TIMING OF IMPULSES FROM THE CENTRAL AMYGDALA AND BED NUCLEUS OF THE STRIA TERMINALIS TO THE BRAINSTEM

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Running head: BNST and CEA projections to brainstem

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The amygdala and bed nucleus of the stria terminalis (BNST) are thought to subserve distinct functions with the former mediating rapid fear responses to discrete sensory cues and the latter longer “anxiety-like” states in response to diffuse environmental contingencies. Yet, these structures are reciprocally connected and their projection sites overlap extensively. To shed light on the significance of BNST-amygdala connections, we compared the antidromic response latencies of BNST and central amygdala (CE) neurons to brainstem stimulation. Whereas the frequency distribution of latencies was unimodal in BNST neurons (~10 ms mode), that of CE neurons was bimodal (~10 and ~30 ms modes). However, after stria terminalis (ST) lesions, only short-latency antidromic responses were observed, suggesting that CE axons with long conduction times course through the ST. Compared to the direct route, the ST greatly lengthens the path of CE axons to the brainstem, an apparently disadvantageous arrangement. Since BNST and CE share major excitatory basolateral amygdala (BL) inputs, lengthening the path of CE axons might allow synchronization of BNST and CE impulses to brainstem when activated by BL. To test this, we applied electrical BL stimuli and compared orthodromic response latencies in CE and BNST neurons. The latency difference between CE and BNST neurons to BL stimuli approximated that seen between the antidromic responses of BNST cells and CE neurons with long-conduction times. These results point to a hitherto unsuspected level of temporal coordination between the inputs and outputs of CE and BNST neurons, supporting the idea of shared functions.

**Key words:**

Extended amygdala; synchronization; anxiety; fear; antidromic.
Behavioral findings indicate that the central nucleus of the amygdala (CE) and bed nuclei of the stria terminalis (BNST) subserve different functions. In particular, lesion (Hitchcock and Davis 1987, 1991; LeDoux et al., 1988; Campeau and Davis, 1995) and local drug infusion studies (Kim et al. 1993; Wilensky et al. 2006) have revealed that CE is critically involved in the rapid expression of conditioned fear responses to discrete sensory cues, functions that are left intact by BNST lesions (LeDoux et al. 1988; Walker and Davis 1997; Gewirtz et al. 1998; Sullivan et al. 2004). Instead, BNST lesions interfere with the development of longer “anxiety-like” states in response to more diffuse environmental contingencies, responses that often persist after termination of the threat (reviewed in Walker et al. 2003). For instance, BNST lesions were reported to disrupt corticosterone and freezing responses to contextual stimuli associated with aversive outcomes (Sullivan et al. 2004).

In contrast with these behavioral findings however, these two structures exhibit similar anatomical properties. For instance, CE and BNST neurons send robust projections to an overlapping set of autonomic and motor brainstem nuclei thought to generate components of fear/anxiety responses (Hopkins and Holstege 1978; Veening et al. 1984; Holstege et al. 1985; Dong et al. 2000; Dong and Swanson 2004, 2006a-c). Moreover, both receive strong glutamatergic inputs from the basolateral amygdala (BL; Krettek and Price 1978a,b; Pare et al. 1995; Savender et al. 1995; Dong et al. 2001). In fact, these overlapping connections of CE and BNST, coupled to similarities in neuronal morphology and transmitter content (reviewed in McDonald, 2003), have led to the proposal that the BNST and CE constitute one anatomical entity termed the extended amygdala (Alheid and Heimer 1988; deOlmos and Heimer 1999).

In further support of this idea, there are strong reciprocal connections between CE and BNST (Krettek and Price 1978b; Price and Amaral 1981; Sun and Cassell 1993; Veinante and
Freund-Mercier 2003; Dong et al. 2001; Dong and Swanson 2006a-c). According to these tracing studies, BNST projections to CE mostly originate in its anterolateral and anteromedial divisions and the same regions receive the bulk of CE outputs. A puzzling property of amygdalo-BNST connections disclosed in the above studies is that there is tremendous heterogeneity in the course taken by these axons to reach their target. Some follow a direct route, through and around the substantia innominata (ventral amygdalofugal pathway). Others follow a circuitous path, via the stria terminalis, that lengthens their trajectory several fold, raising questions as to the significance of this peculiar anatomical arrangement.

