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Lesions of the laterodorsal tegmental nucleus disrupt prepulse inhibition of the acoustic startle reflex

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Abstract

The purpose of the present studies was to determine the effects of bilateral lesions of the laterodorsal tegmental nucleus (LDTg) on prepulse inhibition (PPI) of the acoustic startle reflex under conditions of varying prepulse intensity and interstimulus interval (ISI) durations. Rats with bilateral ibotenic acid lesions of the LDTg were evaluated for changes in PPI and startle amplitude in comparison with an unoperated group, sham-LDTg lesioned group and a group with bilateral ibotenic acid lesions of the subcoeruleus nucleus, a brainstem nucleus approximately 1 mm ventrolateral to the LDTg. Bilateral lesions of the LDTg produced a robust decrease in PPI with no effect on startle amplitude as compared with the three control groups. In contrast, bilateral lesions of the subcoeruleus produced no effect on either PPI or startle amplitude. The effects of bilateral lesions of the LDTg on PPI were observed across prepulse intensities of 5, 10 and 15 dB above background and ISI durations of 30, 100, 300 and 1000 ms without significantly decreasing startle amplitude in either test paradigm as compared with the sham-LDTg lesioned group. Our data provide evidence for a role of the LDTg in modulating PPI.

Keywords: Laterodorsal tegmental nucleus; Prepulse inhibition; Acoustic startle reflex; Ibotenic acid lesions

1. Introduction

Previously, we reported that the muscarinic cholinergic system is involved in the modulation of prepulse inhibition (PPI) of the acoustic startle reflex (Jones and Shannon, 2000a,b). PPI is functionally defined as the reduction in startle response produced by a low-intensity stimulus presented prior to a high-intensity, startle-inducing stimulus and is believed to represent a mechanism for the gating or filtering out of irrelevant or distracting stimuli (Graham, 1975; Ison and Hammond, 1971; Swerdlow and Geyer, 1998; Swerdlow et al., 1994). In particular, we reported that the nonselective muscarinic receptor antagonist scopolamine, but not its quaternary analog *N*-methyl scopolamine, produced a dose-dependent disruption of PPI which was reversed by the muscarinic receptor agonist oxotremorine, indicating that scopolamine disrupts PPI through antagonism of central muscarinic receptors (Jones and Shannon, 2000b). In addition to the direct modulation of PPI by the muscarinic cholinergic system, we have also demonstrated that PPI is modulated by interactions between the muscarinic cholinergic and dopaminergic systems (Jones and Shannon, unpublished observations). While these findings indicate that there is a critical balance between the muscarinic cholinergic and dopaminergic systems in modulating the magnitude of PPI, the muscarinic cholinergic and dopaminergic circuitries involved in mediating this interaction remain unknown.

One possible site for the interaction between the muscarinic cholinergic and the dopaminergic systems in modulating PPI may involve the regulation of the mesocorticolimbic dopamine circuitry by cholinergic innervation from the laterodorsal tegmental nucleus (LDTg). Anatomically, the LDTg is a primarily cholinergic brainstem nucleus supplying cholinergic projections to multiple structures known to be involved in sensory information-processing functions, including PPI. In particular, the

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LDTg sends direct cholinergic projections to both mesocortical and mesolimbic dopaminergic structures, including the prefrontal cortex and the ventral tegmental area (VTA; Bolton et al., 1993; Cornwall et al., 1990; Hallanger et al., 1987; Satoh and Fibiger, 1986) as well as cholinergic afferents to the caudal pontine reticularis (PnC), a region of the reticular formation that forms part of the primary startle reflex circuitry (Fendt and Koch, 1999). Functionally, Blaha et al. (1996) demonstrated that lesions of the LDTg attenuate the excitatory effects of direct infusions of neostigmine into the VTA on dopamine efflux in the nucleus accumbens. Moreover, electrophysiological studies have demonstrated that muscarinic agonists, such as carbachol, increase the discharge rate of VTA dopamine neurons (Gronier and Rasmussen, 1998; Lacey et al., 1990) and increase dopamine release in the nucleus accumbens and prefrontal cortex (Westerrink et al., 1996). Taken together, there appears to be functional interactions, as well as anatomical connections, between the cholinergic LDTg and the mesocorticolimbic dopaminergic circuitry that might serve as one of the possible sites for interactions between the muscarinic cholinergic and the dopaminergic systems in modulating PPI.

