odor–shock association but then forgot it over the 24-hr retention interval (i.e., infantile amnesia). Experiment 3 examined these two ideas. This experiment was based on the numerous demonstrations of conditioned odor avoidance in preweanling rats (e.g., Kucharski & Spear, 1984; Sullivan & Wilson, 1991). That is, if the training procedures used in Experiments 1 and 2 are ineffective for producing the odor–shock association in rats younger than 23 days of age, then they should fail to avoid the odor.

Method

Twenty experimentally naive 16-day-old rats were used. Half of the rats were given 15 pairings of the grape odor with shock, and the others were given the same number of odor and shock presentations but in an explicitly unpaired fashion (see General Method). Twenty-four hours after training, all rats were tested for avoidance of the grape odor (see General Method).

Results and Discussion

Rats in the unpaired condition showed a slight preference for the grape odor (M ± SEM = 60% ± 10% of test in odor zone), whereas rats in the paired condition showed a marked avoidance of this odor (28% ± 7% of test in odor zone). Analysis of these data yielded a significant group difference, t(18) = 2.74, p = .013. Clearly, the training procedures were effective at producing an association between the odor and shock, and the 16-day-olds did not forget this association over the 24-hr retention interval. Therefore, the failure to observe conditioned OPS in rats younger than 23 days of age in Experiments 1 and 2 cannot be attributed to age differences in learning or retention of the odor–shock association.

Experiment 4

The primary purpose of this experiment was to examine what would happen if rats were trained at 16 days of age and then tested at 23 days of age. That is, if rats are trained at an age where they do not exhibit the OPS effect but then tested at an age where they do, what result would be obtained? Would the rats respond in a manner appropriate to the age of training or in a manner appropriate to the age of testing? We predicted that the rats would respond in a manner appropriate to the age of testing. That is, we predicted that rats trained at 16 days of age and tested at 23 days of age would exhibit the OPS effect. Because of potential forgetting over the long retention interval, half of the rats in this experiment were tested on the odor avoidance procedure. Comparison of the amount of odor avoidance by these subjects to that observed in Experiment 3 would allow us to determine the degree of forgetting of the odor–shock association over the 7-day retention interval.

Method

Forty-eight experimentally naive 16-day-old rats were used. Half of the rats were in the paired condition, and the other half were in the unpaired condition. Further, half of the rats in each condition were tested on the startle test, and the other half were tested on the avoidance test (ns = 12). All rats were tested 7 days after training.

Results and Discussion

Startle test. There did not appear to be any differences in baseline startle amplitude between rats in the paired condition (M ± SEM = 31.68 ± 4.80) and those in the unpaired condition (36.13 ± 4.70). Statistical analysis confirmed this, t(22) = 0.66, 

<table>
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<th>Condition</th>
<th>17-day-olds</th>
<th>20-day-olds</th>
<th>23-day-olds</th>
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<tbody>
<tr>
<td>Paired</td>
<td>26.2 (5.2)</td>
<td>44.2 (8.7)</td>
<td>66.8 (10.5)</td>
</tr>
<tr>
<td>Unpaired</td>
<td>17.5 (4.1)</td>
<td>46.5 (10.0)</td>
<td>63.1 (10.8)</td>
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the presence of the odor. This failure to observe the OPS effect was not due to forgetting of the odor-shock association over the 7-day retention interval. That is, rats tested on the avoidance procedure exhibited strong conditioned performance. More specifically, the rats in the paired condition spent much less time in the odor zone (mean ± SEM = 35.5 ± 7.7) than did rats in the unpaired condition (65.4 ± 5.3). This difference was statistically significant, t(22) = 3.2, p = .004. Further, comparison of these means with those obtained in Experiment 3, in which the retention interval was only 1 day, indicates that little forgetting occurred across the 1-week retention interval. Indeed, a post hoc comparison of odor avoidance by the paired rats in Experiments 3 and 4 was not significant, t(20) = 0.76, p = .45.

The results of this experiment were not as predicted. Rats given odor-shock pairings at 16 days of age and then tested for OPS at 23 days of age did not exhibit a potentiated startle response in the presence of the odor. This failure to observe the OPS effect was not due to (a) a failure to acquire the odor-shock association or (b) the forgetting of that association across the 7-day retention interval, because rats tested on the avoidance procedure exhibited a substantial avoidance of the odor. Indeed, the odor avoidance was as strong after a 7-day retention interval as it was after a 1-day retention interval.

General Discussion

The results of this study replicate recent demonstrations of conditioned OPS in adult rats (Richardson et al., 1999; Vishney & Richardson, in press) and extend this phenomenon to 23-day-old rats. However, the present results also show that the OPS effect does not occur in rats younger than 23 days of age. This failure to observe the OPS effect in younger rats was not due to (a) a failure to acquire the odor-shock association or (b) the forgetting of that association over the retention interval, because rats trained at 16 days of age exhibited a marked avoidance of the odor. Previous research on the development of conditioned FPS found that an auditory CS was effective by 23 days of age and a visual CS by 30 days of age (Hunt et al., 1994). Because of the evidence that learned fear emerges in a sensory-specific sequence (see Hunt & Campbell, 1997), we predicted that an olfactory CS would be effective in potentiating the startle response in rats younger than 23 days of age. This result was not observed, and conditioned OPS was first observed in 23-day-old rats.

