Prepulse Inhibition Following Lesions of the Inferior Colliculus: Prepulse Intensity Functions

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LI, L., L. M. KORNGUT, B. J. FROST AND R. J. BENINGER. Prepulse inhibition following lesions of the inferior colliculus: Prepulse intensity functions. PHYSIOL BEHAV 65(1) 133–139, 1998.—The magnitude of the acoustic startle response can be reduced by a relatively weak sound presented immediately before the startle-eliciting sound; this phenomenon has been termed prepulse inhibition (PPI). Previous studies reported that PPI was present in the decerebrate rat, indicating that the primary neural pathways mediating PPI are located in the brainstem. The present study investigated the effects of focal excitotoxic lesions of the inferior colliculus (IC) on acoustic PPI in rats. In the first part, startle magnitudes were measured in six normal rats as the interstimulus interval (ISI) between the prepulse and startle-eliciting sounds varied between 10 and 100 ms. Prepulse-inhibited startle changed in an ISI-dependent manner with the most effective ISI at 50 ms. In the second part, 21 rats were assigned to three groups: normal unoperated, cortical lesion, and IC lesion. With the ISI fixed at 50 ms, as the prepulse sound level increased from 29 to 49 dB SPL, startle responses decreased quickly in both normal and cortical lesion rats. However, rats with unilateral IC lesions made with ibotenic acid had significantly lower PPI but did not display any increase in startle magnitude. These data suggest that the IC is an important structure in the neural circuit mediating acoustic PPI. © 1998 Elsevier Science Inc.

THE ACOUSTIC startle response is a rapid contraction of the facial and skeletal muscles evoked by a sudden and loud acoustic stimulus. The magnitude of the acoustic startle response can be reduced by a relatively weak sound presented immediately before the startle-eliciting sound (8,14,18,20,21–23). This phenomenon has been termed prepulse inhibition (PPI) or prestimulus inhibition and seems to be a useful model for studying sensorimotor gating that is impaired in certain neuropsychiatric disorders such as schizophrenia (41). Although it has become evident that the caudal pontine reticular nucleus (PnC) is the obligatory relay station in the primary startle pathway that contains only three or four central synaptic delays (28,45,47), the neural circuits that mediate acoustic PPI are less well known. However, the existence of PPI in the rat with decerebration at the pretectal level (8,14,30) implies that the primary neural pathways mediating PPI are located in the brainstem.

The inferior colliculus (IC) is critical for processing acoustic information. The ascending auditory pathways that diverge from the cochlear nucleus into multiple ascending tracts largely converge in the IC and make obligatory synapses there. An earlier study reported that after bilateral large radio-frequency destruction of the IC and surrounding areas, including the caudal part of the superior colliculus (SC), periaqueductal gray, and some tegmental structures, the baseline acoustic startle was markedly potentiated and acoustic PPI was eliminated (27), suggesting that the IC might be part of a neural circuit for startle magnitude reduction by acoustic prestimuli. However, because both the SC (13) and certain tegmental areas (25,40) are essential for PPI, further studies are needed to verify the role of the IC in PPI. The present study examined the contribution of the IC to acoustic PPI. Lesions restricted to the IC were made in rats by local injection of the excitotoxin, ibotenic acid. To assess the effects of prepulse sounds at a variety of pressure levels on startle magnitudes, we compared prepulse intensity functions among the three animal groups: normal, cortical lesion, and IC lesion. The cortical lesion rats, whose cortical areas overlying the IC received comparable injection of ibotenic acid, served as anesthetic, surgical, and lesion controls.

The IC is critical for initiation and propagation of audiogenic seizures in rats (11,16,35). The IC-SC connection may be involved in the sensorimotor transduction necessary for audiogenic seizures (36,43). Bilateral injection of an excitotoxin into the auditory brainstem causes severe seizure activity after rats recover from
anesthesia (24,31). Accordingly, we only made unilateral lesions of the IC in the present study to minimize any potential seizure-induced changes in the SC that is also important to PPI (13).