The purpose of the present study was to investigate the role of the LDTg and its cholinergic projections on modulation of PPI by evaluating the effects of bilateral lesions of the LDTg on both PPI and the amplitude of the startle reflex. In the present study, ibotenic acid was used to lesion the LDTg because of its relatively selective toxic effects on cholinergic neurons located in brainstem structures, including the LDTg (Blaha et al., 1996; Inglis and Semba, 1997). To evaluate the selectivity of the LDTg lesions and their subsequent behavioral changes on PPI and startle amplitude, three control groups were used for comparison: an unoperated group, a sham-LDTg lesioned group in which vehicle was infused into the LDTg, and a group with bilateral ibotenic-acid-induced lesions of the subcoeruleus nucleus, a noncholinergic brainstem nucleus approximately 1 mm ventrolateral to the LDTg. The effects of bilateral lesions of the LDTg on both PPI and startle amplitude were initially evaluated using a single prepulse intensity of 77 dB (27 dB above a 50-dB ambient noise background) and a startle stimulus of 106 dB. To determine if bilateral lesions of the LDTg produced changes in the relationship between the prepulse intensity and startle stimulus, that is, the signal-to-noise ratio, LDTg lesioned and control groups were evaluated for changes in PPI and startle amplitude under conditions of varying prepulse intensity (5, 10 and 15 dB above background). In addition, to determine if bilateral lesions of the LDTg produce changes in the temporal relationship between the prepulse and startle stimuli, all lesioned and control groups were also evaluated for changes in PPI and startle amplitude under conditions of varying interstimulus interval (ISI) durations: 30, 100, 300 and 1000 ms.

2. Methods

2.1. Subjects

Adult male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN), weighing 325–350 g, were housed in pairs in a large colony room under a 12-h light/dark cycle (lights on at 0600 h). Each rat was maintained on food and water ad libitum. Test sessions were performed between 0800 and 1800 h. All experiments were conducted in accordance with the NIH regulations of animal care covered in *Principles of Laboratory Animal Care*, NIH publication 85-23, revised 1985 and were approved by the Institutional Animal Care and Use Committee.

2.2. Apparatus

Sessions were conducted in a Coulbourn acoustic startle apparatus (Coulbourn Instruments, Allentown, PA) consisting of two ventilated, sound-attenuated chambers with four force transducer platforms per chamber. Each rat was placed in a testing holder $16.5 \times 8.5 \times 7.6$ cm with a top made of aluminum rods 0.5 cm in diameter spaced 1.25 cm center-to-center which allowed full exposure to acoustic stimuli. Each holder was positioned on an individual force transducer platform. The background decibel level in each chamber was determined to be 50 dB[A] using a Radio Shack Digital Sound Level Meter (cat. No. 33-2055). Sound levels were calibrated in each chamber using a five-point calibration curve. There was no significant difference between the two chambers on sound delivery or response amplitude. Data were recorded on-line utilizing a Compaq Deskpro 386 computer (Compaq Computer) and Lablinc interface modules (Coulbourn Instruments), with two hundred 1-ms readings collected beginning at trial onset.

2.3. Surgical procedure

Rats were anesthetized with 2% isoflurane and placed in a stereotaxic frame. Bilateral lesions of the LDTg were made by injecting 200 nmol/site of ibotenic acid (Research Biochemicals International, Natick, MA; 0.2 μl, 0.1 M) through 30 gauge stainless steel tubing. Ibotenic acid was dissolved in a phosphate-buffered saline, pH 7.4, and the final pH of the solution was adjusted with 1 N NaOH to 7.4. The infusion rate was 0.02 μl/10 s with an additional 5 min in situ to allow for diffusion before retraction of the needle. The sham-LDTg lesioned group was injected with 0.2 μl of phosphate-buffered saline alone. The following stereotaxic coordinates were used for the sham-LDTg lesioned and LDTg lesioned groups: −8.8 mm from bregma; lateral, +3.3 mm from midline: ventral: −7.4 mm from skull surface with skull level; the injection needle was angled 20° from vertical. The following stereotaxic coordinates were used for the subcoeruleus lesioned group: −8.8 mm from bregma; lateral, +1.8 mm from midline: ventral: −9.2

mm from skull surface with skull level (Paxinos and Waston, 1986). All rats treated with ibotenic acid were treated with a 5-mg/kg dose of diazepam at the end of the surgery to prevent any ibotenic-acid-induced seizures. The unoperated and sham-LDTg lesioned groups were not treated with diazepam to avoid heavy sedation. Rats were allowed to recover from surgery for 2 weeks prior to behavioral testing.

2.4. Behavioral testing

Two weeks postlesion, all rats were adapted to the startle chambers for 30 min on two consecutive days. On the third day, rats were placed in the startle chambers and, after a 5-min acclimation period, presented with a test session consisting of eight counterbalanced presentations of the following four trial types (total of 32 trials/session): no stimulus, startle pulse alone (106 dB, 20-ms broad band burst), prepulse tone alone (77 dB; i.e., 27 dB above background, 20 ms, 10 KHz) and prepulse plus startle pulse. The intertrial interval (ITI) was varied pseudorandomly between 15 and 45 s. The ISI duration was 120 ms. An ambient background noise of 50 dB was present throughout the test session.

