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MIDBRAIN PATHWAYS FOR PREPULSE INHIBITION AND STARTLE ACTIVATION IN RAT

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Abstract—The midbrain is essential for prepulse inhibition (PPI) of the startle reflex, but the exact neural circuits for PPI are not yet determined. Electrical stimulation of the superior colliculus (SC) or pedunculopontine tegmentum was used to characterize the neurons and pathways that mediate PPI and the activation of startle that also occurs at higher currents in the same sites. Startle was inhibited by prepulses in most, but not all SC sites, with the lowest intensity sites in intermediate layers of SC. PPI latencies in SC sites were 4–6 ms longer than in inferior colliculus, intercollicular nucleus or pedunculopontine sites. Contrary to previous serial models, there must be two parallel midbrain pathways for PPI, a faster auditory pathway from inferior colliculus to pedunculopontine tegmentum, and a slower multimodal SC output for PPI. Double-pulse stimulation of SC sites shows that PPI results from direct stimulation of neurons with moderate refractory periods (0.4–1.0 ms), similar to SC neurons that mediate contraversive turning responses. By contrast, startle activation occurring at higher currents in all SC sites (even sites where PPI could not be elicited) results from stimulation of very short refractory period neurons (0.3–0.5 ms) and very long refractory period neurons (1.0–2.0 ms), with startle inhibition often found from 0.5–1.0 ms. Startle activation appears to result from stimulation of short refractory period neurons in deep SC layers that mediate fear-potentiated startle, plus long refractory period substrates in more dorsal SC sites. **© 2006 Published by Elsevier Ltd on behalf of IBRO.**

Key words: pedunculopontine, colliculus, tectospinal, tectopontine, refractory period, approach/avoidance.

The functional organization of the vertebrate superior colliculus (SC) in sensory-motor processing has been well understood for many years (e.g. Hess, 1946; Ingle, 1983; Stein, 1984; Sparks, 1986; Dean et al., 1989; Redgrave et al., 1993). Activation of neurons concentrated in intermediate SC layers, or stimulation of the crossed tectoreticulospinal axons arising from these layers, results in contraversive eye and head turns in rats and cats (Stein, 1984; Tehovnik and Yeomans, 1986; Redgrave et al., 1993). These contraversive turning responses orient the animal toward small, moderate-intensity stimuli from the contralateral sensory field in line with retinotopic, somatotopic and auditory maps in the SC (Stein, 1984; Sparks, 1986).

Activation of neurons concentrated in deep SC layers, or stimulation of the uncrossed tectopontine axons arising from these layers, results in ipsiversive eye and head turns that orient rats away from large, high-intensity or threatening stimuli (Yeomans and Buckenham, 1992; Redgrave et al., 1993). Stimulation of the rostromedial SC, corresponding to the dorsal sensory field in rats, leads most often to avoidance turns, while stimulation of the caudolateral SC leads to approach turns (Sahibzada et al., 1986; Dean et al., 1989).

The startle reflex is strongly inhibited when moderate intensity prepulses (either acoustic, visual or tactile) are delivered 20–800 ms before the acoustic or tactile startling stimulus (Hoffman and Ison, 1980). The SC is important for acoustically elicited prepulse inhibition (PPI), since lesions of the SC reduced PPI resulting from an acoustic prepulse and an acoustic startling pulse (Fendt et al., 1994, 2001). Lesions of the inferior colliculus (IC), however, completely blocked acoustic PPI (Leitner and Cohen, 1985), and lesions of the pedunculopontine tegmental nucleus (PPT) were also more effective than SC lesions (Koch et al., 1993; Swerdlow and Geyer, 1993). Furthermore, electrical stimulation of the IC, SC or PPT can be used as a prepulse to inhibit startle elicited by trigeminal stimulation (Li and Yeomans, 2000; Li et al., 1998). Based on lesion and stimulation data, a serial circuit model for acoustic PPI was proposed, in which the IC activates the SC, which activates the PPT, which inhibits the pontine reticular formation (Fendt et al., 1994, 2001). This model further proposed that visual and tactile prepulses are mediated via the SC rather than the IC.

The serial model of PPI has two problems, however. First, complete lesions of SC reduced acoustic PPI by only 45% (Fendt et al., 1994). Second, a preliminary test of PPI latencies using brain stimulation was inconsistent with the serial model that placed IC before SC (Li and Yeomans, 2000). We used the same stimulation methods in this study to estimate PPI latencies and conduction times more accurately in many SC and PPT sites, and, based on the latency analysis, we propose a new circuit model for PPI.

Furthermore, startle responses can be activated by intense electrical stimulation of the SC (Li and Yeomans, 2000; Lin et al., 2002). Startle responses elicited from the midbrain result from activation of uncrossed, fast axons originating in the amygdala (Hitchcock and Davis, 1986; Rosen and Davis, 1988; Yeomans and Pollard, 1993;

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Abbreviations: AP, anteroposterior; C-T interval, the time between the two tectal stimulating pulses; DV, dorsoventral; IC, inferior colliculus; ICN, intercollicular nucleus; ISI, interstimulus interval, i.e. the time between the first tectal pulse and the trigeminal stimulating pulse; ML, mediolateral; PPI, prepulse inhibition; PPT, pedunculopontine tegmental nucleus; SC, superior colliculus.

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Frankland and Yeomans, 1995). Ibotenate lesions or GABA agonist infusions at this midbrain level block fear-potentiated startle (Frankland and Yeomans, 1995; Meloni and Davis, 1999). Fear-potentiated startle was blocked more precisely with localized AMPA blockers in the deep layers of the SC (Zhao and Davis, 2004). Therefore, startle potentiation appears to be critically mediated in the deep layers of SC.

