Caudal Pontine Reticular Formation of C57BL/6J Mice: Responses to Startle Stimuli, Inhibition by Tones, and Plasticity

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Carlson, Stephanie and James F. Willott. Caudal pontine reticular formation of C57BL/6J mice: responses to startle stimuli, inhibition by tones, and plasticity. J. Neurophysiol. 79: 2603–2614, 1998. C57BL/6J (C57) mice were used to examine relationships between the behavioral acoustic startle response (ASR) and the responses of neurons in the caudal pontine reticular formation (PnC) in three contexts: 1) responses evoked by basic startle stimuli; 2) the prepulse inhibition (PPI) paradigm; and 3) the effects of high-frequency hearing loss and concomitant neural plasticity that occurs in middle-aged C57 mice. 1) Responses (evoked action potentials) of PnC neurons closely paralleled the ASR with respect to latency, threshold, and responses to rapidly presented stimuli. 2) ‘Neural PPI’ (inhibition of responses evoked by a startle stimulus when preceded by a tone prepulse) was observed in all PnC neurons studied. 3) In PnC neurons of 6-mo-old mice with high-frequency (>20 kHz) hearing loss, neural PPI was enhanced with 12- and 4-kHz prepulses, as it is behaviorally. These are frequencies that have become ‘overrepresented’ in the central auditory system of 6-mo-old C57 mice. Thus neural plasticity in the auditory system, induced by high-frequency hearing loss, is correlated with increased salience of the inhibiting tones in both behavioral and neural PPI paradigms.

INTRODUCTION

The acoustic startle response (ASR) is a behavioral reflex evoked by abrupt, intense sounds, readily elicited in a wide variety of animal species including humans (Landis and Hunt 1939). The ASR has a short latency, with the whole-body response evident 9–12 ms after the onset of a startle stimulus (e.g., mouse) (Parham and Willott 1988). As a relatively simple acoustico-motor behavior, the ASR is amenable to neurobehavioral research on human and nonhuman subjects (Davis 1984). The ASR has several distinct properties (not shared by most other auditory behaviors) that can be studied at both behavioral and neural levels. For example, auditory stimuli must have a high-intensity (e.g., >70 dB SPL) and relatively slow rate of presentation (e.g., ≈0.2/s) to effectively evoke ASRs (Davis 1984; Flesher 1965; Willott et al. 1979). Another aspect of startle is its value as a behavioral “probe” to study the modulation of behavior by other stimuli or environmental manipulations (Davis 1984; Hoffman and Ison 1980). One example is prepulse inhibition (PPI): when a relatively low-intensity “prepulse” stimulus (S1) precedes the startle stimulus (S2) by an interval of ~5–200 ms, the amplitude of the startle response evoked by the S2 is reduced (“inhibited”). This inhibition presumably results from descending inhibitory circuit(s) activated by the S1. Finally, the fact that the startle response is modulated by other neural circuits suggests that it can be used as a behavioral indicator of neural plasticity within the modulating circuits.

The present study used selected auditory stimuli to examine the relationships between the behavioral ASR and the responses of neurons in the caudal pontine reticular formation (PnC), a brain region thought to be a key component of the “startle circuit(s)” (Davis et al. 1982; Groves et al. 1974; Hammond 1973; Koch et al. 1992; Lee et al. 1996; Leitner et al. 1980; Lingenhöhl and Friauf 1992; Wu et al. 1988; Yeomans and Cochrane 1993; Yeomans et al. 1989) and site of reticulospinal neurons that trigger the motor responses (Davis et al. 1982; Lingenhöhl and Friauf 1994; Mitani et al. 1988a,b; Tohyama et al. 1979; Valverde 1961; Wu et al. 1988; Yeomans et al. 1989). C57BL/6J (C57) mice were used to address these relationships by comparing behavioral and neural responses in three contexts: responses evoked by basic startle stimuli; the PPI paradigm using several different S1s; and the effects of high-frequency hearing loss and concomitant neural plasticity that occurs in middle-aged C57 mice.

C57 mice hear normally as young adults (1–2 mo of age) but exhibit progressive high-frequency hearing loss (basal cochlear pathology) beginning between 2 and 3 mo of age. By 5–6 mo of age, high-frequency hearing loss (>20 kHz) is substantial, and this is accompanied by alterations (neural plasticity) in tonotopic organization of the inferior colliculus (IC) and auditory cortex; neurons in the normally high-frequency regions come to respond better to the still-audible middle frequencies (4–16 kHz). Their thresholds become similar to neurons in middle-frequency regions, and more vigorous suprathreshold responses are evoked by middle-frequency tones (Willott 1984, 1986; Willott et al. 1988, 1991, 1993).