In the prepulse intensity studies, after a 5-min acclimation period, rats were exposed to six counterbalanced presentations of the following six trial types (total of 36 trials/session): no stimulus, startle pulse alone (106 dB, 20-ms broad band noise burst), prepulse tone alone (65 dB, 20 ms, 10 KHz) and three prepulse (55, 60 or 65 dB; i.e., 5, 10 and 15 dB above background, 20 ms, 10 KHz) plus startle pulse combinations. The ITI was varied between 15 and 45 s, the ISI duration was 120 ms, and the ambient background noise was 50 dB.

For the ISI studies, after a 5-min acclimation period, rats were exposed to seven counterbalanced presentations of each of the following seven trial types (total of 49 trials/session): no stimulus, startle pulse alone (106 dB, 20-ms broad band noise burst), prepulse tone alone (77 dB; i.e., 20 dB above background, 10 KHz), and prepulse plus startle pulse trials with the following four ISI durations: 30, 100, 300 and 1000 ms. ISI duration was defined as the interval from the prepulse tone offset to the startle pulse onset. The ITI was varied between 15 and 45 s and the ambient background noise was 50 dB.

All rats were evaluated under each of the behavioral test paradigms, in the order described above, with 3 days between each test day.

2.5. Histochemical analysis

After the final testing day, the rats were sacrificed, and the brains were removed and immediately frozen on dry ice. After freezing, two parallel sets of 50-μm sections were cut through the regions of the LDTg and subcoeruleus on a cryostat. One set of sections was stained for Nissl substance using cresyl violet for determination of the general neuronal damage and lesion area and the other set was stained for acetylcholinesterase using a modified Karnofsky technique according to Saper and Chemlimsky (1984) for determination of damage to the cholinergic neurons in the LDTg. Outlines of the lesioned areas were drawn onto representative sections of the rat brain and the maximum and minimum areas of each lesion were identified.

2.6. Data analysis

Startle amplitude was defined as the peak of the two hundred 1-ms readings. For the surgical-treatment response studies, data were analyzed by a between-groups ANOVA with comparison to the vehicle control group using a Dunnett's test when there was a significant main effect of treatment ($P < .05$). For the interaction studies, data were analyzed by a repeated-measures two-way ANOVA with surgical treatment as a between-subjects factor and prepulse intensity or ISI as a within-subjects factor. If there was a main effect of treatment, the data were further analyzed separately at each prepulse intensity or ISI by a one-way ANOVA followed by a comparison to the sham-operated group using Dunnett's test. PPI was calculated using the following equation: $100 \times [(\text{mean startle amplitude in startle pulse} - \text{mean startle amplitude in prepulse plus pulse trials}) \div \text{mean startle amplitude in startle pulse trials}]$. Calculations were performed using JMP v 3.2 (SAS Institute, Cary, NC) statistical software.

3. Results

Fig. 1 shows the schematic representation of the maximum and minimum regions of general neuronal damage detected by Nissl staining (left coronal sections) and specific cholinergic neuronal damage determined by acetylcholinesterase staining (right coronal sections) of the bilateral lesions of the LDTg and the subcoeruleus nuclei. Together, these two series of coronal sections demonstrate that there was no overlap between the lesions of the LDTg and the subcoeruleus nuclei. Both general neuronal damage of the LDTg, as well as specific cholinergic neuronal damage, was relatively contained within the periaqueductal grey with limited damage extending immediately above and below the superior cerebellar peduncle. Because the majority of cholinergic cell bodies are located in the dorsal region of the LDTg, ibotenic acid lesions of the LDTg appear to have almost completely destroyed the cholinergic neurons in this region (shaded areas outlined in the right coronal sections). The subcoeruleus nucleus does not contain a large population of cholinergic neurons, thus, detection of changes in cholinergic neurons was negligible and only general neuronal damage is shown (shaded areas outlined in the left series of coronal sections).

In the initial characterization of the effects of bilateral lesions of the LDTg or the region of the subcoeruleus nucleus on PPI and startle amplitude, a basic test session consisting of 106-dB startle stimulus trials, prepulse tone (77 dB) alone trials, and prepulse plus startle stimulus trials,