Here, we studied startle inhibition and activation using electrical stimulation of the SC and underlying structures. First, we mapped low-threshold sites for PPI in SC, and related these to retinotopic maps for approach and avoidance responses. Second, to test the serial circuit model, we varied the interval between prepulses and startling pulses to estimate the latency of PPI from different midbrain sites. Third, to distinguish the neurons that mediate PPI and startle activation, we used double pulses to estimate the refractory periods of the neural substrates in SC (Yeomans, 1990, 1995). We found a narrow range of refractory periods for PPI, and two separate ranges of refractory periods for startle activation. The refractory periods for PPI were then related to the neural systems mediating contraversive turns in SC (Sahibzada et al., 1986; Yeomans and Tehovnik, 1988), and refractory periods for startle activation were related to those for ipsiversive turns and fear-potentiated startle (Yeomans and Buckenham 1992; Yeomans and Pollard, 1993).

EXPERIMENTAL PROCEDURES

Subjects

Fourteen male adult Wistar rats (*Rattus norvegicus*) from Charles River Canada (Dorval, QC, Canada) were tested. All rats were housed at 21±1 °C in individual cages on a 12-h light/dark cycle with lights on at 7 a.m. and food and water available *ad libitum*. All procedures were approved by the University of Toronto Animal Care Committee, following the guidelines of the Canadian Council on Animal Care. All efforts were made to minimize the number of animals used and their suffering.

Surgery

Stimulating electrodes were implanted under anesthesia induced by sodium pentobarbital (60 mg/kg, i.p.) following pretreatment with atropine sulfate (0.4 mg/kg, i.p.). A state of areflexia was maintained throughout surgery by supplemental injections of sodium pentobarbital (10–20% of initial dosage). Buprenorphine was used as a postsurgical analgesic.

Stimulation electrodes were constructed with stainless steel 00 insect pins (BioQuip Products, Gardena, CA, USA), insulated with Epoxylite (St. Louis, MO, USA) to within 100 μm of their exposed tips, which had a diameter of 100 μm. Each electrode was connected to a male Amphenol pin connector (Electrosonics, Toronto, ON, Canada), which was inserted into a socket (Molino and McIntyre, 1972) that was mounted on the skull with both anchoring screws and dental cement. Current return was provided by a ground electrode attached to the skull screws.

For 12 rats, bilateral electrodes were implanted in principal nucleus of the trigeminal nerve, SC and PPT sites. In addition, two animals had bilateral electrodes implanted only in the caudal SC adjacent to the intercollicular nucleus (ICN) which were used to test only the refractory periods for startle in these sites. Electrodes were stereotaxically aimed at the following four brain structures, based on the coordinates provided by the Paxinos and Watson (1998) atlas: (i) trigeminal nucleus: anteroposterior (AP) −3.4 mm, mediolateral (ML) ±2.9 mm, dorsoventral (DV) −10.3 mm relative to lambda, with the electrodes tilted backward 11° relative to the coronal plane; (ii) ICN: AP −7.8 mm, ML±1.7 mm, DV −4.8 mm relative to bregma; (iii) SC: AP +2.5 mm, ML ±1.5 mm, DV −5.2 mm relative to lambda; (iv) PPT: AP +1.2 mm, ML ±6.0 mm, DV −5.9 mm relative to lambda, with the electrodes tilted 30° relative to the sagittal plane. Rats were allowed 1 week to recover from surgery.

Apparatus

A metal stabilimeter cage (15×8×8 cm) was placed inside a sound-attenuated chamber (46×41×41 cm) with a Plexiglas window (Cassella and Davis, 1986). A white-noise generator produced a constant background masking noise of 65 dB inside the chamber. Under the cage, a force transducer sensitive to rat startle-like movements produced an electrical signal that was amplified by an Endevco (San Juan Capistrano, CA, USA) signal conditioner, and visualized on an Hitachi (Scarborough, ON, Canada) digital storage oscilloscope (Model 6025A) from which peak startle responses could be read (Li and Yeomans, 1999). Electrical stimuli were delivered by a Grass Instruments (Quincy, MA, USA) model S-88F stimulator connected to the rat by two photoelectric constant-current stimulus isolation units (Grass model PSIU6).

Procedure

Startle reflex testing took place during the light part of the light/dark cycle of the rats, and in a brightly lit room. Prior to testing, each rat was allowed to habituate to the cage and chamber for at least 20 min. A test pulse (0.2 ms duration) was delivered via one trigeminal electrode every 30 s at increasing currents (20–400 μA), until a startle-like response of 1 V or above was reliably elicited on several trials (see Li and Yeomans, 1999). This intensity was then held constant during further habituation, in which moderately startling pulses were delivered every 30 s.

Next, startle thresholds for midbrain sites were tested similarly. Twin pulses, 0.2 ms in duration, were delivered with a conditioning-test (the time between the two tectal stimulating pulses, C-T) interval of 1.75 ms and delivered to the caudal or rostral SC, or the PPT using the method of Li and Yeomans (2000). The current for each electrode was increased (from 100 to 800 μA) until a weak startle response (0.1–0.4 V) was reliably observed. Prepulse current level was then set 10–60 μA below this “threshold” level.