One interpretation of the failure to observe OPS in rats younger than 23 days of age is that the pathway that mediates this particular behavioral expression of learned fear has not matured. This interpretation fits well with current models of the neural circuitry of learned fear (e.g., Davis, 1992; LeDoux, 1994), in which projections from the central nucleus of the amygdala to various structures downstream (e.g., central gray, lateral hypothalamus, or PnC) are critical for the various behavioral expressions of learned fear (e.g., freezing, changes in heart rate, potentiated startle). From this perspective, the projection from the central nucleus of the amygdala to the lateral hypothalamus must be functional in the 16-day-old rat because an odor paired with shock elicits conditioned changes in heart rate (Sananes et al., 1988), but the projection from the central nucleus of the amygdala to the PnC must not be functional because an odor paired with shock does not potentiate the startle response in rats this age (Experiments 1 and 2). According to this view, the amygdala–PnC projection would become functionally mature sometime between 20 and 23 days of age. Tracing studies are clearly needed to explicitly address this issue. Such studies may also provide unique insights into the role of the direct projection from the amygdala to the PnC (e.g., Rosen, Hitchcock, Sananes, Miserendino, & Davis, 1991) and the disynaptic pathway from the amygdala to the central gray and then to the PnC (e.g., Fendt, Koch, & Schmittler, 1994) in conditioned FPS.

If the failure to observe conditioned OPS in rats younger than 23 days of age is due to the immaturity of the pathway mediating this effect, then one might have predicted that rats trained at 16 but tested at 23 days of age would exhibit conditioned OPS. That is, because the startle potentiation pathway is mature by 23 days of age and because these rats remember the odor–shock association (as shown by their continued avoidance of the odor), they should exhibit the OPS effect. This result was not observed, however. One possibility here is that at the time of training, neural plasticity needs to occur both in the amygdala and in the structure mediating the specific form of learned response that is being measured. If the projection from the amygdala to the PnC is not functional in the 16-day-old rat, then changes in the PnC do not occur during training and these rats will not exhibit FPS even when tested at 23 days of age (but see Krupa, Weng, & Thompson, 1996, for evidence counter to this general proposition). In any case, these findings are relevant to the question of whether subjects respond in a manner appropriate to the age of training or in a manner appropriate to the age of testing. Johanson and Hall (1984), for example, have reported data supporting the view that young rats respond in a manner appropriate to their age at testing rather than their age at training (see also Stanton et al., 1998, for another relevant study). More specifically, in studies on the acquisition of an odor–milk association, Johanson and Hall showed that rats trained at various ages (e.g., 3, 6, or 9 days) exhibited different conditioned response profiles to the odor. That is, 3-day-old rats exhibited substantial behavioral activation to the odor, whereas older rats displayed more constrained mouthing and probing responses. Of central relevance to the present study is their finding that rats trained at 3 days of age, but then tested at 9 days of age, exhibited a response profile appropriate to the 9-day-old rats (i.e., they exhibited discrete mouthing responses to the odor but not an intense behavioral activation). These results were taken as evidence that the rat had acquired a stimulus–stimulus association rather than a specific stimulus–response association during pairings of the odor with milk. Indeed, in discussing their findings, Johanson and Hall stated that “as pups grow older, specific responses may not be retained and elicited by a conditioned stimulus, though the stimulus may elicit other behaviors more appropriate to the pups’ present age” (p. 154). Hence, given that 23-day-old rats exhibit the conditioned OPS effect (Experiments 1 and 2), we expected that rats trained at 16 and tested at 23 days of age would exhibit the startle potentiation effect so long as they remembered the odor–shock association. This outcome was not found, however (Experiment 4). It will be important to determine those situations in which animals
respond in a manner appropriate to their age at test (e.g., Johanson & Hall, 1984) and those situations in which they respond in a manner appropriate to their age at the time of training (the present results).

In summary, the present study showed that conditioned OPS is not observed in rats younger than 23 days of age. This failure to observe the OPS effect was not due to a failure to acquire the odor–shock association or to a failure to remember this association over the retention interval. When 16-day-old rats were tested on an odor avoidance procedure, they exhibited pronounced avoidance of the odor. This finding that conditioned OPS is a relatively late-developing effect supports the idea that the secondary circuit responsible for fear modulation of startle is relatively slow to mature (also, see Richardson & Vishney, 2000). However, the most surprising finding of the present study was that rats trained at 16 days of age, but tested at 23 days of age, still did not exhibit the OPS effect, even though they retained the odor–shock association. Future research is needed to more fully explore those situations in which the developing rats respond in a manner appropriate to the age at which they were trained and those situations in which they respond in a manner appropriate to the age at which they are tested.

References