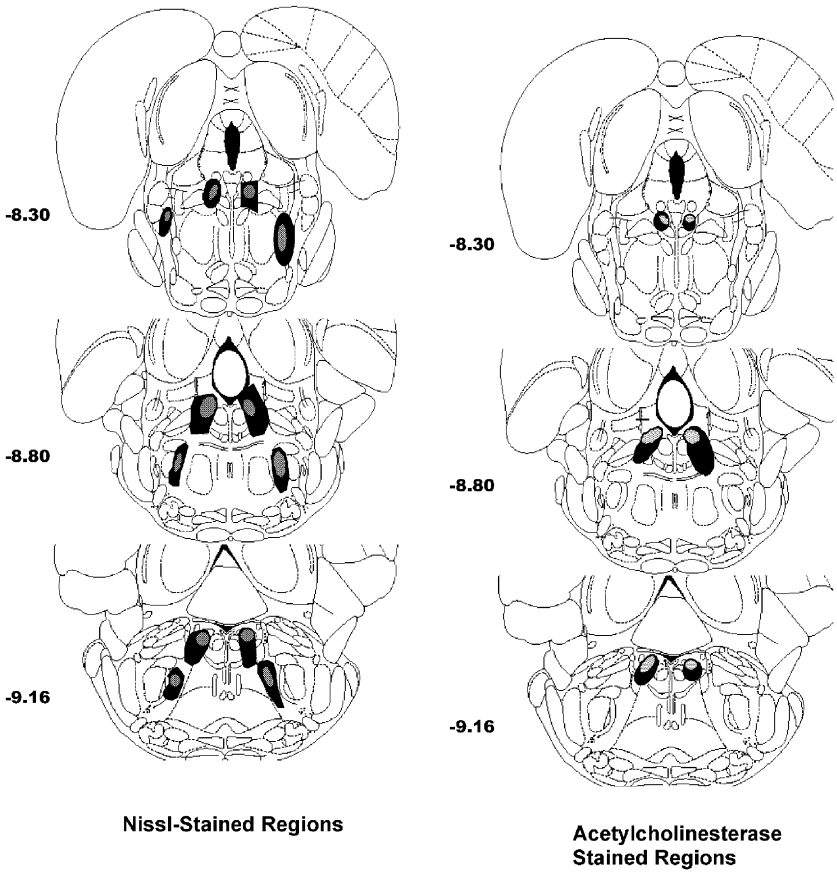

Fig. 1. Schematic representation of the maximum (black area) and minimum (cross-hatched) bilateral ibotenate-induced lesions of the LDTg (upper shaded areas in both series of sections) and the subcoeruleus nuclei (lower shaded areas in Nissl-stained coronal sections). General neuronal loss identified by Nissl staining is outlined in the left series of coronal sections. Presumed loss of cholinergic neurons identified by loss of acetylcholinesterase staining is shown in the right series of coronal sections.

and no stimulus trials was used. Throughout the present studies, the sham-LDTg lesioned group was not different from the unoperated group. Under this basic test paradigm, there was a significant effect of surgical treatment [$F(2,16) = 12.7$, $P < .001$]. In particular, bilateral lesions of the LDTg produced a significant decrease in PPI when compared with the sham-LDTg lesioned group by a Dunnett's comparison (Fig. 2, upper panel). In contrast, bilateral lesions of the subcoeruleus nucleus had no effect on PPI (Fig. 2, upper panel). In addition, there was no effect of bilateral lesions of the LDTg or the subcoeruleus nuclei on startle amplitude as compared with the sham-LDTg lesioned group. (Fig. 2, lower panel).

To further characterize the disruption of PPI by bilateral lesions of the LDTg, all treatment groups were evaluated in a test paradigm designed to determine the effects of the lesions on the signal-to-noise relationship between the prepulse intensity and the startle stimulus in which prepulse intensity was modulated across three different prepulse intensities (5, 10 and 15 dB above background). In all treatment groups, there was an increase in the magnitude of PPI as prepulse intensity was increased from 5 to 15 dB relative to the 50-dB ambient background noise (Fig. 3, upper panel). Under the prepulse intensity modulation paradigm, there was a main effect of treatment [$F(3,96) = 9.88$, $P < .0001$]. In particular, bilateral lesions of the LDTg resulted in a significant decrease in PPI at each of the three prepulse intensities as compared with the sham-LDTg lesioned group by a Dunnett's comparison (Fig. 3, upper panel). Moreover, the magnitude of the disruption of PPI by bilateral lesions of the LDTg was similar in magnitude across the differing prepulse intensities as the Treatment × Prepulse Intensity interaction term was not significant. In contrast, bilateral lesions of the subcoeruleus nuclei had no effect on PPI across the three prepulse intensities relative to the sham-LDTg lesioned group (Fig. 3, upper panel). There was no effect of bilateral lesions of the LDTg or the subcoeruleus on startle amplitude alone as compared to the sham-LDTg lesioned group (Fig. 3, lower panel).

In an additional experiment designed to determine the effects of the LDTg lesions on the temporal relationship between the prepulse intensity and the startle stimulus, all treatment groups were evaluated in a test paradigm in which the ISI duration between the prepulse and startle stimuli was varied from 30 to 1000 ms. For this experiment, the prepulse stimulus was 20 dB above background in all treatment groups; this prepulse stimulus (70 dB) was below startle