Experiment 1: PPI timing curves

For PPI timing experiments, three pulses of 0.2 ms duration were delivered, two pulses to midbrain sites at a C-T interval of 1.75 ms as above, followed by a single pulse delivered to trigeminal nucleus sites as above. In this experiment, the interstimulus interval (ISI) between the first midbrain pulse and the trigeminal pulse was varied. Before formal testing and after habituation, further small adjustments of the prepulse current were made at an ISI of 20 ms to determine the prepulse current that reduced startle response maximally. This current was then held constant throughout further testing.

In formal PPI timing tests, the ISI was varied pseudo-randomly between trials. For caudal SC placements, ISIs of 0, 2.5, 5, 7.5, 10 and 20 ms were used. For middle SC placements, ISIs of 0, 2, 4, 6, 8, 10, 12, 15, 20 and 30 ms were used due to the longer latencies observed. Four to six trials were taken for each ISI, and six to 10 trials were taken for baseline startle responses elicited by trigeminal stimulation.

Experiment 2: refractory periods for PPI in SC sites

The aim of this experiment was to test whether neurons mediating PPI in SC are similar in refractory periods to neurons previously found to mediate contraversive turning responses (Tehovnik and Yeomans, 1986). For PPI refractory period experiments, two prepulses were delivered to each SC site, and the C-T interval between the two prepulses was varied from 0.2–2.0 ms, while the ISI was held constant at 20 ms. We measured thresholds in the same manner described above, but the duration of the SC pulses was set at 0.1 ms in all tests to improve temporal resolution of refractory periods. For PPI refractory periods, however, a criterion of 1.5–3.0 V instead of 1.0–1.5 V was used for trigeminally elicited baseline responses, in order to increase the size of the PPI effect. Prepulse currents were then adjusted to maximize the difference in inhibition induced by a single prepulse as compared with twin pulses at the 1.75 ms C-T interval.

In the formal experiments, the C-T interval was varied pseudo-randomly at intervals of 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.3 and 1.75 ms. Inhibition induced by single pulses (no T pulse) was used as a baseline for comparison with double-pulse induced inhibition. Startle response were measured on six trials at each C-T interval, and six to 10 trials for single pulses.

Experiment 3: refractory periods for startle elicitation in midbrain sites

For startle refractory period experiments, current was adjusted so that twin pulses spaced 2.0 ms apart would elicit a large startle response (5.0 V or above), but single pulses would only elicit a negligible startle response (0.1–0.4 V). As in PPI refractory period experiments, the duration of each pulse was held at 0.1 ms.

In the formal experiments, the C-T interval was varied pseudo-randomly using intervals of 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.5 or 2.0 ms. Startle responses were measured in six trials at each C-T interval and six to 10 trials for single pulses.

Histology

At the end of behavioral testing, rats were deeply anesthetized with an overdose of sodium pentobarbital. Microlesions were made via the stimulation electrodes by an anodal DC current of 500 μA with a duration of 10 s. Rats were then perfused intracardially with physiological saline followed by 10% formalin. The skulls were dissected away and the brains removed. Brains were stored in 10% formalin with 30% sucrose until they sank and then were sectioned coronally into 40 μm sections in a cryostat at −20 °C. Sections were mounted directly onto slides and stained with Cresyl Violet for histological verification of electrode sites.

RESULTS

Histology

Hindbrain electrode sites where startle responses were elicited were located in the ventrolateral medulla near the principal trigeminal nucleus, spinal trigeminal nucleus, pars oralis, or the facial tract, as reported previously (Li and Yeomans, 1999; Scott et al., 1999). To reach the criterion startle response of 1.5 V, currents from 40 to 220 μA (one 0.2 ms pulse) were needed, with the lowest thresholds in the most ventral sites in the trigeminal nucleus (data not shown).

Electrode placements for 26 tectal and six PPT sites are shown in Fig. 1 on coronal sections from the Paxinos and Watson (2005) atlas. Startle was inhibited by prepulses in SC sites at currents from 60 to 250 μA, and in PPT sites at currents from 32 to 60 μA in experiment 1. In experiment 2, SC currents ranged from 60 μA (site 2L) to 300 μA (site 7R), indicating low-threshold sites for PPI. PPI could not be obtained in many other sites shown in Fig. 1. Many of these negative sites were found in the rostralmost 0.8 mm of the SC, where no positive sites were found, and near the lateral and caudal edges of the SC. The lowest threshold SC sites were all found in the intermediate layers.

Effective PPI sites in rostral and mid-SC were concentrated in a cluster between 6.2–7.5 mm caudal to bregma, and 0.8–2.2 mm lateral to the midline (Fig. 1). The lowest threshold sites for PPI were located in the middle of this cluster, in retinotopic areas of SC receiving input from visual fields ventrolateral to or near the optic disc (Siminoff et al., 1966). Sites where PPI was not obtained were found in most cases near the rostral and caudal edges of the SC, corresponding to dorsal or peripheral visual fields.

In three caudal SC sites, however, low threshold PPI sites (100–120 μA) were found near the ICN (7.3–8.3 mm behind bregma) as previously reported by Silva et al. (2005). In deeper sites near the PPT (7.8 mm behind bregma) PPI thresholds were by far the lowest (30 μA for 17R and 52 μA for 17L). In experiment 1 below, the currents required for PPI in three ICN sites and two PPT sites ranged from 30 to 120 μA, with the low currents indicating localization of the substrates for PPI near the electrode tips.

Full startle-like responses (amplified responses greater than 1 V) could be elicited at currents between 500 and 1000 μA in all sites, even those where PPI could not be elicited at 250 μA.