Thus, the present study aimed to shed light on the functional significance of BNST-amygdala connections using extracellular recordings of BNST and central amygdala (CEA) neurons in rats anesthetized with isoflurane. Our results point to an unexpected level of coordination in the timing of BNST and CE outputs relative to BL inputs.
MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use committee of Rutgers State University, in compliance with the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services). Male Sprague-Dawley rats (225-250 g) were anesthetized with a mixture of ambient air, oxygen, and isoflurane. Atropine (0.05 mg/kg, i.m.) was administered to prevent secretions. The body temperature was maintained at 37-38°C with a heating pad. The level of anesthesia was assessed by continuously monitoring the electroencephalogram and electrocardiogram.

A local anesthetic (bupivacaine, 0.1 ml) was injected subcutaneously in the region of the scalp to be incised. Ten minutes later, the scalp was cut on the midline. The bone overlying the regions of interest was removed and the dura mater opened. Under stereotaxic guidance, groups of two or three tungsten stimulating electrodes (inter-tip spacing of ~1 mm) were inserted in the BL nucleus of the amygdala (Fig. 1A1), the stria terminalis, as well as just dorsal to the substantia nigra (Fig. 1A2) where CE and BNST axons en route to the brainstem form a compact bundle (Hopkins and Holstege 1978; Holstege et al. 1985).

For the placement of stimulating and recording electrodes, the following stereotaxic coordinates were used (all relative to the bregma and in mm). For BL, antero-posterior (AP) –2.3, medio-lateral (ML) 5.0, dorso-ventral (DV) 8.7, and AP –2.8, ML 4.8, DV 8.7. For CE, the coordinates were AP –2.6, ML 4.1, DV, 8.0 and AP –1.8, ML 3.6, DV 7.8. For BNST, the coordinates were AP –0.8. ML 1.7, DV 6-7.5 and –0.2, ML 1.6, DV 6-7.5. For brainstem, three electrodes were inserted at the same AP level (-6.0), three different ML levels ML 1.6, 2.1, 2.6, and DV positions 7.7, 7.3, 6.9, respectively.
Evoked responses were recorded in CE and BNST with high-impedance (10-12 MΩ) tungsten microelectrodes (FHC, Bowdoin, ME). The positions of the microelectrodes were adjusted independently with micromanipulators. A subset of rats was prepared with electrolytic lesions of the stria terminalis. Such lesions were performed by applying 1 mA for 10 sec.

We only considered neurons generating spikes with a high signal to noise ratio (≥3). As the electrodes were lowered toward the structures of interest, electrical stimuli (0.1-0.5 mA, 0.1-0.3 ms) were delivered in the brainstem, in search of antidromically responsive neurons, indicating that they are brainstem-projecting cells. To be classified as antidromic, evoked unit responses had to meet at least two of the following three criteria (Lipski 1981): (1) stable latency (< 0.3 ms jitter), (2) collision with orthodromically evoked or spontaneously occurring spikes and (3) ability to respond faithfully to high frequency stimuli (300 Hz). Neuronal activity was observed on a digital oscilloscope, digitized at 20 kHz and stored on disk for offline analysis.

At the end of experiments, the animals were administered an overdose of sodium pentobarbital (100 mg/kg, i.p.) and select recording sites in the BNST (Fig. 1B1) and CE (Fig. 1B2) were marked with electrolytic lesions (0.6 mA for 5-10 sec). The brains were then extracted from the skull, fixed in 2% paraformaldehyde and 1% glutaraldehyde, sectioned on a vibrating microtome (at 100 µm), and stained with cresyl violet to reveal electrode placements, as shown in figure 1. The microelectrode tracks were reconstructed by combining micrometer readings and histology. In order to be included in the analysis, cells had to be histologically confirmed as being located in the regions of interest. Analyses were performed offline with commercial software (IGOR, WaveMetrics, Lake Oswego, OR; Matlab, Natick, MA) and custom-designed software running on personal computers. Spikes were detected using a window discriminator after digital filtering of the raw waves. All values are expressed as means ± SE.
RESULTS

Database

A total of 130 CE and 96 BNST neurons that were spontaneously active and/or responsive to electrical stimuli delivered in the BL or brainstem were recorded from 48 intact rats in this study. Histological controls (Fig. 1B2) revealed that our sample of CE cells included 102 and 28 neurons recorded in the medial (CEm) and lateral (CEl) parts of CE, respectively. For BNST cells (Fig. 1B1), most were recorded in the anterolateral region (n=83), as defined by Ju and Swanson (1989), with the rest in the posterior (n=13) region.