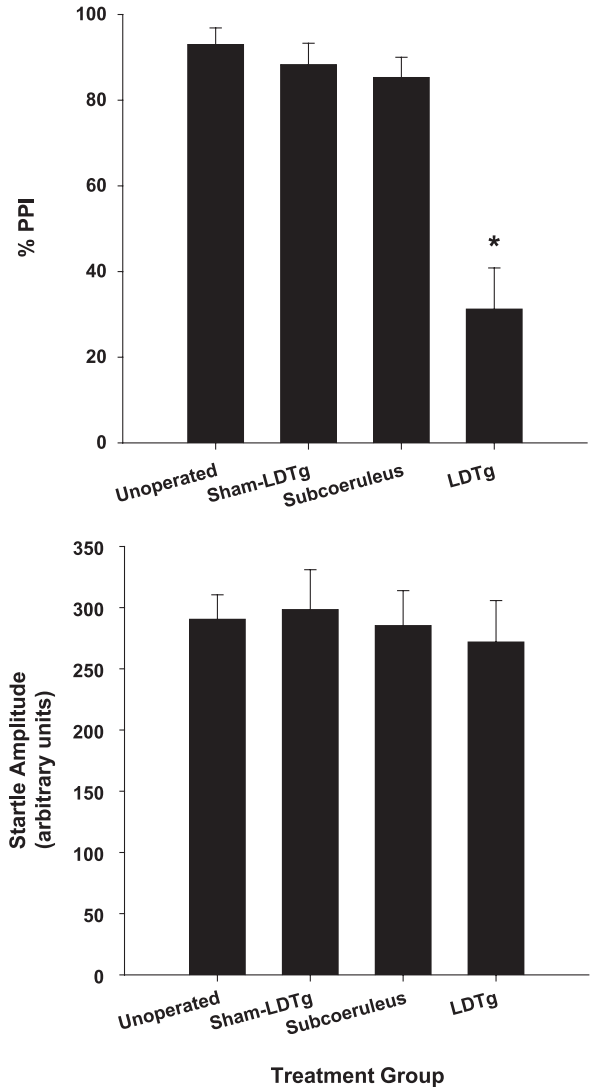

Fig. 2. Bilateral lesions of the LDTg produced a significant decrease in PPI as compared with the sham-LDTg lesioned and other control groups. Each bar represents the mean of five rats (upper panel). Bilateral lesions of the LDTg had no affect on startle amplitude as compared with sham-LDTg lesioned rats. Each bar represents the mean of five rats. Vertical lines represent ± 1 S.E.M. Abscissa: treatment groups. Ordinates: percent PPI or startle amplitude (arbitrary units).

threshold and did not produce a startle response in any of the four groups (data not shown). The magnitude of PPI decreased as ISI duration was varied from 30 to 1000 ms (Fig. 4, upper panel). Bilateral lesions of the LDTg produced a significant decrease in PPI at each of the ISI durations when compared with the sham-LDTg lesioned group by a Dunnett's comparison (Fig. 4, upper panel). There was also a main effect of treatment [$F(3,96) = 6.1$, $P < .0007$]. The magnitude of the disruption of PPI by bilateral lesions of the LDTg was similar across the ISI durations as the Treatment × ISI interaction term was not significant. Under the ISI test paradigm, startle amplitude was not significantly decreased in the bilateral LDTg lesioned rats as compared with the sham-LDTg lesioned group (Fig. 4, lower panel). Bilateral lesions of the subcoeruleus nuclei had no effect on PPI across the ISIs relative to the sham-LDTg lesioned group (Fig. 4, upper panel). Moreover, bilateral lesions of the subcoeruleus nucleus had no effect on startle amplitude as compared to the sham-LDTg lesioned group (Fig. 4, lower panel). Startle amplitude was decreased, but not significantly, in the sham-LDTg, subcoeruleus and LDTg lesioned groups as compared with the startle amplitude of the unoperated group.

4. Discussion

The present findings demonstrated that bilateral lesions of the LDTg, but not the adjacent subcoeruleus nucleus, produced disruption of PPI. LDTg lesions produced significant disruption of PPI across prepulse intensities of 5, 10 and 15 dB, as well as 27 dB, above background and across ISI durations of 30, 100, 300 and 1000 ms. Moreover, bilateral lesions of the LDTg had no significant effect on