METHODS

Animals

Experiments were conducted on 27 male adult Wistar rats (Rattus norvegicus) (300–450 g) obtained from Charles River Canada, St. Constant, Quebec. Rats were housed individually on a 12-h light:dark cycle (lights on at 0700 hours) with food and water freely available.

Startle Apparatus

All testing was carried out in a dark and soundproof chamber. Detailed descriptions of the sound delivery and calibration systems have also been published elsewhere (29). Briefly, broad-band noise bursts (40 Hz–20 kHz; 10 ms in duration with 3 ms rise and decay times; one per 30-s repetition rate) were generated by the software developed in our laboratory, amplified by an ultra wide-band integrated amplifier (Model PM635, Harman Kardon), and presented through two stationary speakers (83 cm above the animal). One speaker produced startle-eliciting sounds and the other produced low-intensity, non-startling prepulse sounds. Interstimulus intervals (ISIs, the time interval between the onset of the prepulse sound and the onset of the startle-eliciting sound) were modulated with a digital delay (Model DN716, Klark-Teknik). Sound levels of the two speakers were modulated independently by two passive attenuators (Model 350B and 350D, Hewlett Packard) and measured at the center of a Plexiglas cage (28.0 cm in length, 15.0 cm in width, and 12.7 cm in height) in which the rat was placed for testing. Calibration of the sound intensity was made in sound pressure level (SPL) (re 0.0002 dynes/cm²) using a 1/2 inch microphone leading to a sound-level meter (Model 2235, Bruel & Kjær). The ambient chamber noise level was around 23 dB SPL.

Startle responses were detected via a Piezo Ceramic transducer placed under the Plexiglas cage. As the rat was startled, the signal on the vibration of the Plexiglas cage was transferred by the transducer and then amplified and filtered with a custom-made electronic circuit. The amplified signal was electronically compared to a fixed trigger level provided by a window discrimination circuit whose output pulses during the period from the onset of the startling sound to 500 ms after this onset were counted with an IBM computer. Both the cage vibration signals and the fixed trigger level could be viewed with an oscilloscope. Data were stored to hard disk and analyzed at a later time.

Lesion Surgery

Ibotenic acid (RBI, Natick, MA) lesions were made with animals under deep anesthesia induced by sodium pentobarbital (60 mg/kg, i.p.). A state of areflexia was maintained throughout the surgery by supplemental injection of sodium pentobarbital (1/6–1/5 of the initial dosage). The animal was wrapped in a towel to decrease loss of body heat. Immediately before surgery, the external ears and tympanic membranes were examined and determined to be free of infection and/or obstruction. The animal was placed in a head holder that left the external meatus free to avoid damage of the tympanic membrane.

A midline incision was made in the scalp, and the skin and temporal muscles were retracted laterally. The head of the animal was positioned with bregma and lambda at the same horizontal plane. Craniotomies were made on the dorsal surface of the skull to permit insertion of a stainless-steel cannula into the IC or overlying cortex.

The injection cannula (0.3 mm in diameter) containing ibotenic acid dissolved in Locke’s solution (10 μg/μL) was tilted 30° relative to the sagittal plane, and the coordinate was referenced to a point 4.6 mm lateral and 0.3–0.4 mm rostral to lambda. The cannula was lowered to a depth of 5.3 mm from the reference point to reach the IC or to a depth of 3.5 mm from the reference point to reach the cortex overlying IC. A total of 1.0–1.5 μL ibotenic acid solution was injected by pressure into the IC or cortex unilaterally at the rate of 1 μL per min. The cannula was then left in place for 5 min to allow for diffusion of the chemical. After the cannula was pulled out of the brain, holes on the skull were filled with bone wax, wounds were sutured, and the animal was then returned to its cage and allowed 1 week to recover from the general effects of surgery.

Startle Testing Procedure

All testing was undertaken between 0900 and 1700 hours. The rat was placed in the Plexiglas cage and 5 min later presented with 60 trials (a session) of acoustic stimuli at 30-s intertrial intervals.