Because this hearing loss–induced (HLI) plasticity entails the strengthening of neural responses to some still-audible (middle-frequency) sounds, it might be expected that some of their behavioral effects would be augmented as well. We have employed the PPI paradigm to test this hypothesis because a substantial body of literature has shown that the magnitude of PPI is related to the behavioral salience of the prepulse (e.g., Hoffman and Ison 1980; Young and Fechter 1983). Various lesion-behavior, anatomic, and physiological experiments (Davis 1984; Fox 1979; Leitner and Cohen 1985; Marsh et al. 1976; Parham and Willott 1990; Swerdlow and Geyer 1993; Willott and Demuth 1986) indicate that, in the PPI paradigm, the S1 activates neural circuits in the upper brain stem and forebrain during the 10- to 200-ms interval between S1 and S2. Descending components of these circuits inhibit the startle circuit in the lower brain.
stem when the startle response is evoked by S2 (cf. Huffman and Henson 1990; Parham and Willott 1990). The inhibition appears to act directly or indirectly on neurons of the PnC, the interface between auditory and motor segments of the startle reflex (Davis et al. 1982; Koch and Friauf 1995; Lingenhöhl and Friauf 1992, 1994). The IC (and perhaps the auditory cortex via projections to IC) plays a critical role in PPI (Davis and Gendelman 1977; Fox 1979; Leitner and Cohen 1985). Some neurons in the IC (particularly the external cortex) have direct connections to the PnC (Lingenhöhl and Friauf 1994), and damage to the lateral IC interferes with inhibition of startle (Leitner and Cohen 1985; Parham and Willott 1990). The IC also projects to the lateral tegmentum, which has been implicated in PPI (Bell et al. 1964; Fendt et al. 1994; Gallager and Pert 1978; Leitner et al. 1979; Powell and Hatton 1969; Saito et al. 1987; Shamma-Lagnado et al. 1987; Sverdlow and Geyer 1993). Thus HLI plasticity in the IC (see also Kazee et al. 1995) and cortex is likely to affect the descending modulation of PnC neurons that produce PPI (Willott et al. 1994).

We have used the PPI paradigm with C57 mice to demonstrate a close correlation between HLI plasticity and increased behavioral salience of still-audible sounds. Willott et al. (1994) and Carlson and Willott (1996) presented tone S1s to mice 100 ms before an intense startle-evoking S2. Compared with 1 mo olds, 5- to 6-mo-old C57 mice (with HLI plasticity) exhibited PPI that was significantly enhanced when S1 was a 12- or 16-kHz tone (50–80 dB SPL), marginally enhanced when S1 was a 4- or 8-kHz tone, and diminished when S1 was a 24-kHz tone. In another study (Willott and Carlson 1995), the duration of inhibition produced by middle-frequency S1s was found to be prolonged in 5-mo-old C57 mice. In normal-hearing mice, an S1-S2 interval of ≥200 ms produces little or no PPI (inhibition has waned), whereas PPI is strong at S1-S2 intervals of 500 ms in the 5 mo olds. To summarize, PPI provides a behavioral indicator of HLI plasticity and suggests that the neural circuit that underlies PPI should be affected by HLI plasticity as well. Thus evaluation of the physiological properties of the PPI circuit provides a unique opportunity to investigate the neurophysiological events linking HLI plasticity to changes in behavior.

It is important to note that high-frequency hearing loss does not appear to result in augmented neural responses in the basic startle circuit itself. In 6-mo-old C57 mice, for example, startle amplitude declines for high-frequency stimuli as expected from threshold elevations but is not increased for middle frequencies (Carlson and Willott 1996; Parham and Willott 1988). The lack of ASR enhancement is presumably due to the fact that the ASR is mediated by projections from the cochlear nucleus (CN) to PnC (Davis et al. 1982; Lingenhöhl and Friauf 1994; Yeomans and Frankland 1996; Yeomans et al. 1989). Augmented responses are observed in the upper auditory brain stem and cortex but have not been observed in the CN (Willott et al. 1991). Thus the occurrence of HLI plasticity in the upper auditory system would be expected to affect PPI because of changes in the descending pathways that modify startle, not in the auditory limb of the startle pathway per se (e.g., CN to PnC).

The present study tested the following hypotheses. If the PnC is the site of initiation of the ASR via reticulospinal projections, a population of PnC neurons in C57 mice should have thresholds around 70 dB SPL, respond well to stimuli presented at a relatively slow rate (e.g., 0.1 Hz) but not at a fast rate (e.g., 1 Hz), and have latencies slightly shorter than the behavioral ASR (this also implies longer latencies in 6-mo-old C57 mice than in 1 mo olds, as occurs behaviorally). Furthermore, if PPI occurs because PnC neurons are inhibited by circuits activated by an S1, the same PnC neurons should be inhibited by S1s that do not evoke an ASR but do produce behavioral PPI, be inhibited when the S1-S2 interval is between 10 and 200 ms, but not when the interval is only 2 ms. Finally, if HLI plasticity in the upper auditory system affects the descending modulation of the ASR circuit, PnC neurons should be more effectively inhibited by middle frequency tones (e.g., 4 and 12 kHz) in hearing-impaired 6-mo-old mice, compared with normal-hearing 1 mo olds.

This work was part of Dr. Carlson’s doctoral dissertation.

METHODS

Subjects

C57BL/6J mice of either sex were obtained from Northern Illinois University’s Psychology Department rodent colony. The mice were first to third generation offspring of stock procured from Jackson Laboratory (Bar Harbor, Maine). After weaning, the animals were separated into same-sex cohort groups and raised in wire mesh cages. Access to tap water and Purina Rodent Chow was provided ad libitum. The room temperature was maintained between 23 and 24°C and a 12-h light/dark schedule was used with light onset at 7:00 a.m.

Two groups of C57 mice were categorized by age at the start of testing: 1) 1 mo, the age at which hearing sensitivity is optimum (n = 12), and 2) 5–6 mo, the age at which high-frequency sensitivity is declining (n = 9). Hearing sensitivity is adultlike by 1 mo of age in C57 mice (Hunter and Willott 1987; Li and Borg 1991; Saunders and Hirsch 1976).

The present study complied with the ethical standards of the National Institutes of Health for the treatment of animals and was approved by the Northern Illinois University Institutional Animal Care and Use Committee.

