Baroreflexes of the rat. IV. ADN-evoked responses at the NTS

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Tang X, Dworkin BR. Baroreflexes of the rat. IV. ADN-evoked responses at the NTS. Am J Physiol Regul Integr Comp Physiol 293: R2243–R2253, 2007. First published September 26, 2007; doi:10.1152/ajpregu.00142.2007.—In a long-term (7–21 days) neuromuscular blocked (NMB) rat preparation, using precise single-pulse aortic depressor nerve (ADN) stimulation and stable chronic evoked response (ER) recordings from the dorsal-medial solitary nucleus (dmNTS), two different response patterns were observed: continuous and discrete. For the continuous pattern, activity began ~3 ms after the stimulus and persisted for 45 ms; for the discrete pattern, two complexes were separated by a gap from ~17 to 25 ms. The early complex was probably transmitted via A-fibers: it had a low stimulus current threshold and an average conduction velocity (CV) of 0.58–5.5 m/s; the high threshold late (HTL) complex had a CV = 0.26–0.58 m/s. The average stimulus amplitude-ER magnitude transduction curves for the A and HTL complexes were sigmoidal. For individual rats, in the linear range, mean \( r^2 = 0.96 \pm 0.03 \) for both complexes. The average stimulus amplitude vs. the systolic blood pressure change (\( \Delta sBP \)) transduction curve was also approximately linear; however, for individual rats, the relationship was not consistently reliable: mean \( r^2 = 0.48 \pm 0.19 \). Approximately 90% of recording sites had respiratory, and 50% had cardiac synchronism. The NMB preparation is useful for studying central baroreflex mechanisms that operate on a time scale of days or weeks, such as adaptation and other kinds of neural plasticity.

baroreceptors; aortic depressor nerve; C-fiber; nucleus of the solitary tract; cardiac synchronism

WITHOUT BAROREFLEXES OF APPROPRIATE gain, the blood pressure has a large amount of variability in the form of low-frequency noise. The source(s) of the noise is (are) not known; however, for the most part, it is endogenous (9). Previously, using a long-term neuromuscular-blocked (NMB) rat preparation, we determined the overall cardiovascular transfer function for electrical stimulation of the aortic depressor nerve (ADN) (8, 9) and showed that the quantitative properties of the transfer function accurately predicted the effect of the baroreflexes on the low-frequency noise. The present study extends our analysis of the rat ADN, from cardiovascular effects to evoked responses (ERs), in the primary sensory nucleus of the baroreceptors, the dorsal-medial NTS (dmNTS). This was accomplished by combining the open-loop stimulation methods (8, 9) with stable long-term (7–21 days) recordings of multunit brain stem activity. Using electrophysiology, pharmacology, and histology, we verified that the central recordings were from second- or higher-order dmNTS baroreflex neurons, and we identified an evoked response complex that, on the basis of threshold and latency, is due to activity propagated in A fibers of the ADN (27).

In the rat, electrical activation of the ADN elicits purely baroreflex responses (8, 9, 20). The stretch-sensitive receptors of the ADN are in the interstitium of the aortic arch, as well as in the carotid and brachial-cephalic arteries. The cell bodies are in the nodose ganglion, and the axons terminate in the dmNTS (2). The rat ADN is composed of myelinated and nonmyelinated axons (A- and C-fibers): the receptors of the A-fibers have a lower stretch threshold than those of the C-fibers (23–25), and the A-fibers have a lower current threshold for electrical activation than C-fibers. We recorded multiunit activity, over 7–21 days, from the same site, in the dmNTS, using a Ta-Ta2O5 capacitative stimulating electrode on the ADN and a glass-insulated tungsten microelectrode in the dmNTS. Previously, direct observation of baroreceptor-evoked activity in the brain had been limited to acute experiments done at surgical levels of anesthesia.

Blood pressure regulation depends upon appropriate gain, and the neurophysiological mechanisms that adjust gain, which operate on a time scale of several hours to many days, are not well understood. Our specific goal was to achieve a stable long-term quantitative measurement of baroreflex activation, with analgesic-level anesthesia (0.5% isoflurane), as a basis for analyzing the mechanisms that adjust the baroreflex gain at key anatomical nodes (1). In this article, we will describe the properties of the ADN-elicited evoked responses in the dmNTS. Using the same methods, in a companion paper, we will describe the effect of a C-fiber tetanus on the magnitude of the A-fiber-evoked responses and explain how this previously unreported effect might be important for regulating blood pressure (22).