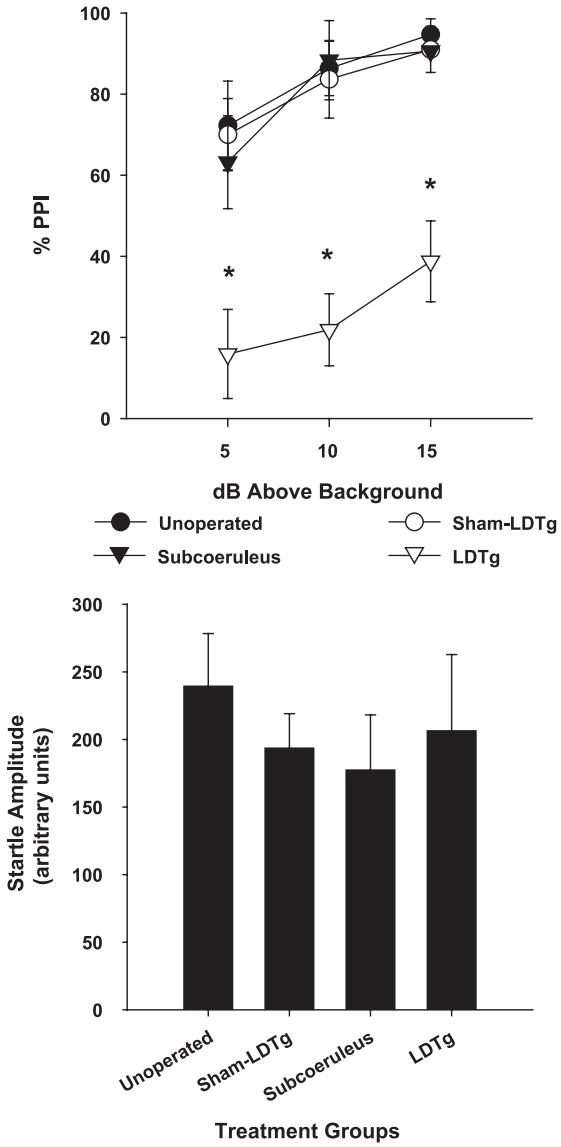

Fig. 3. Bilateral lesions of the LDTg significantly decreased PPI across prepulse intensities as compared with sham-LDTg lesioned and other control groups. Across all groups, PPI increased in magnitude as prepulse intensity increased above background. Each point represents the mean of five rats. The vertical lines represent ± S.E.M. and are absent when less than the size of the point. Abscissa: dB(A) above background. Ordinates: percent PPI or startle amplitude (arbitrary units). * $P < .05$ versus vehicle (Dunnett's).

startle amplitude as compared with the sham-LDTg lesioned group. The bilateral lesions of the LDTg also produced substantial decreases in acetylcholinesterase staining, indicating degeneration of cholinergic neurons in the LDTg. Our data indicate that the LDTg is an important region for mediating PPI, and neuronal loss in this nucleus, specifically cholinergic neuronal loss, appears to produce disruption of PPI in rats.

Our findings represent the first report of decreases in LDTg cholinergic neurons and corresponding decreases in PPI produced by bilateral lesions of the LDTg. Previously, Inglis and Semba (1997) reported that ibotenic acid, as compared with other excitotoxins, such as AMPA, NMDA and quinolinate, produced relatively discrete lesions of LDTg resulting in an 80–90% loss of nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase-positive, presumably cholinergic, neurons with little or no effect on fibers of passage or on the neighboring region of the primarily noradrenergic locus coeruleus. Furthermore, Blaha et al. (1996) demonstrated that 20 nmol of ibotenic acid injected unilaterally into the LDTg produced a 48% decrease in NADPH staining in the region of the LDTg and an attenuation of the dopamine release in the nucleus accumbens after intra-VTA infusions of neostigmine. In the present study, a dose (200 nmol/site) of ibotenate, similar to the dose (100 nmol/site) used by Inglis and Semba (1997), was used and produced a decrease in acetylcholinesterase staining, reflective of cholinergic neuronal loss, similar in magnitude to the cholinergic neuronal loss reported by Inglis and Semba (1997). Thus, we interpret the loss of cholinergic neurons in the LDTg to be a causal factor in the observed disruption in PPI in the LDTg lesioned rats. The hypothesis that a loss of cholinergic functional tone via loss of cholinergic afferents from the LDTg results in PPI deficits is consistent with our previous findings that systemic administration of muscarinic receptor antagonists produced disruptions in PPI (Jones and Shannon, 2000a,b). Furthermore, the present findings that the loss of LDTg cholinergic neurons did not affect startle amplitude are consistent with our previous data that muscarinic cholinergic antagonists had no effect on startle amplitude (Jones and Shannon, 2000a,b). However, acetylcholinesterase or NADPH diaphorase are not necessarily selective stains for cholinergic versus noncholinergic neurons, such as glutamatergic and GABAergic neurons, or the fibers of passage through the LDTg. Nevertheless, the present data are consistent with the interpretation that a loss of primarily cholinergic neurons within the LDTg and/or cholinergic afferents from the LDTg resulted in disruption of PPI. Thus, the cholinergic neurons in the LDTg or its cholinergic projections may be one of the sites of action for muscarinic receptor antagonists in modulating PPI.