Behavioral screening

Behavioral PPI was evaluated before neurophysiological examination of PnC neurons. This procedure was employed to confirm that the mice involved in the experiment exhibited patterns of PPI behavior that were consistent with previous research.

The startle stimuli (S2) were 100 dB SPL (re: 20 μPa), 4-kHz tone pips (10 ms duration, 1 ms rise-fall). Prepulse stimuli (S1) were tone pips (10 ms duration, 1 ms rise-fall time, 70 dB SPL) of 4, 12, or 24 kHz emitted by a Radio Shack Super Tweeter. S1 and S2 were separated by a 100-ms interval (onset to onset) and were produced using hardware and software from Modular Instruments (Southeastern, PA). A mouse’s movements within the startle test chamber produced voltages whose amplitudes were measured with a digital storage oscilloscope. A startle was characterized by spikelike voltage changes typically beginning 9–12 ms after S2 onset. Startle amplitude was defined as the largest peak-to-peak voltage deflection occurring within 30 ms after S2 presentation (the initial peak of the spike usually occurred within 2–3 ms of the onset latency). A full description of behavioral procedures can be found elsewhere (Carlson and Willott 1996). ASRs were obtained for 10 S1-S2 presentations for each S1 frequency and 10
S2s alone (all presented in random order) before the neurophysiologic experiments. PPI for each frequency of S1 was expressed as a percentage of the mean S2-only startle measure (S1-S2 trials divided by the mean of S2-only trials).

Neurophysiological experiments

Surgery. A mouse was anesthetized with pentobarbital sodium (Nembutal, 60 μg/g ip) and chlorprothixene (Taractan, 12.5 μg/g ip). Xylocaine hydrochloride (Lidocaine) was applied to tissue in the initial surgical incision as an additional humane precaution. The mouse’s head was secured by inserting its upper incisors into a stainless steel bite bar; this fixed the head in a specific horizontal position so that electrode penetration angles were consistent across all subjects. The nose was braced using a steel nosebar attached to the skull with dental cement and an anchor screw. A dental drill was used to remove a portion of the skull to expose the area of the brain overlying the PnC. The mouse’s temperature was maintained at 37°C during subsequent experimental manipulations by a circulating-water heating pad lying beneath the animal. The animal was checked periodically during the experiment for signs of arousal. When necessary, additional drugs were administered to ensure a deep level of anesthesia. Supplemental drug dosages were ~25% of the original dose of either pentobarbital sodium or chlorprothixene.

Recording. For PnC recordings, mice were placed in a sound-attenuated 2.1 m² room. The walls were covered with Sonex sound-absorbing foam, and the floor was carpeted. Ambient noise levels measured for frequencies of 2–31.5 kHz ranged from 21 dB SPL (100 Hz) to 37 dB SPL (12 kHz) with S1-S2 intervals of 2, 5, 10, 20, 50, and 200 ms. The various S1-S2 intervals were presented in a random order.

Histology

After recordings had been obtained from a penetration, the microelectrode was retracted to a position above the PnC, and a small Xylocaine hydrochloride (Lidocaine) was applied to tissue to the skull with dental cement and an anchor screw. A dental drill removed the skull so that electrode penetration angles were consistent across all subjects. The nose was braced using a steel nosebar attached to the skull with dental cement and an anchor screw. A dental drill was used to remove a portion of the skull to expose the area of the brain overlying the PnC. The mouse’s temperature was maintained at 37°C during subsequent experimental manipulations by a circulating-water heating pad lying beneath the animal. The animal was checked periodically during the experiment for signs of arousal. When necessary, additional drugs were administered to ensure a deep level of anesthesia. Supplemental drug dosages were ~25% of the original dose of either pentobarbital sodium or chlorprothixene.

Data analysis

Several dependent measures were used in evaluations of the data: 1) the S2-only response (total number of action potentials produced by a series of 10 S2-alone presentations); 2) the S1 response (total number of action potentials produced by a series of 10 S1 presentations); 3) the S1-S2 response (the total number of action potentials produced by a series of 10 S2s each preceded by an S1) divided by the S2-only response; this number reflects, in a percentage, the extent to which neural responses evoked by an S2 are modified by S1 stimuli (neural PPI); 4) the latency (time from the onset of the S2 stimulus until the onset of action potentials evoked by S2) of neural responses evoked by S2 in both S2-only and S1-S2 trials; 5) the “threshold” of PnC neuron responses to a 12-kHz stimulus. Threshold was defined in this instance as the lowest intensity of the stimulus that is capable of eliciting any action potentials during a series of 10 stimulus presentations. The traditional definition of threshold (the minimum intensity of a stimulus to which the responses of at least 50% of the stimuli) was not used in the present experiment because PnC neurons often do not respond to every stimulus, even at high intensities. 6) The last of the dependent measures used was the threshold for neural PPI using a 12-kHz S1 stimulus. Threshold in this in-
stance is defined as the lowest intensity of S1 that is still capable of reducing the number of action potentials evoked by S2, producing a neural PPI score of <75%.

Statistics

Behavioral and neural PPI were evaluated using $2 \times 3$ (Age group $\times$ S1 frequency) analyses of variance (ANOVA). Significant Main Effects and interactions were investigated further using t-tests for independent samples. Neural PPI was also evaluated as a function of medial/lateral location of the neuron in the PnC (those located within 600 $\mu$m of the midline were considered “medial;” neurons that were more laterally placed were considered “lateral”) using a $2 \times 2 \times 3$ (Age group $\times$ Location $\times$ S1 frequency) ANOVA. Significant effects were followed up using t-tests for independent samples.