METHODS

Fourteen female Sprague-Dawley rats (230–270 g) were studied one at a time, while being monitored and attended around the clock. All acute surgery, or any possibly irritating manipulation, such as replacement of the bladder cannula, or removal of feces was done with surgical levels of anesthesia.

General Surgery

Surgery required 2 days and was done with a sterile technique. During all procedures, the anesthetic level was >1.5%, ensuring that 1) the EEG was synchronized and dominated by high-voltage slow-wave activity, 2) mean BP was <100 mmHg, and HR was <420 beats/min, and 3) there were no evident EEG, BP, or HR responses to

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manipulations. During the first day, for recording the EEG, silver wires were attached to two 0–80 screws, inserted into the left parietal bone, 2 mm left of the midsagittal suture, and 1 mm from the lambdoid or coronal suture. For the precordial ECG, two silver wires were implanted subcutaneously. For arterial blood pressure (ABP), an abdominal aortic catheter (28-gauge, Teflon) was inserted via the right femoral artery, and for administering parenteral solutions and recording of venous blood pressure (VBP), a silicone (0.025” ID × 0.047” OD) catheter was threaded into the inferior vena cava from the right femoral vein. A silicone cannula was inserted into the bladder to allow drainage and measurement of urine production rate. For ventilation, a per os coaxial tracheal cannula was inserted and to allow an adequate surgical field for the ADN and nucleus of the solitary tract (NTS) preparation, moved as far to the right as possible. Core temperature was measured by a thermistor inserted into the vagina and was servo-regulated at 37°C. Following induction of paralysis with a 75 μg iv bolus of α-cobotoxin, the rats were ventilated with positive pressure at 72 breaths/min (I:E 1:2), at volume of 180–200 cc/min of 48.5% O2, 47% N2, and 3% CO2, and 1.5% isoflurane.

During the second surgical day, an ~3-mm segment of left ADN, was dissected caudal to the carotid bifurcation and placed on an anodized Ta-Ta2O5 capacitance electrode (8), which was driven by an optically isolated constant current unit (CCIU-8, FHC, Bowdoin, ME), controlled by Spike2 (Cambridge Electronic Design, Cambridge, UK). The ADN identity was confirmed, under 1.5% isoflurane anesthesia, by electrical stimulation (5–70 Hz, 50 μA, 2 ms pulse width), which elicited a monotonoc, current-dependent, depres sor response that included bradycardia and hypotension and did not convert to a pressor pattern at >30 μA. The field surrounding the nerve was thoroughly dried, and an embedding compound (Kwik-Sil, WPI, Sarasota, FL) was injected under and around the nerve and electrode. After ADN embedding, a 1–2 MΩ glass-insulated tungsten microelectrode was positioned at the dmNTS to record ERs elicited by ADN stimulation. Detailed procedures are given below in the stereotaxic surgery section.

During days 3 to 4, after all surgery was completed, the isoflurane concentration was gradually reduced to an analgesic level of 0.5% and maintained at that level for the remainder of the experiment, except during electrode manipulations or maintenance procedures, when it was restored to a surgical level of >1.5%. Normally, experimental protocols and data acquisition started between day 4 and 5. For each rat, several protocols were scheduled: these included measuring the transduction curve, evaluating the effects of high-frequency activation of A+C-fibers on A-fiber-evoked responses at NTS [see the companion paper (22)], and some additional observations, concerning the relationship between EEG arousal and ER magnitudes, which will be reported at a later time. The protocols all required a stable physiological and consistent baseline. Normally, experiments terminated when planned protocols were completed; on several occasions, experiments terminiated earlier because of deterioration of the preparation. Ordinarily, 11–13 days (including the surgery and poststereotomy stabilization days) were required to complete the planned protocols; the actual experiment durations were from 7 to 21 days, with a mean of 12 days; in some experiments, the additional days were used to gather supplementary data concerning the relationship between EEG arousal and ER magnitude.