In the first part of this study, ISI functions in six normal rats were measured. The intensity of the startle-eliciting sound was fixed at 98 dB SPL, and the intensity of non-startling prepulse sound was set at 44 dB SPL. This part of the study had two testing sessions for each of the normal rats and 60 trials for a session. The delay between the two sessions was 1 day. One session included the following six stimulation conditions: 1) startling sound alone, 2) the same startling sound and a prepulse sound at the ISI of 10 ms, 3) 20 ms, 4) 50 ms, 5) 70 ms, and 6) 90 ms. The other session included the following six stimulation conditions: 1) startling sound alone, 2) the same startling sound and a prepulse sound at the ISI of 10 ms, 3) 30 ms, 4) 50 ms, 5) 70 ms, and 6) 100 ms. For each session, ten trials were assigned to each stimulation condition (ten trials to the startling-sound-alone stimulation condition and 50 trials to the five paired-stimulation conditions), and the order of stimulus presentation in a testing session was arranged randomly. An ISI function combined data from two testing sessions.

In the second part of the study, prepulse intensity functions were measured in the remaining 21 rats that were randomly assigned to three groups: normal (n = 7), cortical lesion (n = 7), and IC lesion (n = 7). The ISI was fixed at the most effective interval (50 ms) obtained from the first part of the study, the intensity of the startle-eliciting sound was fixed at 98 dB SPL, and the intensity of
the non-startling prepulse sound was changed in the range of 29–49 dB SPL. The prepulse intensity function was made by presenting the following six stimulation conditions: 1) startling sound alone (98 dB SPL), 2) the same startling sound and a prepulse sound at a level of 29 dB, 3) 34 dB, 4) 39 dB, 5) 44 dB, and 6) 49 dB SPL. Ten trials were assigned to each stimulation condition, and the order of stimulus presentation was arranged randomly.

To make data comparable across animals, prepulse-inhibited responses for each animal were normalized relative to the individual’s response to the startling sound. The following equation was used to calculate the percent response:

\[
\text{Percent response} = 100\% \times \left( \frac{\text{spikes to paired sound}}{\text{spikes to startling sound}} \right)
\]

**Histology**

At the end of testing, all rats that had received an injection of ibotenic acid were anesthetized deeply with an overdose of sodium pentobarbital and perfused transcardially with saline followed by 10% formalin. The brains were removed, stored in 10% formalin with 30% sucrose until they sank, and then sectioned at 50 μm in the frontal plane on a freezing microtome. Sections through the IC were stained with cresyl violet for histologic verification.

**RESULTS**

**Anatomical Results**

Injection of ibotenic acid into the IC or overlying cortex resulted in neural loss, gliosis, and some cavitation in the affected area. Restricted cortical lesions were made in the seven rats, and no neural damage was found in any subcortical structure (Fig. 1). The histologic reconstruction for each rat with IC lesions are shown in Fig. 2. No damage was found beyond the IC that received injection of ibotenic acid.

**General Post-Surgical Observations**

Rats receiving unilateral injection of ibotenic acid into the IC had brief and minor seizures shortly after surgery. During the surgical recovery and testing periods, no difference in general
activity was noticeable between lesioned and normal rats. Both IC and cortical lesion rats maintained normal body weight.

Startle Responses

The first part of this study was carried out in six normal animals to determine the most effective ISI for PPI. As shown in Fig. 3, with the intensities of startling and prepulse sounds fixed at 98 and 44 dB SPL, respectively, the systematically changed ISI in the range of 10 to 100 ms produced a "U" type of timing function with the maximum inhibition of startle at the value of 50 ms. A one-way ANOVA with repeated measures on the ISI revealed a significant effect, $F(9, 45) = 12.8, p < 0.001$.

The second part of the study was conducted to examine the effects of IC or cortical lesions on the prepulse intensity function when the ISI was fixed at 50 ms. As shown in Fig. 4, for both the normal and the cortical lesion groups, acoustic startle responses declined quickly as the intensity of the prepulse sound was increased from 29 to 49 dB SPL. For the IC lesion group, the inhibition of startle produced by prepulse sounds was reduced. The data were examined with a 3 (the three animal groups) × 6 (the six stimulation conditions) ANOVA, with repeated measures on the six stimulation conditions. The analysis revealed a significant interaction, $F(10, 90) = 2.2, p < 0.05$. For the main effect of prepulse sound level, louder prepulse sounds resulted in significantly lower startle magnitudes than weaker prepulse sounds, $F(5, 85) = 46.3, p < 0.001$. There were also significant differences among animal groups, $F(2, 18) = 6.9, p < 0.01$. Due to the significant interaction, we subsequently conducted a one-way ANOVA for each prepulse sound level to test differences among animal groups.