Neural PPI under various S1-S2 intervals was examined using a $2 \times 5$ (Age group $\times$ S1-S2 interval) ANOVA. Significant effects were followed up using a Tukey test.

The effect of Age group on the latency shift of an S2 response, produced by the presence of an S1 was examined using t-tests for each S1 frequency.

A correlational analysis (Pearson’s $R$) was used to investigate the relationship between PPI and the number of unit responses evoked by S1 in 1-mo-old animals for 4- and 24-kHz S1 stimuli.

S2 responses to stimuli presented at slow (0.1/s) and fast rates (1/s) were evaluated using a $2 \times 2$ (Age group $\times$ S2 presentation rate) ANOVA. Significant Main Effects and interactions were investigated further using a t-test for paired groups.

The frequency distributions of the latency of an S2-only response for 1- and 6-mo-old mice were compared using a $\chi^2$ test of independence.

Thresholds for a response to S2 stimuli and for neural PPI using a 12-kHz S1 were examined using a t-test for independent samples to compare the two age groups.

Results

Behavioral screening

All mice exhibited behavioral PPI when screened before the recording experiments. As seen in Fig. 1, 6-mo-old mice showed better PPI using low- and middle-frequency S1 stimuli and slightly worse PPI using a 24-kHz S1 stimulus than 1-mo-old mice. The pattern of PPI obtained in the present study is similar to those found in previous studies (Carlson and Willott 1996; Willott et al. 1994). No animals had to be excluded from the study on the basis of abnormalities in behavioral PPI scores.

Location and number of recordings

Recordings were obtained from 81 PnC neurons in 1-mo-old mice, and 80 neurons in 6-mo-old mice. The neurons sampled were scattered evenly throughout the rostrocaudal, mediolateral, and dorsoventral dimensions of the PnC, described earlier.

The PnC neurons reported on here were not spontaneously active, and action potentials were evoked by startle stimuli (S2s) presented at the 0.1/s rate. Typically, the neurons did not respond to every S2; when they did respond it was with a burst of one to several action potentials. We focused on neurons that were not spontaneously active because the ASR only occurs when an acoustic stimulus is presented. Spontaneously active neurons were present in the PnC, but these were not studied in detail. Some of these were mildly excited or inhibited for a brief time after the presentation of S1 or S2 stimuli, but most exhibited little or no change in discharge rate after the presentation of an S2 stimulus.

Response latencies

Figure 2 is a frequency distribution, plotting latency of the response of each neuron evoked by an S2-only stimulus for each age group. As can be seen in the figure, a greater number of neurons demonstrated short-latency (<6 ms) responses in the 1-mo-old C57 mice, when compared with the responses of neurons in 6-mo-old mice (20 vs. 3 neurons demonstrating short latencies). Many PnC neuron response latencies in the 6-mo-old mice were clustered around 8 ms, whereas the responses in the younger mice were distributed more evenly across the shorter latencies. The two age groups

![Figure 1](http://jn.physiology.org/DownloadedFrom)  
**FIG. 1.** Behavioral prepulse inhibition (PPI) in 1- and 6-mo-old mice. Error bars indicate means $\pm$ SE. For 6 mo olds, PPI was significantly better (lower %) when the S1 was 4 or 12 kHz.

![Figure 2](http://jn.physiology.org/DownloadedFrom)  
**FIG. 2.** Frequency distribution of S2-only response latencies of all caudal pontine reticular formation (PnC) neurons in 1- and 6-mo-old mice ($n = 81$ and 80 neurons, respectively). S2s were 4-kHz 90-dB SPL tone pips. Fewer PnC neurons responded with short latencies in 6 mo olds.
Sensitivity of PnC neurons

PnC neurons generally had high thresholds for excitation by auditory stimuli. Many neurons gave no response when S1 stimuli of 4, 12, and 24 kHz were presented at a level of 70 dB SPL. For 1-mo-old animals, 74% of neurons had no response to 4-kHz stimuli (the same frequency as all S2 stimuli used in this experiment). Stimuli of 12- and 24-kHz elicited no response in 5 and 40% of neurons, respectively. For 6-mo-old animals, 63% of neurons had no response to 4-kHz stimuli presented at 70 dB SPL. Stimuli of 12- and 24-kHz elicited no response in 6 and 96% of neurons, respectively.

The 12-kHz S1 elicited the greatest number of action potentials, regardless of the age of the animal. The lowest intensity of a 12-kHz stimulus that evoked action potentials in PnC neurons was determined in 13 neurons for each age group. Threshold was defined as at least one action potential evoked by 10 S2 stimuli. The mean thresholds were 63 and 57 dB SPL for 1- and 6-mo-old mice, respectively \[t(24) = 1.29, P > 0.213\]. These values are 30 ± 40 dB higher than thresholds of IC neurons in C57 mice at the respective ages (Willott 1986).

Responses of PnC neurons using the PPI paradigm

Neural PPI was defined as the number of action potentials evoked by the S2 when the S1 was present divided by the number of action potentials evoked by S2-only (S1-S2) / S2-evoked in 1-mo-old mice, \[t(159) = 2.89, P < 0.005\]. Only.