Experiment 1: PPI timing curves

To study the timing of PPI onset, the interval between tectal stimulating pulses was held constant, and the ISI between the first tectal pulse and the trigeminal startling pulse was varied from 0 to 30 ms. Results for six low-threshold mid-SC sites (located between 6.2 and 7.0 mm caudal to bregma) are shown in Fig. 2A. The 100% baseline level is the mean startle response elicited by trigeminal stimulation alone. At ISIs from 0 to 6 ms, the effects of tectal and trigeminal stimulation summed to produce a larger startle response (95–168% of baseline, mean 130% across all intervals). At ISIs from 10 to 30 ms, startle was strongly inhibited by tectal stimulation, with PPI increasing gradually as ISIs increased from 10 to 20 ms.

To describe the PPI latency for each curve with a single statistic, the maximum PPI was determined (at an ISI of 20 or 30 ms, whichever produced maximum inhibition) and the half-maximum PPI was estimated by the startle level halfway between the 100% baseline level and the maximum inhibition. These half-maximum inhibition levels were near 60% for most curves.

The ISI at which half-maximum PPI was achieved in mid-SC sites ranged from 11.2–17.5 ms, with a mean of 13.4±2.2 ms across all mid-SC sites. By contrast, in five caudal sites (three sites near the ICN and two deeper sites near the PPT), PPI latency was between 7.8 and 10.0 ms

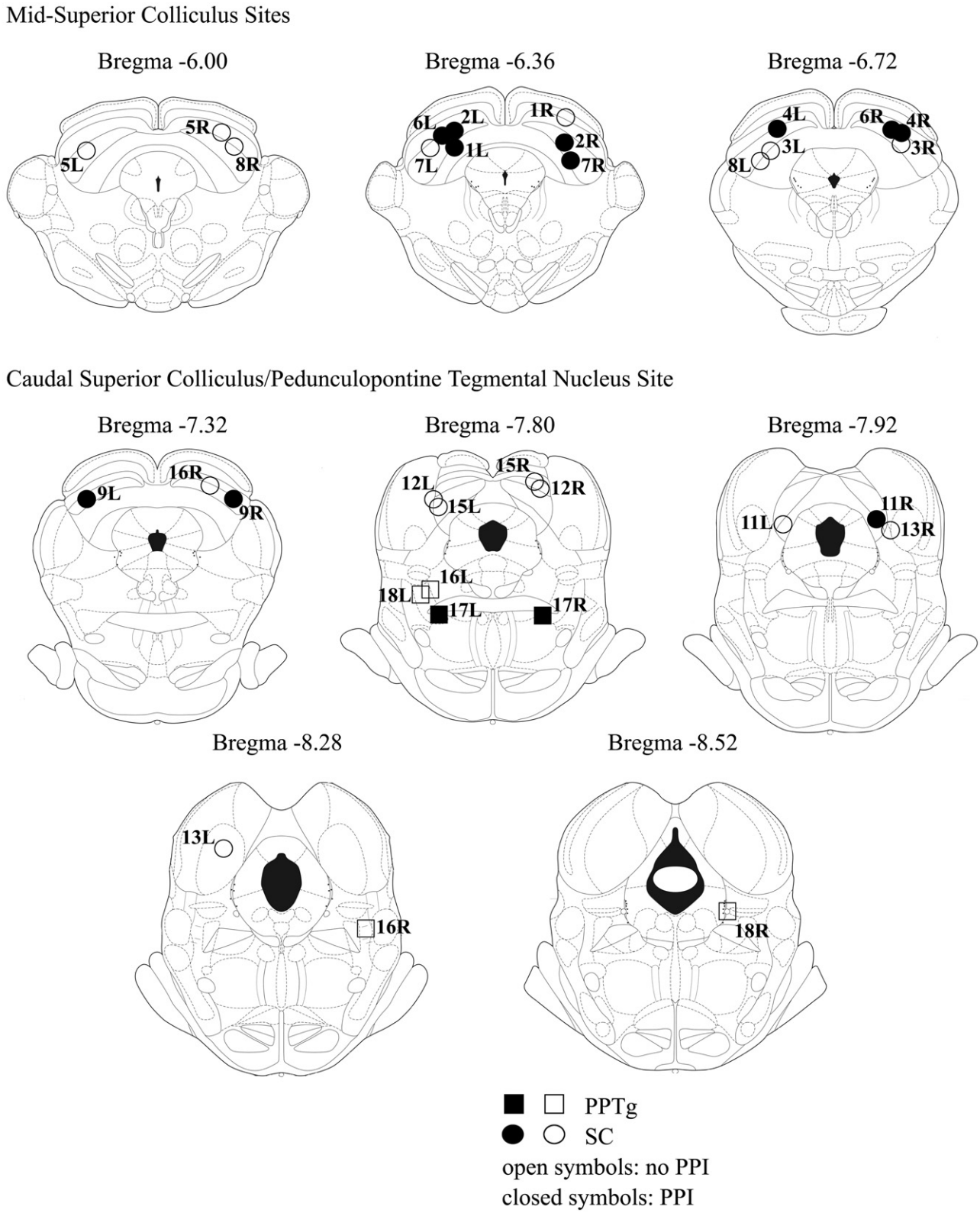

Fig. 1. Histology sections from Paxinos and Watson (2005) atlas, showing SC and PPT sites. Sites where PPI could not be obtained are shown with open circles or squares. SC sites where PPI was obtained are shown with solid circles. PPT sites where PPI was obtained are shown with solid squares.