When the prepulse sound intensity was 49 dB SPL, a one-way ANOVA revealed a significant group effect, $F(2, 18) = 11.0, p < 0.01$. Post hoc Scheffé's $F$ tests (with the significant level at 0.05) indicated that the significant difference was between the normal and IC lesion groups, between the cortical lesion and IC lesion groups but not between the normal and cortical lesion groups.

When the prepulse sound intensity was 44 dB SPL, a one-way ANOVA also revealed a significant group effect, $F(2, 18) = 7.3, p < 0.01$. Post hoc Scheffé’s $F$-tests indicated that there was no significant difference between the normal group and the cortical lesion group, but that the IC lesion group significantly differed from both the normal group and the IC lesion group.
When the prepulse sound intensity was 39 dB SPL, a one-way ANOVA revealed that the group effect was not significant, $F(2, 18) = 2.8, p > 0.05$.

When the prepulse sound intensity was 34 dB SPL, a one-way ANOVA revealed that the group effect was significant, $F(2, 18) = 6.7, p < 0.01$. Post hoc Scheffé’s $F$-test indicated that no significant difference was between the normal and cortical lesion groups, but that significant differences were between the IC lesion and normal groups and between the IC lesion and cortical lesion groups.

When the prepulse sound intensity was 29 dB SPL, a one-way ANOVA revealed that the group effect was not significant, $F(2, 18) = 3.1, p > 0.05$.

We also evaluated the effect of IC or cortical lesions on habituation of startle within a testing session. As shown in Fig. 5, mean responses to the startling-eliciting sound for each group declined gradually with repeated presentation of the stimulus. A 3 (the three animal groups) $\times$ 10 (the ten presentations of the startling-eliciting sound in a testing session) ANOVA revealed a significant decrease of startle across the ten presentations of the startling-eliciting sound, $F(9, 216) = 5.9, p < 0.001$, and no significant differences among groups, $F(2, 24) < 1$. The interaction was not significant, $F(18, 216) = 1.1, p > 0.05$. The data indicated that neither IC nor cortical lesions had significant influence on baseline startle and short-term habituation.

**DISCUSSION**

The results of the present study indicate that the IC makes an important contribution to acoustic PPI. The startle suppression that is normally augmented with an increase in prepulse sound level is reduced by unilateral restricted lesions of the IC. In contrast, damage to the cortex overlying IC has no effect on PPI. The present data are consistent with previous observations of the effects of radio-frequency lesions of the IC and surrounding areas (27) and, therefore, support the view that the IC is an important relay station for PPI. The present study made an important improvement in IC lesion method: excitotoxic lesions were restricted to the IC.

The degree of PPI largely depends on the ISI that separates the prepulse stimulus and the startle-eliciting stimulus (8,21,23). The present study shows that when the duration of both prepulse and startle-eliciting sounds is 10 ms, the most effective ISI that produces the maximum PPI is 50 ms, which is within the optimum range of ISI, 40–60 ms, as reported in earlier studies (21,23). Our recent studies (Li and Yeomans, manuscript in preparation) show that unilateral electrical stimulation of the IC has a strong inhibitory effect on acoustic startle, with the most effective ISI between 15 and 30 ms, which is shorter than the most effective ISI (50 ms) obtained from the present study in which IC neurons were driven by sounds. This shift of the most effective ISI further confirms the position of the IC in the PPI pathways and suggests that the ISI function is determined by signal conduction in the PPI pathways.