By modulating the intensity of the prepulse stimulus relative to background, we investigated whether the deficits in PPI produced by bilateral lesions of the LDTg were a result of a change in the signal-to-noise ratio between the prepulse stimulus and the background noise. If the lesions of the LDTg decreased PPI primarily by decreasing the signal-to-noise ratio, then it would be expected that increasing prepulse intensity relative to background would reverse or surmount the lesion-induced disruptions of PPI. In the present study, bilateral lesions of the LDTg produced similar magnitudes of disruption in PPI across all prepulse intensities tested relative to background with no effect on startle amplitude. Thus, increasing the intensity of the prepulse stimulus over the range of prepulse intensities tested did not surmount the deficits produced by LDTg lesions. In con-

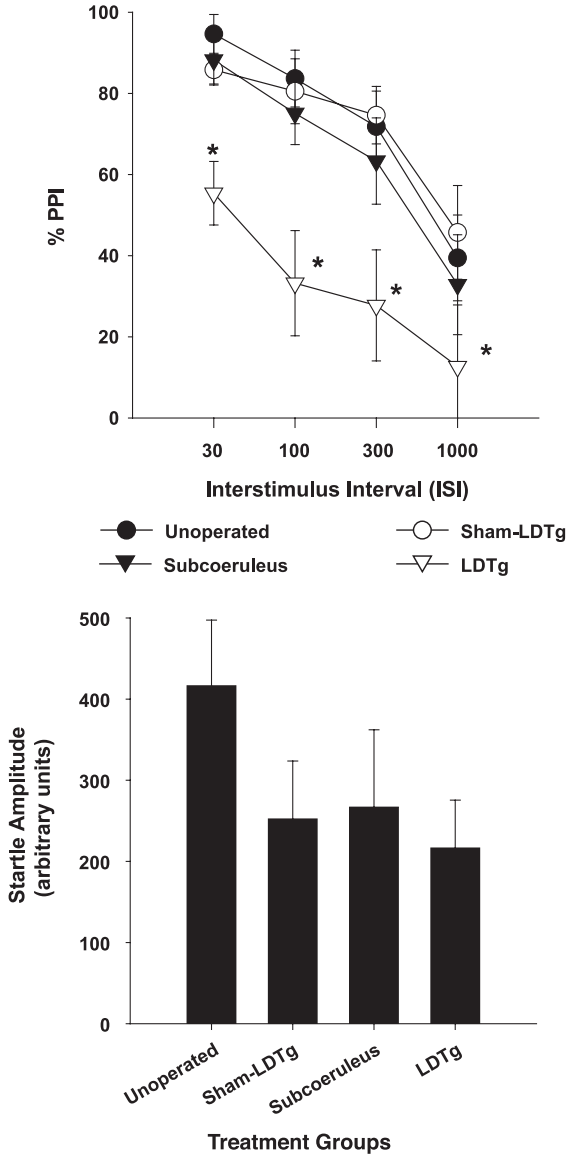

Fig. 4. Bilateral lesions of the LDTg significantly decreased PPI across ISIs as compared with sham-LDTg lesioned and other control groups. Across all groups, PPI decreased in magnitude as ISI increased from 30 to 1000 ms. Each point represents the mean of five rats. The vertical lines represent ± S.E.M. and are absent when less than the size of the point. Abscissa: ISI. Ordinates: percent PPI or startle amplitude. $*P<.05$ versus vehicle (Dunnett's).

trast, the disruption of PPI produced by the muscarinic receptor antagonist scopolamine was surmounted by increasing the prepulse intensity, suggesting that the muscarinic cholinergic system is primarily involved in modulating the signal-to-noise ratio for the detection of the prepulse stimulus (Jones and Shannon, 2000a,b). The reasons for the insurmountable disruption of PPI by increasing prepulse intensity in animals with LDTg lesions compared with the surmountable disruption in animals administered scopolamine are not entirely clear, but may be due to differences resulting from a chronic irreversible loss of all cholinergic neurons and their projections versus an acute, reversible loss of muscarinic cholinergic tone induced by pharmacologic antagonism. Moreover, the surmountable nature of the scopolamine-induced disruption of PPI may also reflect the involvement of one or more nicotinic cholinergic receptor subtypes, because previous studies have demonstrated that the nicotinic cholinergic system is also involved in the mechanisms of PPI (Stevens and Wear, 1997).

Interestingly, Grillon et al. (1992) demonstrated that individuals with schizophrenia displayed consistent disruption of PPI over a range of prepulse intensities from 5 to 20 dB above background. The consistent disruption of PPI over the range of prepulse intensities in both individuals with schizophrenia and in rats with LDTg lesions makes it tempting to speculate that the LDTg and its cholinergic projections may be functionally compromised in individuals with schizophrenia.

Another way of modulating the magnitude of PPI is to alter the ISI duration, thereby modulating the temporal relationship between the prepulse and startle stimuli. In general, PPI is largest in magnitude at ISI durations of approximately 100 ms and decreases with increasing ISI duration (Braff et al., 1978; Ison and Hammond, 1971). In the present study, bilateral lesions of the LDTg produced similar magnitudes of disruption in PPI across all ISI durations. In particular, the significant effects of LDTg lesions on the 30-ms ISI duration suggest that lesioning the LDTg may interfere with the detection of the prepulse stimulus. The present data are in contrast to the effects of scopolamine that did not significantly decrease PPI at the 30-ms ISI duration (Jones and Shannon, 2000a,b). The reasons for the differences in the observed effects of LDTg lesions and muscarinic receptor antagonists are not entirely clear, but again may be due to differences in chronic irreversible loss of cholinergic neurons and their projections by LDTg lesions versus an acute, reversible loss of only muscarinic cholinergic tone induced by pharmacologic antagonism. Further studies are needed to better understand these differences in PPI under varying ISI durations.