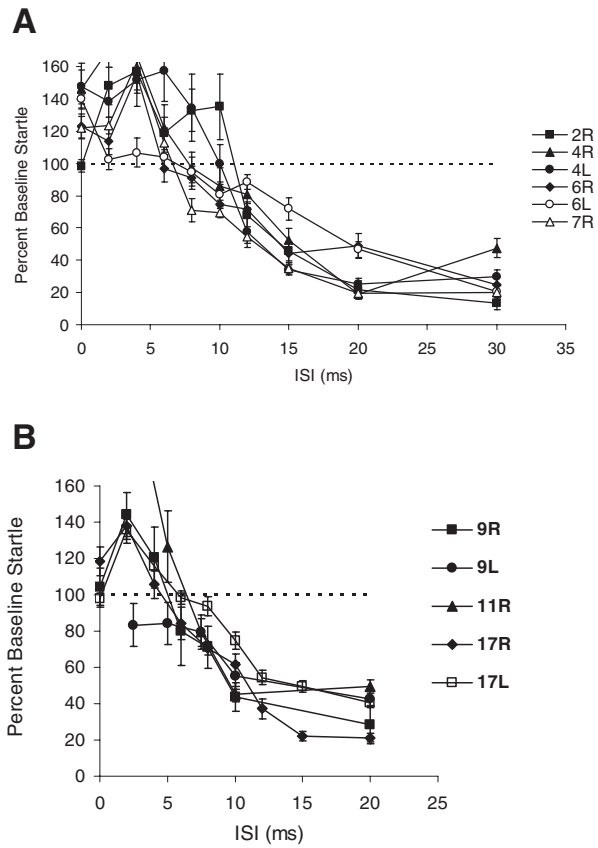

Fig. 2. PPI latencies. Startle responses evoked by trigeminal stimulation without prepulses were used as the baseline. Percent baseline startle was calculated as follows: %Baseline Startle=[startle response with prepulse]/[mean baseline startle response]×100%. ISI is the interval between the onset of the first prepulse (delivered in the midbrain) and the onset of the startling pulse (delivered in the medulla). Prepulses were delivered to rostral SC sites in panel A, or caudal SC, ICN, or PPT sites in panel B. Latency was estimated by finding the time corresponding to half-maximum PPI (see results). Error bars in all figures represent ±S.E.M.

in all sites (mean 8.9±1.2 ms) (Fig. 2B). Therefore, PPI latencies for caudal sites were always earlier than for mid-SC sites, with a mean difference of 4.5 ms.

Experiment 2: refractory periods for PPI in SC sites

To estimate the refractory periods of the neural substrates mediating PPI, the interval between the two SC prepulses was varied from 0.3–1.75 ms. SC pulse durations were shortened to 0.1 ms to improve temporal resolution of refractory periods. The 100% baseline in Fig. 3 is the startle response elicited by a single SC prepulse and a single trigeminal startling pulse.

The effect of adding the second SC prepulse (the T pulse) was to inhibit the startle response further. For all five sites, at C-T intervals of 0.3 and 0.4 ms startle responses were similar to the baseline level. The inhibitory effect of the T pulse increased strongly as the C-T interval increased from 0.4–0.6 ms, and then increased more gradually as the C-T interval increased from 0.6–1.0 ms. As C-T intervals increased from 1.0–1.75 ms, no further inhibition was obtained. These results indicate a range of refractory periods from 0.4–1.0 ms for directly stimulated SC neurons mediating PPI.

Experiment 3: refractory periods for startle elicitation in midbrain sites

When the currents were increased to 500 μA in SC and ICN sites (two identical stimulating pulses at a C-T interval of 1.75 ms, no trigeminal stimulation), startle responses were reliably elicited at short latencies. To measure the refractory periods of the neural substrates responsible for the startle reflex, the current of both SC pulses was held constant so that one stimulating pulse elicited a weak startle response (0.1–0.4 V). The C-T interval was varied from 0.2–2.0 ms.

In all rostral (Fig. 4A) and caudal (Fig. 4B) SC sites, the startle responses increased sharply as the C-T interval increased from 0.3–0.4 ms. In many sites there was also an increase as the C-T interval increased from 0.4–0.5 ms. In most sites, there was a decline in startle or no change as the C-T interval increased from 0.5–1.0 ms. The decline in startle was clearest for two rostral SC sites (9L and 9R from Fig. 4A), three caudal SC and ICN sites (12L and two 15R in Fig. 4B), and least for the PPT site (17R) and one ICN site (11L) in Fig. 4B. As C-T intervals increased from 1.0–2.0 ms, another increase in startle (10–50% in Fig. 4) was observed for most sites, but was not seen in the PPT site (17R).

In conclusion, three ranges were seen in these startle refractory period curves: 1) a sharp rise from 0.3–0.4 or 0.5 ms; 2) no rise or a small decline from 0.5–1.0 ms, followed by 3) another rise from 1.0–2.0 ms in tectal but not tegmental sites.

DISCUSSION

Identifying tectal substrates for startle has been difficult because opposing responses of startle activation and inhibition can often be evoked in the same sites as a function of current, and lesions can inhibit both fear-potentiated

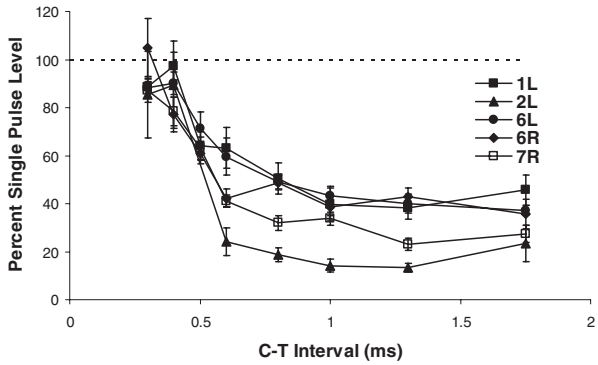

Fig. 3. Refractory periods for PPI in rostral SC sites. The C-T interval between two prepulses delivered to rostral SC sites was varied. A trigeminal startling pulse was delivered at a 20 ms ISI after the first prepulse. Percent single pulse level was calculated as follows: [Double-pulse response at each C-T interval]/[Single pulse response at 20 ms] ×100%.