Responses to fast (1/s) or slow (0.1/s) presentation rates

All neurons responded well to the relatively slow rate of presentation (0.1/s) used in the recording protocols. However, they typically did not respond well when a more rapid rate (1/s) was employed. The majority of neurons tended to respond to the first of a train of rapidly presented startle stimuli. Subsequent stimuli elicited fewer action potentials or no responses at all. Figure 3 shows examples of both response patterns, plotting the number of action potentials for each stimulus presentation elicited by rapidly or slowly presented stimuli in two representative neurons. In both neurons, action potentials were evoked by 9 of 10 S2 stimuli presented at the slow rate. The neuron represented in the top panel responded to the first stimulus presented at the rapid rate, and subsequent responses to the stimuli were diminished (fewer action potentials were evoked). The neuron represented in the bottom panel responded to the first stimulus presented at the rapid rate and ceased to respond to subsequent S2s in the stimulus train.

The relationship between presentation rate and the number of action potentials evoked by 10 S2 stimuli in PnC neurons in which quantitative rate data were examined (1-mo-old, \[n = 13\]; 6-mo-old, \[n = 15\]) is shown in Fig. 4. The number of action potentials evoked by S2-only stimuli was measured using fast and slow stimulus presentation rates. Neither an effect of Age nor an Age × Rate interaction was present. A Main Effect of Rate was significant \[F(1.26) = 58.48, P < 0.001\]. The fast presentation rate produced fewer action potentials, regardless of the age of the animal \[t(27) = 7.77, P < 0.001\].
PPI was apparent for all S1 frequencies in both age groups, with the exception of 24 kHz in the 6-mo-old mice. The overall ANOVA indicated a significant Main Effect of Age, S1 Frequency, and an Age × S1 Frequency interaction ($P < 0.001$). Significantly poorer PPI was demonstrated by the 6-mo-old mice in comparison with the 1-mo-old mice when a 24-kHz stimulus was employed ($t(163) = 11.59$). Significantly better PPI was demonstrated by 6-mo-old mice when a 4-kHz S1 was employed. No age differences were found on this measure using a 12-kHz S1. PPI was clearly superior for both age groups when using this S1 frequency. In many neurons 12-kHz S1s produced complete inhibition of the neural response to S2 (1 mo old, 56%; 6 mo old, 58%).

To compliment the mean data shown in Fig. 7, the distribution of neurons demonstrating various percentages of PPI in 1- and 6-mo-old mice is shown in Figs. 8–10. As seen in Fig. 8, more neurons in 6-mo-old mice showed extremely good PPI (i.e., better than 10% PPI score) when a 4-kHz S1 was employed. This accounts for the superior mean PPI scores seen in Fig. 1 for 6-mo-old mice in comparison with 1-mo-old mice. An age difference in PPI is evident when 24-kHz S1s were used (Fig. 9). Good PPI is seen only in the younger mice, and 6-mo-old mice were more likely to demonstrate negligible PPI when compared with 1-mo-old mice.

By contrast, the distributions of PPI when a 12-kHz S1 is employed are very similar for both age groups (Fig. 10).

**Fig. 5.** Representative poststimulus time histograms (PSTHs) showing responses of PnC unit SC12E (1-mo-old mouse) to S1 and S2 stimuli for each frequency of S1. PSTH binwidth, 1 ms. Horizontal lines under each PSTH show the occurrence of the S1 and S2, separated by 100 ms. Open squares show the number of action potentials in each 1-ms time bin evoked by the S2-only; filled squares show the number of action potentials when S1 preceded S2 by 100 ms. For each S1 frequency, the number of responses evoked by S2 was reduced when S1 preceded S2. This unit also responded to the S1 (especially 12 kHz).

Unit SC12e (Fig. 5) shows substantial PPI when each S1 is employed. Thirty responses were initiated by the S2 stimulus alone. This was decreased to 10 responses when the 4-kHz S1 was present (PPI = 67%), 1 response when the 12-kHz S1 was present (PPI = 39%), and 9 responses when the 24-kHz S1 was present (PPI = 71%). Note that all of the S1 frequencies were capable of eliciting action potentials in this unit, although responses to the 12-kHz S1 were the most robust. The response of this neuron to S2 when a 4- or 12-kHz S1 was employed showed a latency shift when compared with the S2-only response. This was commonly seen in PnC neural responses and will be discussed in detail later.

Figure 6 shows a substantial reduction in the response of unit SC9d when S1 stimuli preceded the S2 stimuli. Twenty-seven responses were initiated by the S2 stimulus alone. This was decreased to 14 responses when the 4-kHz S1 was present (PPI = 52%), 1 response when the 12-kHz S1 was present (PPI = 4%), and 16 responses when the 24-kHz S1 was present (PPI = 59%). Note that the only S1 that was capable of eliciting a response in this unit was 12 kHz. Substantial PPI occurred even when the S1 did not evoke a response (4- and 24-kHz S1s). The response of this neuron to S2 when a 12-kHz S1 was employed showed a 5-ms latency shift when compared with the S2-only response.

**PPI as a function of age and S1 frequency**

Figure 7 summarizes the degree of neural PPI in all neurons as a function of Age group and S1 frequency. Neural PPI was apparent for all S1 frequencies in both age groups, with the exception of 24 kHz in the 6-mo-old mice. The overall ANOVA indicated a significant Main Effect of Age, S1 Frequency, and an Age × S1 Frequency interaction ($P < 0.001$). Significantly poorer PPI was demonstrated by the 6-mo-old mice in comparison with the 1-mo-old mice when a 24-kHz stimulus was employed ($t(163) = 11.59$). Significantly better PPI was demonstrated by 6-mo-old mice when a 4-kHz S1 was employed. No age differences were found on this measure using a 12-kHz S1. PPI was clearly superior for both age groups when using this S1 frequency. In many neurons 12-kHz S1s produced complete inhibition of the neural response to S2 (1 mo old, 56%; 6 mo old, 58%).