Consistent with earlier anatomical findings indicating that CEm has more extensive brainstem projections than CEl (Hopkins and Holstege 1978; Veening et al. 1984; Petrovich and Swanson 1997), the incidence of brainstem projecting cells, as identified by their antidromic responses to brainstem stimuli, was significantly higher in CEm than CEl (Fisher exact test, p <0.001). Indeed, as many as 76% of CEm cells (or 78 of 102) were antidromically responsive to brainstem stimuli, compared to 32% of CEl cells (or 9 of 28). In the BNST, all antidromically responsive neurons to brainstem stimuli (30% or 29 of 96) were located in the anterolateral region. Thus, below we focus on these anterolateral BNST neurons.

Latency of brainstem-evoked antidromic responses in CE and BNST neurons

Figure 2 shows representative examples of CE (Fig. 2A) and BNST (Fig. 2B) neurons that generated antidromic spikes in response to brainstem stimulation. As shown in the superimpositions of evoked responses (Fig. 2A1, B1), antidromic action potentials could easily be distinguished from synaptically evoked spikes because they had a fixed latency. Moreover,
antidromic spikes failed when spontaneous action potentials occurred in the collision interval (Fig. 2A2, B2, Collision). Another property common the CE and BNST cells was that the transition between the initial segment and somatodendritic components of antidromic spikes was slower than seen in spontaneously occurring action potentials (Fig. 2A1, B1, insets), often giving rise to clear break between the initial segment and somatodendritic components of the spikes (Fig. 2A1, B1, arrowheads in insets).

Consistent with previous findings in rats (Quirk et al. 2003) and rabbits (Pascoe and Kapp 1985), antidromic response latencies to brainstem stimuli were distributed bimodally in CE neurons with an early mode at 9.7 ± 0.7 ms and late one at 29.4 ± 0.7 ms (Fig. 2A3). Computing the Kolmogorov-Smirnov test for goodness of fit confirmed that the antidromic response latencies of CE neurons were not normally distributed (p<0.01). In contrast, the frequency distribution of brainstem-evoked antidromic response latencies was unimodal in BNST neurons (average of 10.6 ± 0.8 ms; Fig. 2B3).

As mentioned in the introduction, previous tract-tracing studies have revealed that CE axons can reach the BNST directly, via the ventral amygdalofugal pathway, or through a longer round-about path, the stria terminalis. Thus, these findings led us to suspect that the axons of CE cells with longer conduction times to the brainstem might course through the stria terminalis.

To test this idea, 26 rats were prepared with electrolytic lesions of the stria terminalis. Post-hoc histological controls revealed that in twelve of these cases, the stria was successfully lesioned with minimal damage to adjacent structures (Fig. 3A). An additional sample of CE neurons (n=42) was recorded in these rats and the distribution of brainstem-evoked antidromic response latencies was compared to that seen in intact rats (Fig. 3B). For the purpose of statistical comparisons, we used a cut-off of 20 ms to define cells with short vs. long conduction
times. In intact rats (Fig. 3B, black), our sample of antidromically responsive CE cells (n=87) was divided equally between cells with short (47%) vs. long (53%) conduction times. By contrast, in rats prepared with lesions of the stria terminalis (Fig. 3B, red), our sample of antidromically responsive CE cells (n=15) was mostly comprised of cells with short conduction times (80% of cells). Using a Fisher exact test, the differing incidence of CE neurons with short vs. long conduction times to the brainstem in intact vs. stria terminalis lesioned rats was found to be statistically significant (p = 0.034).

**Latency of BL-evoked orthodromic responses in CE and BNST neurons**

Compared to the ventral amygdalofugal pathway, the stria terminalis lengthens the path of CE axons to the brainstem several fold, raising questions as to the significance of this apparently disadvantageous arrangement. Since the BNST and CE both receive major excitatory inputs from the BL nucleus, we reasoned that lengthening the axonal path of some CE neurons might allow synchronization of BNST and CE impulses to the brainstem when they are both activated by BL inputs. To test this idea, we applied electrical stimuli in the BL nucleus and compared orthodromic response latencies in CE and BNST neurons.