**Stereotaxic Surgery for Recording NTS Baroreflex Neurons**

On day 1, the rat was positioned supine on a stereotaxic frame (Benchmark Deluxe Digital, MyNeurolab.com, St. Louis, MO), with its head rigidly fixed according to the atlas standard orientation (16); on day 2, following isolation and embedding of the ADN, the NTS was accessed ventrally via the foramen magnus. Because the ventral approach, which was required for accurate chronic ADN stimulation, precluded use of skull surface landmarks, a removable precision-positioned zero bar was used as the working reference. Before each surgery, the zero point of the bar was calibrated to ear bar zero of the stereotaxic frame. All coordinates cited are referenced to the zero bar. Multunit recording was with 1–2 MΩ glass-insulated tungsten microelectrodes (Alpha-Omega, Alpharetta, GA) mounted in a modified remotely controlled FHC hydraulic probe drive (50–16-1, FHC), which was mounted to a stereotaxic carrier. The electrode was advanced through a close-fitting stainless steel guide tube; and the tip of the guide tube was the “zero” reference of the digital stereotaxic; the DV “zero” of the hydraulic drive (the electrode tip) was microscopically aligned with the end of the guide tube. In practice, the guide tube tip was moved to the target AP (anterior-posterior) and ML (medial-lateral) coordinates, and near the ventral surface of the brain stem (1 mm from ear bar zero), and the microdrive used to advance the electrode to the target DV (dorsal-ventral) coordinate. The guide tube shielded the electrode, and stabilized its axial travel.

On day 2, before dissection of the ADN, the foramen was exposed by a ventrodorsal blunt dissection at approximately AP = −6.0 mm; ML = −0.7 mm; gently retracting muscle along this path produced a clear field with no bleeding. The path was then filled with a damp cotton plug, and the ADN preparation was completed, following which, the tough membrane covering the foramen was removed, exposing the brain surface. At AP = −5.6 mm, ML = −0.6 mm, the guide tube was lowered until the tip was at DV = 1.0 mm, which was below the level of the bone ridge and membrane, but not, yet, into the brain tissue. With breach of the meninges, cerebrospinal fluid escaped, and the brain sank ~1 mm toward the dorsal skull surface. Using the FHC controller, the microelectrode was advanced into the brain. The preamplifier (XCELL-3 X 4, 40-#40-8B, FHC) gain was 20 k, and the signals were bandpass filtered at 0.3–1.0 kHz and digitized at 10 kHz using the Spike2 and a Power 1401 data acquisition system (Cambridge Electronic Design, Cambridge, UK). The multiunit spike data were archived and used for generation of histograms.

In addition to atlas coordinates, the baroreflex area in the NTS was located by reference to the somatotopic features of the gracile nucleus. At the search coordinates, gracile nucleus begins 50–150 μm dorsal to the baroreflex cells of the dmNTS. Initially, the electrode was moved to DV = 2.0 mm, from where it was advanced in 10-μm steps. Using the map of Tracey and Waite (26) as a guide, we stimulated the left flank and foot with a cotton-tipped applicator, and while listening to the activity, estimated the actual ML and AP coordinates of the site (In the correct location, the receptive fields of the knee, dorsal surface of the foot, and the footpads appear sequentially, as the electrode advances dorsally). With the ML and AP location thus confirmed, we withdrew the electrode to the ventral edge of gracile nucleus and stimulated the left ADN (30–50 μA, 300 μs, 0.17 Hz), while averaging evoked responses (ERs) for twenty 0.1 s sweeps, at each 10-μm ventral step, until the ensemble average was stable and showed a distinct pattern in the range of 3–45 ms, which usually occurred within 200 μm ventral to the edge of gracile nucleus. The longer (>20 ms) latency part of the complex always had a higher threshold and is referred to as the High Threshold Late (HTL) complex.

**Protocols**

**Effects of kynurenic acid block on the ERs.** 1-Glutamate is the neurotransmitter of baroreceptor afferents in the NTS (14), and blocking glutamatergic synaptic transmission blocks baroreflex activation; thus, to verify that the group ERs were recorded from the second- or higher-order neurons, and not from ADN fibers in the solitary tract, in two separately prepared rats, we infused the broad-spectrum excitatory amino acid (EAA) antagonist, kynurenic acid (KYN) into the fourth ventricle. ERs from the second- or higher-order NTS neurons should be diminished by KYN, whereas, those from fiber bundles should not be affected.

For KYN application, one end of a 30-cm 29-gauge hypodermic tube was stereotaxically placed into the 4th ventricle (ML = −0.10
mm; AP = −3 mm; DV = 7.5 mm), and cemented to the dorsal surface of the skull during the first surgery day. The other end of the tube was connected to a microsyringe pump (100 μl Hamilton; UMP2, WPI) containing 56.25 μg/μl KYN (Sigma Aldrich, St. Louis, MO) in PBS, buffered to pH 7.4. In an initial dose-ranging experiment (rat IG), 0.3 μl KYN injected over 3 min, reduced the ER by 40%. On the basis of this, in rat IZ, we infused 8 μl KYN over 2 min. This procedure was repeated 3 times, and the time between each infusion was 8 h. ADN stimuli were single pulses (interpulse interval = 6 ± 1 s; 300 μs, 100 μA for IG; 25 μA for IZ).