Our data that bilateral lesions of the LDTg disrupt PPI corroborate and extend previous evidence for the involvement of the reticular formation and adjacent brainstem structures in modulating PPI and the startle reflex. Previously, Davis et al. (1982) established, using lesioning techniques, that the primary startle circuit in rats is located exclusively in the brainstem and consists of the auditory nerve, ventral cochlear nucleus, nuclei of the lateral lemniscus, nucleus reticularis caudalis (PnC), spinal interneuron, lower motor neurons and muscles. Our data indicate that the LDTg could be a critical component of the circuitry that is involved in PPI of the acoustic startle reflex. The finding that bilateral lesions of the LDTg disrupted PPI, but had no effect on startle amplitude, suggests that the LDTg functions mainly as a part of the inhibitory neural circuitry that modulates the startle reflex and not as part of the primary startle reflex circuitry itself. Moreover, because bilateral lesions of the adjacent subcoeruleus nucleus had

no effect on PPI, it appears that the function of the LDTg in modulating PPI is anatomically specific. However, previous studies have demonstrated that both electrolytic- and quinolinic-acid-induced lesions of the pedunculopontine tegmental nucleus (PPN), another predominately cholinergic nucleus in the brainstem, also produced disruption of PPI (Koch et al., 1993; Swerdlow and Geyer, 1993). Although the lesions in the present study did not appear to produce collateral damage to the PPN, such damage cannot be ruled out entirely. Moreover, there are extensive interconnections between the LDTg and the PPN (e.g., Bolton et al., 1993; Satoh and Fibiger, 1986), suggesting that damage to either structure would likely cause perturbations in the innervation of the other and that both structures are important parts of the circuitry modulating PPI.

The mesocorticolimbic dopaminergic system is well known to play a critical role in the modulation of PPI (e.g., Geyer et al., 1990). Cholinergic projections from the LDTg project directly to dopamine neurons in the VTA (Cornwall et al., 1990; Satoh and Fibiger, 1986) and modulate activity of the mesolimbic pathway. Infusions of muscarinic cholinergic receptor agonists into the VTA produce an increased discharge rate of VTA dopamine neurons (Gronier and Rasmussen, 1998; Lacey et al., 1990) as well as increased dopamine release in the nucleus accumbens (Westerrink et al., 1996). Increases in dopaminergic activity via systemic or intranucleus accumbens infusions of dopamine receptor agonists produce disruption of PPI (Swerdlow et al., 1994; Wan and Swerdlow, 1993). However, Blaha et al. (1996) reported that lesions of the LDTg attenuated the stimulatory effects of intra-VTA neostigmine on dopamine release in the nucleus accumbens. Thus, loss of cholinergic tone to the VTA after lesions of the LDTg does not appear to account for the deficits in PPI observed in the present study. However, the LDTg projects to other areas important in either the regulation of the primary startle reflex circuit (e.g., the PPN and PnC) or the inhibition of the startle reflex (e.g., medial prefrontal cortex and mediodorsal thalamic nucleus) and it may be that disruption of one or more of these projections may lead to imbalances in the cholinergic and dopaminergic systems which result in the observed deficits in PPI. It would be of interest to evaluate the role of cholinergic afferents from the LDTg to the VTA, medial prefrontal cortex, PPN or PnC on modulating PPI by determining the effects of direct infusion of muscarinic cholinergic agonists and antagonists into LDTg, VTA prefrontal cortex, PPN and PnC on PPI.

Differences in the LDTg cholinergic neurons between normal controls and patients with schizophrenia have been reported. Garcia-Rill et al. (1995) and Karson et al. (1991) reported that the total number of cholinergic neurons, as labeled by NADPH diaphorase, was larger in the brains of patients with schizophrenia as compared with their age-matched controls in the combined regions of the LDTg and PPN, another primarily cholinergic brainstem nucleus. In addition, Karson et al. (1996) reported a 40–70% reduction in the tissue concentrations of choline acetyltransferase (ChAT), the primary synthetic enzyme of acetylcholine, in both the LDTg and the PPN of brains of patients with schizophrenia compared with age-matched controls. While the relationship between increases in NADPH-diaphorase-labeled neurons and decreases in ChAT levels in the LDTg and PPN of patients with schizophrenia is not entirely clear, both sets of postmortem data provide evidence for changes in the cholinergic neurons of the LDTg in individuals with schizophrenia. Together with the present findings, such changes in cholinergic neurons in the LDTg of patients with schizophrenia suggest that the functional balance between the LDTg and the mesocorticolimbic dopaminergic circuitry could be disrupted and may account, at least in part, for the observed deficits in PPI in patients with schizophrenia.

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