**Figure 4** shows representative examples of BL-evoked orthodromic responses in CE (Fig. 4A1) and BNST (Fig. 4B1) neurons (note different time base) and the corresponding peri-stimulus histograms of neuronal discharges (Fig. 4A2, B2). The incidence of such orthodromic responses to BL stimuli was significantly higher among CE than BNST neurons (CE, 45% or 46 of 102; BNST, 31% or 26 of 83; Fisher exact test, p<0.02). However, the likelihood of observing BL-evoked orthodromic responses was similar for CE neurons with short vs. long conduction times to the brainstem (Fisher exact test, p>0.15).
As shown in the representative examples of figure 4A1-2, CE cells generally responded with a pronounced, but brief period of increased firing probability, lasting 3-6 ms. In contrast, the responses of BNST cells were more distributed in time, lasting 10-17 ms (Fig. 4B1-2; the origin of this difference is considered in the Discussion). The contrasting temporal profile of CE and BNST responses to BL stimuli led us to use two different measures to analyze response latencies: response onset vs. response peak. The latency to response onset was defined as the average of the first two consecutive 1-ms bins of poststimulus time histograms with counts three times higher than seen in the 10 ms period preceding the BL stimulus. In neurons showing no spontaneous activity during the prestimulus period, the latency to response onset was defined as the average of the first two poststimulus bins with counts.

Consistent with the fact that the distance between the stimulation and recording sites is shorter for CE than BNST neurons, both measures yielded shorter latencies for CE than BNST neurons. Indeed, using 1.5 times the threshold BL stimulation intensity (usually around 0.3 mA), the average latency to response onset was 7.6 ± 0.4 ms for CE neurons (n=46; Fig. 4A3) compared to 16.5 ± 0.7 ms for BNST neurons (n=26; Fig. 4B3). The difference between the latency to response onset of CE and BNST neurons was statistically significant (t-test, p<0.001). It should be noted that further increases in stimulation intensity did not appreciably reduce the latency to response onset of CE and BNST neurons.

Similarly, as shown in the average peri-stimulus histograms of Figure 4C, the latency of the response peak was significantly shorter for CE (8.1 ± 0.4 ms; Fig. 4C, thick line) than BNST neurons (23.6 ± 1.1 ms; Fig. 4C, thin line; t-test, p < 0.001). However, the difference between the two cell groups was much larger with this estimate of response latency. In fact, consistent with our timing hypothesis, the difference in latency to peak was of the same order as that seen
between the antidromic responses of BNST cells and CE neurons with long-conduction times. In closing, it should be mentioned that separate analyses of the latency to peak of BL-evoked responses in BNST neurons with (n=8) vs. without (n=15) physiologically-identified projections to the brainstem yielded qualitatively identical results (respectively, 25.1 ± 3.8 ms and 22.8 ± 2.1 ms latencies, t-test, p>0.05).
DISCUSSION

The present study was undertaken to shed light on the functional interactions between CE and BNST neurons with a particular emphasis on the relative timing of their outputs to the brainstem. The interest of this question stems from earlier findings suggesting that despite having similar connections and anatomical properties, CE and BNST play different roles in regulating behavior. Our results point to an unexpected level of coordination in the timing of BNST and CE outputs to the brainstem, relative to BL inputs. Below, we consider the significance of these findings in light of previous anatomical and behavioral studies on the role of the extended amygdala.

Path heterogeneity in CE projections to brainstem

Prior tract-tracing studies have revealed puzzling variations in the path followed by CE axons to the brainstem (Krettek and Price 1978b; Price and Amaral 1981; Sun and Cassell 1993; Dong et al. 2001; Veinante and Freund-Mercier 2003). Although many CE axons reach the brainstem directly, via the ventral amygdalofugal pathway, others follow the stria terminalis over its entire course. Thus, they first course caudally, then arch dorsally and rostrally along the lateral aspect of the thalamus, and later curve ventrally and caudally to merge with axons of the ventral amygdalofugal pathway.

Consistent with this, our analysis of brainstem-evoked antidromic response latencies has revealed that CE output neurons fall into two classes, with short or long conduction times to the brainstem, respectively. The rarity of CE cells with long antidromic response latencies in rats prepared with lesions of the stria terminalis strongly suggests that the neurons with long conduction times correspond to the subset of CE cells whose axons reaches the brainstem via the
striata terminalis. The net consequence of this path heterogeneity is that some CE impulses reach their brainstem targets quickly, in around 10 ms, whereas others take around three times longer.