Because the cannula tended to distort the recording map, this procedure was not done in the rats that were included in reported data. The procedure was designed to reversibly block synaptic transmission throughout the brain stem without risking any movement of the recording electrode, which might have invalidated the procedure. The very large volume rate of flow of cerebral spinal fluid through the 4th ventricle and canal requires a high concentration and volume of infusate to achieve tissue levels adequate for the block.

Transduction curves. Transduction curves were used to characterize the relationships of the ADN stimulation intensity to both the magnitude and latencies of the group ERs at the NTS. Two patterns were used: “ascending,” which consisted of 10 incrementally increasing stimuli (300 μs, interpulse interval = 15 s), and the “symmetric,” which consisted of 20 stimuli, 10 incrementally increasing and 10 symmetrically decreasing (300 μs, interpulse interval = 15 s). The procedure was as follows: by successive approximation, we found the lowest intensity that saturated the HTL complex; set 110% of that value as the pattern maximum, and 10% of that, which was always below the HTL complex threshold, as the pattern minimum. From the minimum, for each step, the intensity was increased (or decreased, for the other 10 stimuli in the “symmetric” pattern) by 10% of the maximum intensity. The “ascending” pattern was repeated 24 times/h and was used in the initial 8 rats. To test for hysteresis effects, we used the “symmetric” pattern, repeated 12 times/h, in four later rats; in fact, hysteresis was minimal, and the two patterns gave comparable results. Determination of the transduction curve required ~8 h.

Termination of the experiments and anatomy. After increasing the isoflurane level to >2.5%, the recording electrode was connected to a current source and a 10-μA anodal current applied for 10 s; 30 min later, euthanasia was achieved with an intravenous bolus of barbiturate. With cardiac arrest and an isoelectric EEG, the tracheal cannula was removed, and a solution of 10% buffered formalin was applied latent (HRP) study of left aortic depressor nerve (ADN) terminations of Ciriello et al. (2) The field of small gray dots represents the areas of HRP density identified by Ciriello: some fibers terminate contralateral to the HRP injection (see the discussion of cardiac synchronization in Anatomy). The inset shows an example photomicrograph of an electrode track and lesion. The arrow indicates the map location of this recording site (open circle). GR, gracile nucleus; ST, solitary tract; Com., central canal; Sm, medial subnucleus of NTS; Sv, ventral subnucleus of NTS; DMV, dorsal motor nucleus of the vagus. The lesions have been collapsed onto the same AP plane, which is approximately −5.1 mm from ear bar zero; in fact, they were distributed over a AP range of ± 200 μM.

Limits of the ERs. To measure the relationship between threshold and latency, we used the above definitions to make “initiation” estimates for an iterative determination of the ER complex limits: for all cases, we used 3 ms as the earliest, and 60 ms as the latest possible ER component; in addition, for the seven discrete cases, we used 20 ms as the initial right-hand limit (RHL) of the A group and left-hand limit (LHL) of the HTL group.

The limits were determined algorithmically from a stimulus-triggered, ensemble-averaged ER trace. After removing the mean, we calculated the SD of the 1-s prestimulus baseline. The LHL of the A response was defined by rightward search from 3 ms after the artifact to the first time point where the amplitude of averaged ER was ± 3.5 SD from zero. The RHL of the HTL response was determined by leftward search, beginning 60 ms after the artifact. To determine the RHL of A and the LHL of HTL complex, for those cases that had discrete complexes, the leftward or rightward search started from 20 ms after the artifact. Means and SE for the time limits were calculated at each stimulus level and were plotted separately for the continuous and discrete patterns. Note that an arbitrary minimum synaptic delay, 1 ms, was systematically subtracted from all calculated latencies in the results.

Magnitudes of the ERs. The magnitude was determined from a stimulus-triggered, ensemble-averaged ER trace. After removing DC, the area between the fiducial LHL and RHL of the ER and the area of the symmetrically reflected interval before the trigger (the baseline) were calculated by integration of the absolute value of the signal. The ER magnitude was defined as the difference between the ER and the baseline areas:

\[ \text{Mag}_{\text{ER}} = \int_{\text{LHL}}^{\text{RHL}} |\text{ER}| - \int_{-\text{RHL}}^{-\text{LHL}} |\text{Baseline}| \]

In the transduction curve protocol, the average magnitudes of the ERs were determined at each stimulus intensity level and given as the percentage of the maximum magnitude elicited by the pattern. Data were pooled across all rats, and means and SE were calculated for each percentage-of-maximum stimulus level.