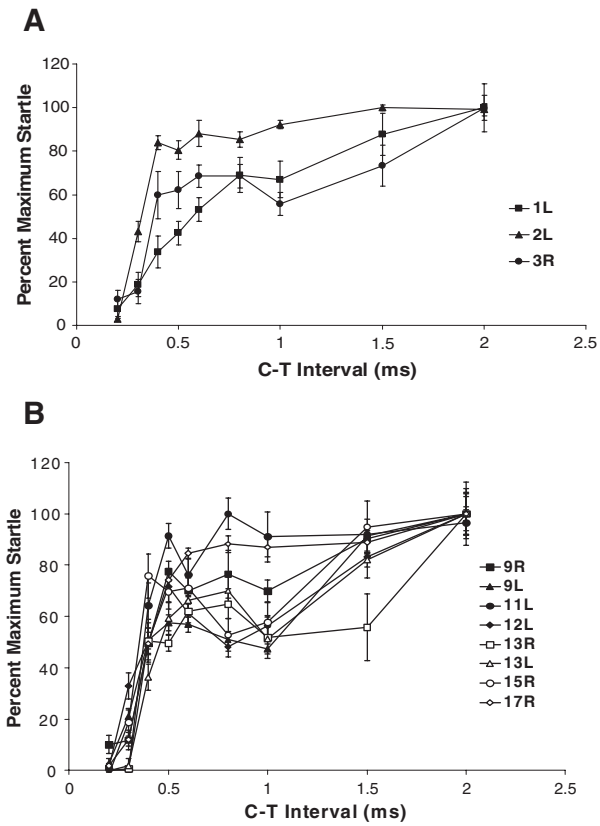

Fig. 4. Refractory periods for startle elicitation in rostral SC sites (A) and caudal SC, ICN or PPT sites (B). Startle response was normalized according to the following calculation: Percent Maximum Startle Response=[Double-pulse response at each C-T interval−Single-pulse response]/[Maximum Double-pulse response−Single-pulse response]×100%. This statistic varies from 0 (the single-pulse response level) to 100% (the maximum double-pulse response level at a C-T interval of 2.0 ms) to show the added effect of the T pulse at each C-T interval.

startle and PPI (Li et al., 1998; Fendt et al., 1994, 2001; Silva et al., 2005; Heldt and Falls, 2003). Here we have separated these opposing substrates in three ways. First, we mapped the effects of different currents in different SC layers to sort out the anatomy of SC substrates for electrically elicited startle, as has previously been accomplished for approach and avoidance turns in rats (Sahibzada et al., 1986; Yeomans and Tehovnik, 1988). Second, we used PPI latency analysis to differentiate PPI substrates in rostral and mid-SC from those in caudal SC, IC and PPT. Third, we used double-pulse stimulation of SC to show different refractory periods for neurons mediating PPI than for startle activation.

Stimulation of many mid-SC sites in the intermediate layers inhibited startle at currents below 250 μA. The lowest threshold SC sites for PPI were near or ventrolateral to the retinotopic location of the optic disc in rats (Siminoff et al., 1966). Previous studies of these SC regions in rats showed that approach responses rather than avoidance responses were activated at similarly low currents (Sahibzada et al., 1986). This suggests that startle is inhibited best by low-current stimuli that activate approach turns, rather than high-current, large-field stimuli ("looming" or threatening stimuli) that activate avoidance turns (Ingle, 1983; Dean et al., 1989). Startle activation occurred only at high currents above 500 μA, in all SC sites, consistent with activation of large sensory fields, in all SC layers, simulating large, threatening stimuli.

Second, the latencies of PPI for the low-threshold SC sites mediating startle inhibition were long (13.4 ms). These PPI latencies were much longer than for caudal SC, ICN or PPT sites (8.9 ms) or for IC sites (9.5 ms) previously studied by Li et al. (1998). These results show that PPI elicited from IC sites inhibits startle more quickly than PPI from mid-SC sites. This shows that the serial circuit model of PPI cannot be correct, and that SC must provide a slower input for PPI, independent of the faster auditory pathway for PPI from IC.

Finally, refractory period tests showed that at least three different neural populations near SC alter startle responses. Neurons mediating PPI at low currents in the intermediate layers of SC had a narrow range of moderate refractory periods (0.4–1.0 ms). Neurons mediating startle at high currents had very short refractory periods (0.3–0.5 ms) in all SC sites. Very long refractory period neurons (1.0–2.0 ms) also activated startle in many SC sites at currents above 500 μA.

Experiment 1: PPI onset latencies

We used the same method as Li and Yeomans (2000) and Li et al. (1998) here, to allow direct comparisons between PPI latencies in the 3 studies. Single-pulse stimulation of trigeminal sites was used to elicit startle with maximal temporal precision, presumably a single volley of action potentials, more precise than an acoustic startling stimulus that reverberates and can elicit several action potentials.