Previous studies have suggested that some forebrain structures, such as hippocampus, nucleus accumbens, and ventral pallidum, have a significant modulating influence on PPI (41). However, PPI is present in decerebrate rats (8,14,30), indicating that the primary neural pathways mediating PPI are located in the brainstem. To date, a detailed description of the PPI pathways is lacking.

In addition to the IC, the pedunculopontine tegmental nucleus (PPTg) is another important structure putatively mediating acoustic PPI. A study with a combination of retrograde axonal tracing and immunohistochemistry suggests that the PPTg may send bilateral cholinergic projections to the PnC (25), the obligatory relay station for acoustic signals producing a startle response (28,45,47). Injection of ACh agonists into the PnC suppresses most of the neurons that respond to acoustic stimulation (25). The role of the PPTg in mediating PPI has been demonstrated by studies in which electrolytic or excitotoxic lesions of the PPTg reduce acoustic PPI in rats (25,40).

In mammals, the IC might be a source that provides auditory inputs to the PPTg by way of the middle and/or deeper layers of the SC, a sensorimotor integrator processing auditory, visual, and somatosensory information (38). Some subregions of the IC, such as the dorsomedial region, external nucleus, and nucleus of the brachium of the IC, have direct projections to the middle and/or deeper layers of the SC (1,5,7,10,19,26,33,37,42,44,46,48). The middle and deeper layers of the SC, in turn, send bilateral (but predominantly ipsilateral) projections to the PPTg (39). The role of the SC in PPI has been reported by the study in which excitotoxic lesions of the SC reduce acoustic PPI without affecting the baseline startle magnitude (13).

The function of the IC and SC in PPI has been further investigated recently by two independent studies: 1) Electrical stimulation of the SC decreased the tone-evoked activity of PnC neurons of anaesthetized rats; chemical stimulation of the SC of awake rats with picrotoxin (a GABA antagonist) facilitated PPI without affecting the baseline startle magnitude (12). 2) Unilateral electrical stimulation of the IC of awake rats strongly inhibited startle-like responses elicited by unilateral electrical stimulation of the trigeminal nucleus (32).

There is a remote possibility that reduction of PPI after lesions of the IC resulted from a lack of short-term habituation. Normally, when animals are repeatedly exposed to a startle-eliciting sound, the overt startle reaction decreases (6,8,22,34). The data of the present study indicate a significant decline of startle with repeated presentation of the startling stimulus. However, rats with IC lesions did not show less habituation compared to normal and cortical lesion rats.
A further question arising from the study of PPI pathways is whether lesions of the IC, SC, and PPTg can increase the baseline startle magnitude. Studies using excitotoxic lesion method have shown that damage to either SC or PPTg does not affect the baseline startle magnitude (13,25); studies using the electrolytic lesion method have shown a potentiation of startle magnitude after damage to the PPTg and surrounding areas (40). The present study indicates that excitotoxic lesions of the IC have little effect on the startle magnitude in the trials of startling sound alone. In contrast, radio-frequency lesions of the IC and surrounding areas cause a four-fold increase in the startle amplitude (27). This inconsistency might be due to differences in lesion profile. First, compared to massive radio-frequency lesions, focal excitotoxic lesions may have less chance to cause damage to fibers of passage near the IC. Damage to some fibers, such as those important for inhibiting fear potentiation of startle, might cause an increase in baseline startle.

In addition, it has been reported that activation of the dorsolateral periaqueductal gray interferes with the expression of fear-potentiated startle (45). In the study using radio-frequency lesions (27), the dorsolateral periaqueductal gray was severely damaged. The four-fold increase in the baseline startle magnitude after radio-frequency lesions might be resulted from damage to structures beyond the IC, such as the dorsolateral periaqueductal gray. Finally, it should be noted that another study has shown that the baseline startle magnitude was not correlated significantly with the extent of electrolytic lesions of the IC but related to damage to either the PPTg or the cuneiform nucleus (40).

In recent years considerable attention has been paid to neural mechanisms of the production and modulation of PPI because PPI is deficient in patients with schizophrenia (2,3,4,9,17). A detailed knowledge of the primary PPI pathways in the brainstem will be the cornerstone for understanding the sensorimotor gating deficits in schizophrenics.

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