Data analysis. All analysis, if not specifically noted, was performed with Spike2 software.

General methods: topography of the ERs. In 7 out of 12 rats, A and HTL complexes of the ADN ERs were discrete and separated by baseline level activity in the interval of ~17 to 25 ms; in the other five rats, the evoked activity began after the artifact and continued uninterrupted for ~45 ms; for these, we used fiducial limits of 3–20 ms for the A complex and 20–45 ms for the HTL complex. In dissected rat ADN nerves, Fan and Andresen (10) measured the in vitro stimulus intensity-dependent recruitment of fibers and found that the conduction velocity of the high threshold, “nonmyelinated C-fibers” was 0.3–0.5 m/s (for low-threshold, “myelinated” fibers it was ~5–10 m/s). In four rats, we stereotaxically measured the Pythagorean distance between the NTS recording and ADN stimulation sites as 11.5–16.5 mm; thus, the C-fiber ER would need at least 11.5 mm to reach the recording site, and any ER components arriving before that are almost certainly not from nonmyelinated fibers. In our analyses, we defined the A-fiber ER latest latency as 20 ms and the HTL complex latest latency as 60 ms.
Stimulus intensities vs. BP responses. For the blood pressure transduction curve, the systolic BP response was calculated, from a stimulus-triggered ensemble-averaged systolic BP trace, as the difference between the minimum systolic value, occurring during the 1–3-s poststimulus period, and the mean systolic value of the 1-s prestimulus period. The magnitudes (in mmHg) were then plotted as the percentage of the maximum magnitude elicited in the pattern. Data were pooled for all 12 rats.

To evaluate the effects of the kynurenic acid block on the ERs. In rat IZ, the KYN infusion was repeated 3 times, with an 8-h rest between infusions; following infusion, a stimulus-triggered ensemble-averaged ER trace was generated every 5 min, beginning from 30 min before the KYN infusion to 240 min after the infusion. The ER magnitudes were calculated for each trace and plotted as the percentage of the mean magnitude of the preinfusion ERs. Data were pooled across the three replications and evaluated by a two-tailed, unpaired Student’s t-test between the ER response magnitudes of the 30-min pre-KYN infusion baseline and those of 90–120 min postinfusion.

RESULTS

Anatomy

Nine brains yielded clear electrolytic lesions. The lesions have been marked on a drawing adapted from the Ciriello et al. (2) horseradish peroxidase map of left ADN terminations in the NTS (Fig. 1). The recording locations coincide with the field of small gray dots of the horseradish peroxidase result (cf. Ref. 2, Fig. 3). The locations are also consistent with the electrophysiological relationship to the gracile nucleus. The inset shows an example photomicrograph of an electrode track and lesion. Because the recording sites were close to the solitary tract, to verify that the ERs were from the second- or higher-order neurons, not from the ADN fibers, synaptic transmission was blocked by infusing KYN, a broad-spectrum EAA antagonist, into the fourth ventricle. A preliminary dose-ranging experiment showed that (rat IG) 16.8 μg KYN (16.8 μg/μl × 56.25 μg/μl × 0.1 μl/min × 3 min ig) reduced the ER to 60% of the baseline and that the maximum effect occurred at 90–120 min. In a second experiment (rat IZ), 450 μg KYN (450 μg/μl × 56.25 μg/μl × 4 μl/min × 2 min) was infused into the same target region: Fig. 2A shows a 20-min baseline ensemble average, and Fig. 2B shows the average of the 100–120 postinfusion ERs.

Fig. 2. The effect of intrathecal kynurenic acid (KYN), an EAA synapse blocking agent, on an ADN-evoked response (ER) in rat IZ. A: 20-min baseline ensemble average, following which KYN was infused (450 μg = 56.25 μg/μl × 4 μl/min × 2 min) into the 4th ventricle (ML = −0.10 mm; AP = −3 mm; DV = 7.5 mm). B: 20-min average ER from 100–120 min postinfusion.

Fig. 3. In rat IZ, the baseline and KYN infusion was repeated 3 times: ERs were averaged in 5-min blocks, and the magnitudes plotted as a percentage of the mean of the 30-min preinfusion baseline. Each point is the mean ± SE of the three complete replications. At 90–120 min post-KYN, the magnitudes of the ERs were reduced to <35% of baseline (df = 4; t = 9.626; P < 0.001). In a preliminary dose-ranging experiment, in rat IG, KYN (16.8 μg = 56.25 μg/μl × 0.1 μl/min × 3 min ig) injected into the same target region reduced the ERs to <60% of baseline. Because of the very high flow rate of CSF, relatively large quantities of KYN were required to achieve the block.
that a substantial component the ER is from fibers of the solitary tract.