Summation between prepulses and startling pulses (indicated by startle responses greater than 100%) was observed in most sites at ISIs from 0 to 6 ms, with mean summation of 130%. These summation effects were not included in the analysis of PPI, because the neural circuits between SC and trigeminal sites that might mediate this summation are not known.

PPI increased sharply as ISIs increased from 5 to 20 ms in all caudal sites, and 10–20 ms in all rostral sites. PPI latencies (measured by half-maximal PPI) were much shorter in caudal SC, ICN and PPT sites here (mean 8.9 ms), and in IC sites previously (9.5 ms) (Li et al., 1998), than in mid-SC sites (13.4 ms). The 8.9 ms required to conduct PPI from PPTg to PnC is consistent with the slow conduction velocity of unmyelinated mesopontine cholinergic axons believed to inhibit PnC giant neurons (Fendt and Koch, 1999). The low currents needed in PPT and caudal SC sites support the idea that short-latency neural substrates for PPI pass through the IC, ICN and PPT (Silva et al., 2005). The similar latencies for PPI in these many sites, suggest a close functional relationship between these sites, with a short conduction time (0.6 ms from IC to PPT) between sites. Whether the same uncrossed axons mediate PPI from IC, ICN and caudal SC sites, or whether

a synapse for PPI is located in ICN or PPT is not tested by this experiment, however.

This suggests a faster auditory pathway mediates PPI from IC to PPT, and a 4–5 ms slower pathway mediates multisensory PPI from mid-SC sites. Consistent with this idea, acoustic stimuli activate PPT neurons in rats at very short latencies (13 ms), long before visual stimuli activate PPT (30–50 ms) (Reese et al., 1995; Garcia-Rill et al., 1996; Pan and Hyland, 2005). Based on this, we propose a new model of PPI (Fig. 5) in which the fast IC auditory pathway relays prepulses quickly to PPT, while the slower multisensory SC pathway takes 4–5 ms more to reach the PPT.

This new model also accounts for the weak effects of SC lesions on acoustic PPI (Fendt et al., 1994). Since most acoustic input for PPI relays quickly from IC to PPT via the fast auditory pathway, SC lesions only block the smaller proportion of auditory information that relays to SC and then via the slower SC pathway to PPT. We predict (following Fendt et al., 2001) that SC lesions will block PPI mediated by visual prepulses, while IC lesions will have no effect.

The powerful inhibiting effect of excitotoxic lesions in PPT on acoustic PPI of startle suggests that glutamatergic synapses near PPT cholinergic neurons are important for the fast auditory PPI pathway (Koch et al., 1993; Fendt et al., 2001; Fendt and Koch, 1999; Swerdlow and Geyer, 1993). Whether this PPI effect is mediated by a crossed pathway (as for turning) or by bilateral pathways (to inhibit both sides of the bilateral startle reflex) cannot be determined by the present data. Also, given the 4.5 ms latency difference, the number of synapses in the PPI circuit between SC and PPT cannot be determined. Since the refractory periods for PPI have been measured here, circuits mediating PPI can now be tested by collision methods (Yeomans 1990, 1995).

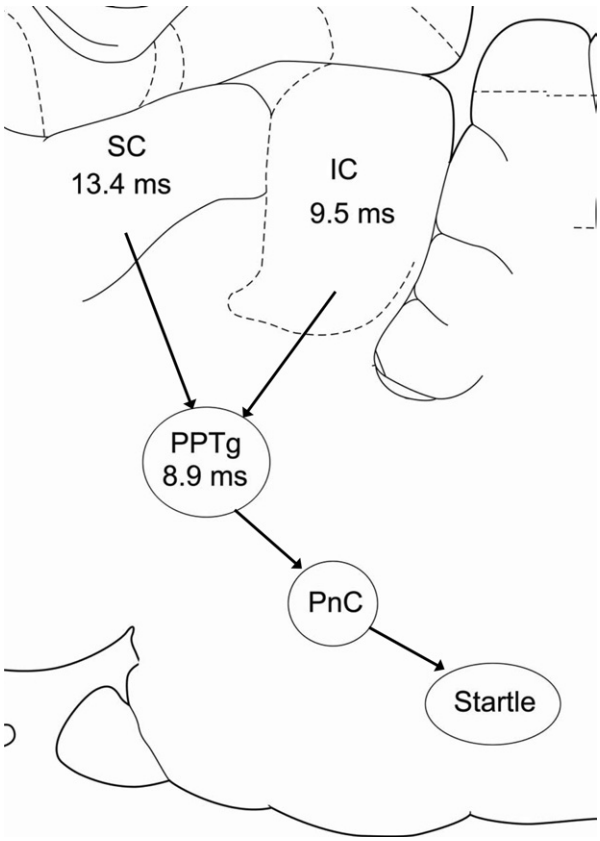

Fig. 5. New model of PPI. The IC to PPT pathway mediates fast acoustic inputs for PPI, while the SC to PPT pathway mediates a slower pathway that integrates visual, acoustic and tactile inputs for PPI. Modified from Paxinos and Watson (1998).

Experiment 2: refractory periods for PPI in middle SC sites

These studies are the first to estimate refractory periods for startle inhibition. Inhibitory refractory periods have been estimated previously in other systems (Dennis et al., 1976; Skelton and Shizgal, 1980). The inhibitory effect of SC stimulation on trigeminally elicited startle increased as C-T intervals increased from 0.4–1.0 ms. These results indicate that refractory periods for SC neurons mediating PPI are concentrated in the moderate range.