What could be the significance of this peculiar arrangement? One possibility is that it serves no special purpose. According to this view, the path heterogeneity would reflect a developmental oddity where some CE cells, subjected to conflicting chemotaxic cues, would be lured into the stria terminalis, whereas others would merge with the ventral amygdalofugal pathway. However, a second possibility, the one we favor, is that this arrangement serves to synchronize CE and BNST outputs to the brainstem when they are activated by BL inputs (Fig. 5). Synchronization of CE and BNST impulses to the brainstem would likely enhance the post-synaptic impact of each input.

Consistent with this possibility, we observed that the latency of peak BL-evoked responses was longer in BNST cells, by about 20 ms, than seen in CE cells. This difference closely approximated the conduction delay introduced by lengthening the path of CE axons to the brainstem via the stria terminalis. Thus, by lengthening the path of some CE axons to the brainstem, the arrival of BL-driven CE impulses would be delayed, allowing for synchronization of BNST and CE impulses on their targets. This idea is further supported by a previous anatomical study showing that the same BL neurons that project to CE also contribute axon collaterals to the BNST (Smith and Millhouse 1985).

However, there is a third possible interpretation for our findings. This view assumes that BNST and CE neurons with slow vs. fast conduction times to the brainstem do not converge on the same brainstem neurons. While the tract-tracing data indicates that the brainstem targets of CE and BNST neurons overlap extensively at a macroscopic level (Hopkins and Holstege 1978; Veening et al. 1984; Holstege et al. 1985; Dong et al. 2000; Dong and Swanson 2004, 2006a-c),
it remains to be demonstrated, at the single cell level, whether convergence occurs for inputs originating from all three cell groups. For instance, it is conceivable that fast-conducting CE neurons contact brainstem neurons involved in the rapid mediation of short-lived fear responses. In contrast, slow conducting CE neurons and BNST cells might contact brainstem targets that are involved in more persistent fear responses. An important challenge for future studies will be to compare the brainstem projection sites of BNST as well as CE neurons with slow or fast conduction times to the brainstem.

A puzzling difference between CE and BNST neurons evidenced in the present study was that BL stimuli evoked a much longer period of increased firing probability in BNST than CE cells. Although differences in the electroresponsive properties of CE and BNST neurons might have contributed to this effect, it is also possible that BL stimuli engaged contrasting polysynaptic influences. In particular, CE neurons receive a strong GABAergic input from intercalated amygdala neurons (Pare and Smith 1993) that also receive inputs from BL (Royer et al. 1999). The excitation of ITC neurons by BL inputs was previously shown to generate a rapid feed-forward inhibition in CE neurons, limiting the duration of BL-evoked EPSPs (Royer et al. 1999). In addition, BL projects to the medial prefrontal cortex (Krettek and Price 1977) that in turn projects to BNST (Vertes 2004). Thus, the excitation of BNST neurons by BL inputs may have been prolonged via the activation of the medial prefrontal cortex.

**Behavioral significance of path heterogeneity**

Our analyses of BL-evoked response latencies and conduction times to the brainstem argue for a tight temporal coordination between CE and BNST outputs. However, this view does
not fit with the lesion and pharmaco-behavioral studies reviewed in the introduction that stress the different functions of CE and BNST. Yet, it remains that some effects of BNST and CE lesions overlap. For instance, ibotenic acid lesions of BNST (Gray et al. 1993) and CE (Van deKar et al. 1991) attenuate the increase in corticosterone associated with the expression of contextually-conditioned fear. More relevant to the theme of this paper are studies where CE and BNST lesions were reported to attenuate behaviors that are thought to depend on parallel projections of these structures to the brainstem. For instance, it was observed that the expression of contextual conditioned freezing responses is attenuated by both, CE and BNST lesions (Van de Kar et al. 1991; Gray et al. 1993; Goosens and Maren 2001; Sullivan et al. 2004). Moreover, CE and anterior BNST lesions prevent the pain-induced increase in vocalization seen following exposure to noxious electrical stimuli (Crown et al. 2000). Both of these effects are thought to depend on parallel projections of CE and BNST to the periaqueductal gray (PAG): the ventral PAG for freezing responses (LeDoux et al. 1988) and the dorsal PAG for the pain-induced increase in vocalization (McLemore et al. 1999; Crown et al. 2000).