**NTS-Evoked Responses**

We stimulated the ADN and recorded stable ERs in the cardiovascular region of the NTS in NMB rats for 7–21 (mean = 12) days. Fig. 4 is a typical ensemble-averaged ER trace \( (n = 589 \text{ sweeps}) \) from a single NTS recording site, which is composed of complexes that arrive at the NTS in the time ranges of 4–20 ms (median \( \sim 8 \text{ ms} \)) and 30–50 ms (median \( \sim 38 \text{ ms} \)). On the basis of nerve length, conduction time, and threshold, the early complex with a short latency is probably due to transmission via A-fibers, and the late HTL complex could be produced by monosynaptic and polysynaptic C-fiber ERs, as well as possibly polysynaptic A-fiber ERs (10). A 360° phase histogram triggered by the ECG R wave (Fig. 4B) [or the inspiratory peak (Fig. 4C)] shows the distribution of spikes within the cardiac (or respiratory) cycles. For generating Fig. 4B and Fig. 4C, an \( \sim 10-\mu \text{V} \) threshold was used for spike detection in the multunit recording. A pronounced cardiac synchronism (cf. Fig. 4B) was found at \( \sim 50\% \) of the sites, and the cardiac synchronism was not attenuated by lesion of the ipsilateral ADN. A distinct respiratory synchronism was evident at \( \sim 90\% \) of the sites (Fig. 4C).

The ERs had one of two topographies. For 7 of 12 rats, the A and HTL complexes were discrete and separated by an \( \sim 7\text{-ms} \) interval of baseline level activity; for the remaining five rats, the ER pattern began \( \sim 3 \text{ ms} \) following the artifact, and with increasing stimulus amplitude, broadened to \( \sim 45 \text{ ms} \). Fig. 5 is the discrete pattern and shows a sequence of averaged \( (n = 48) \) ERs (Fig. 5A) at increasing stimulus amplitudes. At \( \sim 10 \mu \text{A} \), an “A” complex emerged; then, at \( \geq 20 \mu \text{A} \), the longer latency HTL complex emerged. The magnitude and duration of both complexes grew with increasing stimulus amplitude; at \( > 40 \mu \text{A} \), both complexes reached saturation. The magnitude (Fig. 5B) and duration (Fig. 5C) transduction curves for the A and HTL complexes summarize the results for this recording site. The activity at the site had afferent-phase respiratory synchronism (Fig. 5D), which depended on mechanical ventilation, and the spike-derived poststimulus histogram (Fig. 5E).
5E) had discrete peaks that correspond temporally to those in the A and HTL complex regions of the ERs. For generating Fig. 5D and Fig. 5E, the spike threshold in the multiunit recording was 10 μV.

Figure 6 is an example of a continuous pattern. Fig. 6A shows a sequence of averaged ERs at increasing stimulus amplitudes. At ~10 μA, an A complex emerged; at >20 μA, the RHL of the complex extended into the HTL complex range, without an intervening interval of baseline-level activity. The magnitude and duration of the complex grew with stimulus amplitude, until reaching saturation at 35 μA. The magnitude (Fig. 6B) and duration (Fig. 6C) transduction curves for the A and HTL complexes summarize the results for this rat; fiducial limits of 3–20 ms and 20–45 ms were used to define the A and HTL complexes, respectively (see Data Analysis for details). The recording site had afferent-phase respiratory synchronism (Fig. 6D), which depended on the ventilator and the poststimulus histogram (Fig. 6E), has a continuous peak extending from A to HTL complex regions, which corresponds temporally to the ER. For Fig. 6D and Fig. 6E, a threshold of 10 μV was set for spike detection in the multiunit recording.

Figure 7 shows that the different patterns can be related to the recording site rather than nerve or nerve electrode configuration. Using the same ADN stimulation current, in the same rat, and on the same day, we located two NTS recording sites receiving ADN inputs, which were 100 μm apart in AP, and 75 μm apart in ML, the ER had a discrete pattern (Fig. 7A) on the one site and a continuous pattern (Fig. 7B) on the other.