To compliment the mean data shown in Fig. 7, the distribution of neurons demonstrating various percentages of PPI in 1- and 6-mo-old mice is shown in Figs. 8–10. As seen in Fig. 8, more neurons in 6-mo-old mice showed extremely good PPI (i.e., better than 10% PPI score) when a 4-kHz S1 was employed. This accounts for the superior mean PPI scores seen in Fig. 1 for 6-mo-old mice in comparison with 1-mo-old mice. An age difference in PPI is evident when 24-kHz S1s were used (Fig. 9). Good PPI is seen only in the younger mice, and 6-mo-old mice were more likely to demonstrate negligible PPI when compared with 1-mo-old mice.
Most neurons from both age groups were totally inhibited by a 12-kHz S1 stimulus. As the example in Fig. 6 shows, neural PPI occurred whether or not action potentials were evoked by S1. The relationship between PPI and the ability of an S1 to produce action potentials was investigated in greater detail using a correlational (Pearson’s R) analysis. Correlations were determined for neural PPI using 4- and 24-kHz S1s in 1-mo-old mice and the number of action potentials evoked by the same S1. Correlations were not performed on the data obtained using 12-kHz S1s because so many units were inhibited completely (i.e., there was not enough variance in the PPI scores to allow for a meaningful correlation to be calculated). Correlations of unit responses and PPI data for 4- and 24-kHz S1s were not significant, $r_s = -0.0008$ and $-0.155$, respectively. This indicates that the ability of the PnC neurons to be excited by an S1 has little relationship to the ability of that S1 to provide neural PPI.

Further investigation of PPI using a 12-kHz S1 in PnC units demonstrating incomplete inhibition

Because 12-kHz S1s were extremely effective at reducing the neural response to S2 (due to a floor effect), other possible indicators of neural PPI were examined to elucidate age differences that are consistently demonstrated in behavioral experiments using the same stimulus parameters (Carlson and Willott 1996; Willott and Carlson 1995; Willott et al. 1994).

M i e n P S T H S. By considering only neurons that were not completely inhibited by the S1, it is possible to examine the temporal patterns of evoked discharges and see whether they were differentially affected by S1 in the two age groups. To do so, mean PSTHs were computed from neurons showing...
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FIG. 11. Mean PSTHs (responses to S2) for PnC units of 1- and 6-mo-old mice when a 12-kHz S1 was employed. Note: Only units that had nonzero PPI scores (37 of 81 units from 1 mo olds; 34 of 80 units from 6 mo olds) were included in this figure.

S2 responses for each age group, as shown in Fig. 11 (few responses occurred beyond 15 ms in any of these neurons). A sharp modal concentration of action potentials occurs at a poststimulus time of ~7 ms in 1 mo olds, but no such peak is present in the older mice. These data suggest that there is a greater degree of synchrony in S2-evoked discharges in 1 mo olds when the S1 was present. This is particularly striking, considering what was found with S2-only responses (Fig. 2) in which the latency distribution was broader (less synchronized) in 1 mo olds. In summary, these data suggest that when S1 was present, fewer synchronized discharges occurred in 6 mo olds.

LATENCY SHIFT PRODUCED BY S1. As indicated in Figs. 5 and 6, the presence of an S1 often resulted in an increased response latency of the response to a subsequent S2. This latency shift is an additional measure of the response inhibition that is provided by S1. Latency shift was examined as a function of Age group for each S1 frequency (data displayed in Fig. 12). t-Tests indicated that 4- and 12-kHz S1s produced a similar latency shift for both age groups \( t(136) = 0.1, P > 0.05 \) and \( t(69) = 0.6, P > 0.05 \), respectively. S1s of 24 kHz produced a greater latency shift in the 1-mo-old animals than in the 6-mo-old animals \( t(138) = 6.07, P < 0.0001 \).

THRESHOLDS FOR PPI. To determine whether less intense S1s might have a differential impact on the two age groups, the threshold for PPI (defined in this study as the intensity of an S1 that produced a neural PPI <75%) was assessed in 25 neurons using a 12-kHz S1. The mean thresholds for PPI were 47 dB SPL in 1-mo-old mice and 45 dB SPL in 6-mo-old mice. This difference was insignificant \( t(23) = 0.26, P > 0.05 \).

NEURAL PPI WITH DIFFERENT S1-S2 INTERVALS. No differences in neural PPI were demonstrated using a 12-kHz S1 with various S1-S2 intervals (2, 5, 10, 50, 100, 200, and 500 ms). Figure 13 shows approximately equal PPI for each age group. The only significant finding obtained from an ANOVA was a main effect of S1-S2 interval \( F(6, 204) = 58.85, P < 0.001 \). Generally speaking, 2-ms intervals provided no PPI (mean, 102.8%), 5- and 10-ms intervals provided similar degrees of better PPI (means, 75.1% and 58.2%), optimal PPI was seen with 50, 100, and 200 ms intervals (means, 5.6, 7.3, and 13.1%), and slightly poorer PPI was seen with a 500-ms interval (mean, 26.7%).