Previously, electrical stimulation of SC intermediate layers elicited contraversive turning responses at C-T intervals from 0.4–2.0 ms, with 65% of the effect occurring between 0.4–1.0 ms (Tehovnik and Yeomans, 1986). In addition, refractory periods of 11 crossed tectoreticulospinal axons were found to range from 0.4–1.8 ms with 73% in the 0.4–1.0 ms range. These refractory periods were measured by extracellular recording of intermediate layer SC neurons following antidromic stimulation of axons in the contralateral tegmentum (Tehovnik and Yeomans, 1986). Therefore, crossed tectoreticulospinal systems mediating approach turns in rats have similar locations in SC and similar neural refractory periods to those mediating PPI in intermediate layer SC sites.

The low currents used to elicit PPI in middle SC sites (60–300 μA, experiments 1 and 2) provide an estimate of the field of stimulation. Based on Tehovnik and Yeomans' (1986) estimate of the current–distance relationship for tectoreticulospinal axons mediating turning (which have similar excitability properties to those for PPI), the maximum radius of stimulation is 0.75 mm at 300 μA and 0.15 mm at 60 μA (for site 2L, located in the middle of the intermediate layers of SC). This indicates that in the lowest SC threshold sites, the neural substrates for PPI were localized only to the intermediate layers of SC.

We previously proposed that PPI functions to inhibit startle responses during the execution of approach responses activated by SC and PPT neurons (Fendt et al., 2001). For example, foveation of a visual stimulus following SC activation would be disrupted by the eye closure that occurs during startle responses. Stimulation of SC intermediate layers activates responses that turn the animal toward novel, moderate intensity stimuli in the contralateral field via activation of the crossed tectoreticulospinal pathway (Dean et al., 1986; Yeomans and Tehovnik, 1988; Redgrave et al., 1993). The present data suggest that tectal neurons activating approach turns resemble those that mediate PPI. Accordingly, PPI is a useful con-

sequence of SC activation, in that PPI protects processing of sensory stimuli from the disruptive effects of startle.

Experiment 3: refractory periods for startle in SC sites

At currents from 500 to 1000 μA, startle responses were activated in all SC sites. This high-current stimulation is sufficient to activate low-threshold neurons in all SC layers.

When double-pulse stimulation was used, the refractory periods of neurons activating startle were concentrated in two C-T interval ranges, 0.3–0.5 and 1.0–2.0 ms. The rapid rise in startle observed as C-T interval increased from 0.3–0.4 ms was clearly shorter than the refractory periods for PPI, indicating that different neurons in SC mediate startle activation and PPI.

These refractory periods are consistent with the 0.3–0.5 ms refractory period neurons mediating startle elicited by midbrain stimulation (Frankland and Yeomans, 1995; Yeomans and Pollard, 1993; Lin et al., 2002). Zhao and Davis (2004) localized these cells to the deep layers of the SC lateral to periaqueductal gray and dorsal to the deep mesencephalic gray. These neurons have axonal conduction velocities greater than 50 m/s from midbrain to medulla, and mediate fear-potentiated startle via the amygdalofugal pathway to the midbrain (Yeomans and Pollard, 1993; Frankland and Yeomans, 1995). In contrast to the crossed SC axons mediating approach turns (Ingle, 1983; Dean et al., 1986, 1989; Yeomans and Buckenham, 1992), the axons mediating startle potentiation in SC are uncrossed and relay directly from the amygdala to the SC, and then from the SC to the medulla (Hitchcock and Davis, 1986; Yeomans and Pollard, 1993; Frankland and Yeomans, 1995; Lin et al., 2002).

Refractory periods of 1–2 ms were not found for tegmental stimulation (Yeomans and Pollard, 1993; Frankland and Yeomans, 1995) or here for the one PPT site tested. This suggests that the 1–2 ms refractory period effects are due to neurons located more dorsally within the SC. Yeomans and Buckenham (1992) studied ipsiversive, avoidance turns from SC sites following midline knife cuts to the crossed tectoreticulospinal path. They found a wide range of refractory periods (0.45–3 ms), including a population of long refractory period axons (1–3 ms) that conduct from the SC to the ipsilateral pons. The present data, therefore, suggest a possible link between avoidance turns and the long refractory period SC neurons activating startle.

At intermediate refractory periods (0.5–1 ms), startle was unchanged or weakly inhibited in most sites (Fig. 4). We attribute this inhibition of startle to stimulation of the PPI-mediating neurons of the intermediate layers. This effect was not seen by Yeomans and Buckenham (1992) when the crossed tectoreticulospinal axons were cut. The inhibitory PPI effects from 0.4–1 ms in experiment 2 were partially masked in experiment 3 by the startle activating effects from 0.3–0.5 ms, however. Inhibitory effects at 0.5–1 ms were also evident in caudal SC and ICN sites, suggesting that similar medium-refractory-period substrates for PPI pass through these regions on their way to PPT (Fig. 5).

CONCLUSIONS

Our data suggest that PPI is mediated by a fast auditory pathway that passes from the IC to the ICN/caudal SC and PPT, and a slower multisensory pathway that originates in the intermediate layers of the SC. This new model accounts for the latency differences between sites and for the partial effects of SC lesions on acoustic PPI. Refractory periods of the intermediate layer neurons in SC mediating PPI were found from 0.4–1 ms, similar to the crossed tectoreticulospinal neurons mediating contraversive, approach turns. These conclusions support the theory that PPI functions to inhibit startle responses during the several hundred ms required to execute approach turning and arousal responses (Fendt et al., 2001).

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