Transduction Curves
The average stimulus amplitude-ER magnitude (Fig. 8A) and stimulus amplitude-ΔsBP (Fig. 8B) transduction curves were constructed over all 12 rats, using either discrete peaks or fiducial limits to define the A or HTL complex regions of the ERs. To combine rats, the stimulus amplitudes were

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**Fig. 5.** A typical transduction curve, with discrete A and HTL response complexes. A: stimulus triggered (at t = 0) ensemble-averaged ER traces (0.1 s, n = 48 sweeps) at 10 increasing amplitudes (an “ascending” pattern, 300 μs pulse interval = 15 s). B: stimulus amplitude-response magnitude transduction curve. Each point is the mean magnitude (± SE) of 48 sweeps. C: stimulus amplitude-ER latency relationship: the pairs of points that define the gray area are the LHL and RHL for the A and HTL complexes. D: inspiratory peak triggered-phase histogram. There were 8,639 respiratory cycles sorted in 45 bins of total 360° (8°/bin) E: ADN stimulus triggered poststimulus histogram. The spike detection threshold was 10 μV for D and E.
converted to the percent of the maximum amplitude used in the pattern, and the average response magnitudes were converted to the percent of maximum response elicited by the pattern. The maximum pattern amplitude was determined as the minimum current that fully saturated the HTL response complex. The characteristics of the aggregate are similar to the individual results, and there was no obvious difference between rats with discrete and continuous ERs. The threshold of the A complex was between 10 and 20%, and that of the HTL complex was between 20 and 30%, of the respective saturation currents; the magnitudes and durations of the A and HTL complexes increased monotonically with stimulus amplitude. Both the A and HTL complex amplitude-ER magnitude transduction curves (Fig. 8A) are sigmoidal; however, the $\Delta sBP$ curve (Fig. 8B) was approximately linear ($r^2 = 0.97; n = 10$) over the full amplitude range, with a threshold between 10 and 20% of the saturation current. The $\Delta sBP$ response, to 300 $\mu$s, single-pulse ADN stimuli, at 100% amplitude, however, was only $\sim 1.0$ mmHg, and for individual rats, the relationship between amplitude and $\Delta sBP$ was not consistently reliable. For the 12 rats, the mean of the $r^2$ values was 0.96 for both the A and the HTL ERs, and over all rats, for both complexes, the standard deviation of the $r^2$ values was 0.03. In contrast, for $\Delta sBP$ over the same amplitude ranges, the mean $r^2$ values were much smaller and more variable (Table 1): over the A linear range: mean $r^2 = 0.30$, SD = 0.34; over the HTL complex linear range, mean $r^2 = 0.52$, SD = 0.34; taken over the full-stimulus amplitude range, the mean $r^2$ was 0.48, SD = 0.19. Thus, the evoked blood pressure change was less reliable and robust than the evoked NTS response. For each rat, the data sets were identical, as was the number of values of ER or $\Delta sBP$ averaged for each point of the transduction curve.

The relationship between stimulus amplitude and ER latency was plotted separately for the rats with discrete and continuous patterns (Fig. 9). Both have similar outer (A + HTL) envelopes: $\sim 3–45$ ms; however, for the discrete pattern (Fig. 9A), during 17–25 ms, the activity is at baseline.
Synchronism

Approximately 50% of recording sites had cardiac synchronism. To explore whether the cardiac synchronous activity was propagated in the ipsilateral ADN or via some other pathways, in one rat with typical synchronism, we lesioned the ADN by applying a negative (reverse) DC current (10 μA) to the Ta-Ta2O5 ADN electrode for 10 s, while continuing to record both ERs and background activity, and without otherwise disturbing the preparation. The lesion completely eliminated the ERs to the same or stronger ADN stimulus (300 μs, 35–80 μA) (cf. Fig. 10, A and B) but failed to noticeably attenuate the cardiac synchronism (cf. Fig. 10, C and D). The ADN stimulation was reinitiated 20 min after the lesion, and data in Fig. 10 were collected in the subsequent 30 min. If the synchronous activity were propagated over the ADN, by interrupting the nerve, the lesion should have obliterated it.

DISCUSSION

Latency Ranges of the A and HTL Complexes

Two different ER patterns were observed. In 7 of 12 rats, the A and HTL complexes were discrete bursts; in the remaining 5, there was continuous activity following the stimulus artifact. For the discrete pattern, the complexes were separated by baseline-level activity in the interval of 17–25 ms, while in the continuous pattern, activity was sustained from 3–45 ms. In the defined A and HTL complex time ranges, the magnitude of the response was well related to stimulus amplitude, and the A components consistently occurred at lower currents. Fig. 7 shows that both the discrete (A) and continuous (B) patterns can occur with the same nerve at the same stimulation current; however, this does not directly implicate interneurons, because fibers of intermediate latencies may arrive at some sites and not...
others. There were, in fact, several instances in which we found sites that were apparently without A-fiber complexes.