PPI in medial versus lateral penetrations

To determine whether the ability of an S1 to inhibit the response of a unit to an S2 was influenced by the anatomic location of the neuron within the PnC, the anatomic location of the units was considered. The PnC was divided up into dorsal and ventral halves and medial and lateral halves (re: 600 μm from midline). Comparisons of neural PPI revealed no differences in PPI due to dorsal or ventral location of the neurons. However, the medial/lateral location did appear to affect neural PPI. A \( 2 \times 2 \times 3 \) (Age × Location × S1 frequency) ANOVA showed a significant Location × S1 interaction.
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allow sufficient time for the completion of the startle circuit before behavioral startle is initiated, and this was found to be the case. The mean response latencies of neurons in the PnC were 7.5 ms for 1-mo-old animals and 8.5 ms for 6-mo-old animals. Parham and Willott (1988) found that mean startle latencies of 1-mo-old C57s were 9 ms, and those of 6 mo olds were 10 ms. The 1-ms startle latency difference between 1- and 6-mo-old C57s is replicated in PnC neurons, and the absolute latencies of PnC neuron responses to startle stimuli allow 1.5 ms for neural impulses to complete the startle circuit (reticulospinal projections synapse with spinal motoneurons that synapse with muscle fibers).

Stimulus intensities in excess of 70 dB SPL are required to reliably evoke ASRs in 1- and 6-mo-old C57 mice (Parham and Willott 1988; Willott et al. 1979). Therefore it would be expected that thresholds for excitation in PnC neurons would be high as well. Indeed, PnC units did exhibit high thresholds for excitation in both age groups. Many PnC neurons gave no response when stimuli of 4, 12, and 24 kHz were presented at 70 dB SPL. Although the PnC neurons were most responsive to 12-kHz stimuli, thresholds (defined as at least 1 action potential evoked by 10 stimuli) for these neurons were 63 and 57 dB SPL for 1- and 6-mo-old mice, respectively. These values are 30–40 dB higher than auditory thresholds measured from IC neurons in C57 mice of these same ages (Willott 1986). Thus thresholds of PnC neurons are much higher than those within the auditory system per se.

The behavioral ASR is sensitive to stimulus presentation rates. Willott et al. (1979) elicited ASRs in C57 mice, using a fast rate of 1/s or a slower, variable rate of 1/15–30 s. For the fast presentation rate, startle amplitude elicited by the second stimulus was ~30% of the response evoked by the first stimulus. Subsequent responses presented at the fast rate were reduced as well. Startles elicited by the stimuli presented at a slower rate showed no such amplitude reduction. Similarly, PnC neurons involved in the present study...

**DISCUSSION**

This study systematically evaluated prepulse inhibition at the neural level and compared neural responses with behavioral findings. Many characteristics of the behavioral ASR were reflected in the responses of PnC neurons, providing support for the involvement of the PnC in startle and PPI. Thus the hypotheses were generally supported by the findings of the present study.

**Responses of PnC neurons to S2 stimuli**

The latency, threshold, and sensitivity to stimulus presentation rate exhibited in PnC neurons showed close, quantitative parallels with behavioral data on the ASR in C57 mice. These findings support the hypothesis that the responses of PnC neurons would reflect the characteristics of the behavioral ASR.

The latency of the response of neurons in the PnC should...
typically did not respond well when a rapid presentation rate (1/s) was employed. The vast majority of neurons tended to respond well only to the first of a train of rapidly presented startle stimuli. Subsequent stimuli elicited fewer action potentials or no responses at all. Most S2s in a slowly presented stimulus train (1/10 s) were capable of eliciting responses.

Our findings build on the pioneering work by Wu et al. (1988), who measured startle electromyographically from the neck and simultaneously sampled the activity of single units in the pontomedullary reticular formation (including the PnC) of awake cats. High-intensity stimuli were required to elicit action potentials in many of these neurons, and there was a strong correlation between unit response magnitudes and motor response magnitudes. They determined response thresholds for 12 auditorily responsive units; 10 had very high thresholds, and these were similar to the electromyographic (EMG) startle thresholds. Lingenhoël and Friauf (1992) located neurons within the PnC that responded to intense acoustic stimuli with latencies sufficiently short for the ASR. The same researchers (Lingenhoël and Friauf 1994) recorded the intracellular activity of the largest PnC neurons and found them to have high thresholds. The present study demonstrates quantitatively a correspondence between PnC responses and ASRs when both stimulus parameters (threshold, latency, and repetition rate) and subjects' age/hearing sensitivity are varied. Taken together, the past and present findings establish a strong case for the PnC as mediator of the ASR.

Responses of PnC neurons to PPI stimuli

PnC neurons in both age groups of mice demonstrated neural PPI as hypothesized. In every unit examined in the present study, at least one of the three S1 frequencies employed produced neural PPI, in that fewer discharges were evoked by S2 when S1 was present. These findings suggest that PPI is mediated by a reduction in evoked discharges in PnC neurons that trigger the ASR.

Neural PPI data obtained using different S1-S2 intervals provide additional evidence that PnC responses have parallels with behavioral PPI. A 2-ms S1-S2 interval is too short to produce behavioral PPI (Willott and Carlson 1995). Similarly, neural PPI was not evident with a 2-ms S1-S2 interval. Behaviorally, intervals of 5–500 ms do result in PPI in C57 mice with optimal intervals of 50–100 ms (Willott and Carlson 1995). A similar interval range and optimum was observed in the neural PPI function of PnC neurons in the present study (Fig. 13).