Conclusions

Although recent behavioral studies have stressed the differing functions of CE and BNST, others point to overlapping roles of these two structures via their common brainstem projection sites. Consistent with the latter view, our results indicate that the path of BNST and CE axons is such that BL-driven CE and BNST impulses can reach the brainstem simultaneously, maximizing their impact on common targets.
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FIGURE LEGENDS

**Fig. 1** Histological verification of stimulating and recording sites. Coronal sections stained with cresyl violet. (A) Arrowheads point to stimulation sites in the BL nucleus (A1) or dorsal to the substantia nigra (A2). (B) Arrows point to electrolytic lesions in BNST (B1) or CEm (B2), where brainstem-projecting neurons were recorded. Abbreviations: AC, anterior commissure; BL, basolateral nucleus of the amygdala; CC, corpus callosum; CE, central nucleus of the amygdala; cp, cerebral peduncle; CPu, caudate-putamen; H, habenula; LA, lateral nucleus of the amygdala; LG, lateral geniculate nucleus; rs, rhinal sulcus; Th, thalamus; v, ventricle.

**Fig. 2** Physiological identification of brainstem-projecting CE (A) and BNST (B) neurons by antidromic invasion from the brainstem. In A and B, panel 1 shows superimposed antidromic responses to brainstem stimulation, whereas panel 2 shows cases where the antidromic spikes failed because of collision with spontaneous action potentials. The *insets in panels 1* show superimpositions of antidromic (black) and spontaneous (red) spikes. Note that the transition between the initial segment and somato-dendritic components of the spikes is longer for antidromic action potentials. Panel 3 shows a frequency distribution of antidromic response latencies evoked from the brainstem in samples of 87 CE and 29 BNST cells.

**Fig. 3** CE neurons with long conduction times reach the brainstem via the stria terminalis. (A) Coronal section showing electrolytic lesion of stria terminalis (arrow). (B) Frequency distribution of antidromic spike latencies in brainstem-projecting CE neurons. Black (left y-axis) and red (right y-axis) curves respectively show data obtained in intact rats vs. rats prepared with
an electrolytic lesion of the stria terminalis (87 and 15 CE neurons, respectively). Control data replotted from figure 2A3.

**Fig. 4** BL stimulation orthodromically actives CE (A) and BNST (B) neurons. In A and B, panel 1 shows orthodromic responses to BL stimuli, panel 2 shows the corresponding peristimulus histogram of unit discharges, and panel 3 shows the frequency distribution of onset response latencies in samples CE (A3) and BNST (B3) neurons. (C) Average peri-stimulus histogram of neuronal discharges for CE (thick line) and BNST neurons (thin line). Prior to averaging, the data of each cell was normalized by dividing the number of spikes in each bin by the number of stimuli. Note that there was a much larger difference between the timing of the response peaks (16 ms difference) than between response onsets (6 ms).

**Fig. 5** Temporal interactions between BL-evoked activity in CE and BNST neurons and their hypothesized impact on brainstem cells. (A,C) Artificially-generated histograms showing the normalized firing rate (y-axis) of CE (top), BNST (middle), and brainstem (bottom) neurons after BL discharges (arrows). Panel A shows the impact of CE cells with direct projections to brainstem (as depicted on the left side of the scheme in B). Panel C shows the impact of CE cells with axons reaching the brainstem after coursing through the stria terminalis (as depicted on the right side of the scheme in B). The scheme in B shows interconnections between BL, CE, BNST. For clarity, CE cells with direct projections to brainstem are shown on the left whereas those with axons coursing in the stria terminalis are shown on the right. As shown in A, CE cells with direct projections to the brainstem inhibit brainstem cells at an earlier latency than BNST cells. As shown in C, the delay introduced by having CE axons reach the brainstem after
coursing in the stria terminalis allows temporal summation of the inhibitory effects generated by CE and BNST cells in brainstem neurons.
A1: CE
0.4 mV
3 ms

B1: BNST
0.2 mV
5 ms

A2: 
Responsiveness (%)
Time (ms)

B2: 
Responsiveness (%)
Time (ms)

A3: 
Frequency
Latency (ms)

B3: 
n = 26
Avg = 16.5

C: 
Responsiveness (%)
Time (ms)