Earlier work investigated the effects of a prepulse on a few PnC neurons and also found good evidence of inhibition. Wu et al. (1988) tested 11 units for neural PPI using auditory prepulses and observed discharge reductions, ranging from 30 to 90% of baseline responses. EMG recordings obtained by Wu et al. (1988) showed PPI using these parameters as well. A study by Lingenhoël and Friauf (1994) assessed prepulse inhibition in eight PnC neurons and found inhibition in each. The present study is the first to evaluate neural PPI parametrically in a large number of neurons, confirming the preliminary indications that PPI is robust in the PnC. [Note also that Wu et al. (1988) used awake animals; the similarity to the present results suggests that anesthesia used in our study did not fundamentally affect neural PPI].

Neural PPI and HLI plasticity

It was predicted that neural PPI would be better in 6-mo-old mice for 4- and 12-kHz S1s but worse for 24-kHz S1s, as is found in behavioral studies (Carlson and Willott 1996; Willott and Carlson 1995; Willott et al. 1994). The predictions of 4- and 24-kHz S1s were strongly supported because neural PPI provided by 24-kHz S1s was much poorer in 6-mo-old than in 1-mo-old C57 mice, but neural PPI was better for 6-mo-old mice when a 4-kHz S1 was employed.

Support for the predicted age difference for 12-kHz S1s was less clear. PPI was better for the 12-kHz S1 than 4 or 24 kHz in both age groups, as expected from behavioral studies. However, superior performance in 6-mo-old mice was not immediately apparent from the mean percent reduction of action potentials of the two age groups. Neural PPI using 12-kHz S1s was extremely good in both ages of mice, and responses of many PnC units were completely inhibited. Thus it is possible that a floor effect did not allow for the detection of age-related differences in PPI (i.e., it is difficult to demonstrate a lower percent PPI when the baseline is near 0). This may also account for the absence of an age effect and for neural PPI at longer S1-S2 intervals. Behaviorally, middle-frequency S1s are effective for S1-S2 intervals as long as 500 ms in 6-mo-old C57 mice, but PPI wanes by 200 ms in 1-mo-old mice (Willott and Carlson 1995). Whereas the neural PPI was present at 200- to 500-ms intervals in 6-mo-old mice (consistent with the behavioral data), it was equally potent in 1-mo-old animals.

Because age differences for the 12-kHz S1 may be obscured by a floor effect in the mean percent reduction in evoked discharges, additional analyses were used. Responses of neurons that did not show complete inhibition by a 70-dB 12-kHz S1 were examined separately using data other than the mean percentage of PPI. First, the presence of an S1 often resulted in an increased latency of the neural response to S2 (Fig. 12). This latency shift may be an additional measure of the response inhibition by an S1, and it was examined to see whether an Age effect was present. However, there were no significant age differences in latency shift for 12-kHz S1s (despite a trend toward longer latencies in the 6-mo-old mice). Second, thresholds for PPI (lowest intensity of S1 that produced a PPI score <75%) were compared for 1- and 6-mo-old mice using 12-kHz S1s. Thresholds were around 45–50 dB SPL for both age groups. Again, this provides no evidence for enhanced PPI in 6-mo-old mice for 12-kHz S1s. The third approach was to focus on the mean PSTHSs to evaluate the mean temporal discharge pattern in more detail. This analysis did provide evidence for better neural PPI in the 6-mo-old mice (Fig. 11). In the PnC neurons that were not completely inhibited by a 12-kHz S1, there was a sharp peak of action potentials in the averaged PSTH at ~7 ms in the 1-mo-old mice, but this was not present in 6 mo olds, where the PSTH distribution was much flatter. These data suggest that the presence of the S1 in 6 mo olds may have disrupted the synchrony of short-latency evoked discharges to a greater extent than occurred in 1 mo olds. If desynchronization of evoked action potentials results in a diminished ASR (e.g., fewer action potentials evoked at the same poststimulus time to drive motoneurons), this
might account for the improved PPI (smaller ASRs with S1 present) in the older mice.

One other variable that influenced the PPI data was the anatomical location of PnC neurons. There appears to be a crude relationship between mediolateral location within the PnC, S1 frequency, and the efficacy of neural PPI. In young mice, PPI with the 4-kHz S1 tended to be better (greater inhibition) in the lateral PnC than in the medial PnC. By contrast, the 24-kHz S1 produced more inhibition in the medial PnC. This is probably the first evidence for any type of frequency organization in the PnC (the traditional concept of tuning curves is difficult to apply to neurons with high thresholds that are not reliably excited by tones). However, this organization did seem important with respect to the manifestation of HLI plasticity for PPI using 4-kHz S1 stimuli. The age difference was quite clear in the lateral PnC but absent in the medial PnC. Perhaps the descending inputs to the PnC that are affected by HLI plasticity (for 4-kHz stimuli) project primarily to the lateral PnC, causing enhanced behavioral PPI through their influence on cells in this region.

Conclusions

Responses of PnC neurons clearly parallel the ASR with respect to latency, threshold, responses to rapidly presented stimuli, and PPI. In 6-mo-old C57 mice, the inhibition of PnC responses by 4-kHz S1s was enhanced. The very strong neural PPI using 12-kHz S1s in 1-mo-old mice may have masked HLI plasticity for 12 kHz. Nevertheless, evidence of improved PPI is present in the reduced synchrony of discharges in PSTHs, and overall, the findings of the present study support the idea that HLI plasticity influences the responses of PnC neurons. Neural PPI is enhanced for S1 frequencies that have become “over-represented” in the central auditory system of 6-mo-old C57 mice.

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