For the discrete pattern, the complex with the shorter latency was defined as A, and that with longer latency as HTL; the A threshold was consistently lower than the HTL threshold (10). For the continuous pattern, we defined fiducial limits guided by the latencies of the discrete pattern: 3–20 ms for A, and 20 – 45 ms for HTL complex. Again, the A complex consistently had a lower threshold than the HTL complex. In several rats, we tested with ADN currents much above the HTL complex saturation levels, and no longer latency complexes emerged. Although the different latencies of the A and HTL complexes were likely due to different conduction velocities (CVs), the velocity range that we estimated for the A complex is less than that of previous observations (10). In four rats, the stereotaxically measured Pythagorean (minimum) distance from the stimulation site to the recording site was 11.5–16.5 mm, which gives $\sqrt{H11021}/H110210.58 – 5.5 \text{ m/s}$ for the A, and $\sqrt{H11021}/H110210.26 – 0.58 \text{ m/s}$ for the HTL complex. Measurements on in vitro dissected ADN (10) gave 5–10 m/s for A and 0.3– 0.5 m/s for the HTL complex. The Pythagorean CV estimate for the HTL complex is consistent with the CV of C-fiber-propagated group action potentials in rat ADN (6); however, our A range is less than half of what was reported for group potentials. A likely reason is that Fan and Andresen’s measurements (10) were of group potentials on sections of ADN distal to the nodose ganglion, whereas ours were from ERs in second- or higher-order neurons. Although we subtracted a fixed 1-ms synaptic delay from each latency in our latency or CV analyses, for second-order ADN baroreceptor neurons, the latencies of solitary tract-evoked excitatory postsynaptic currents are actually 1.9 – 4.8 ms (3). In addition to the synaptic delay component, arborization and reduced myelination in the terminal axons could substantially slow conduction and increase the net latency (11–13). These factors, which because they add the same small increment to the A- and C-fiber conduction times, disproportionately increase the shorter A latencies and would be expected to produce the observed error in the A-fiber conduction velocities. Other investigators have measured latencies from single NTS neurons and separated them into two distinct ranges: 2–10 and 20 –29 ms (27); these results are similar to the ranges of our discrete pattern (Fig. 9A). Given the above, we

### Table 1. Linear regression of ADN stimulus amplitude with evoked NTS response magnitude or blood pressure decrease

<table>
<thead>
<tr>
<th>Rats</th>
<th>IH</th>
<th>II</th>
<th>IK</th>
<th>IL</th>
<th>IM</th>
<th>IO</th>
<th>IS</th>
<th>IV</th>
<th>IW</th>
<th>IY</th>
<th>IZ</th>
<th>JA</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fit range (A)</td>
<td>20–100%</td>
<td>10–50%</td>
<td>10–70%</td>
<td>10–70%</td>
<td>10–80%</td>
<td>20–60%</td>
<td>10–50%</td>
<td>20–70%</td>
<td>20–70%</td>
<td>20–50%</td>
<td>20–70%</td>
<td>10–50%</td>
<td>0.96</td>
<td>0.03</td>
</tr>
<tr>
<td>$r^2$(A ERs)</td>
<td>0.96</td>
<td>0.92</td>
<td>0.98</td>
<td>0.90</td>
<td>0.99</td>
<td>0.96</td>
<td>0.98</td>
<td>0.94</td>
<td>0.99</td>
<td>0.99</td>
<td>0.98</td>
<td>0.96</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>$r^2$(sBP)</td>
<td>0.96</td>
<td>0.03</td>
<td>0.49</td>
<td>0.46</td>
<td>0.19</td>
<td>0.18</td>
<td>0.42</td>
<td>0.45</td>
<td>0.47</td>
<td>0.69</td>
<td>0.48</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fit range (HTL)</td>
<td>20–50%</td>
<td>20–50%</td>
<td>20–40%</td>
<td>20–50%</td>
<td>40–80%</td>
<td>30–70%</td>
<td>40–70%</td>
<td>20–70%</td>
<td>40–80%</td>
<td>40–60%</td>
<td>40–70%</td>
<td>30–70%</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>$r^2$(HTL ERs)</td>
<td>0.99</td>
<td>0.96</td>
<td>0.98</td>
<td>0.96</td>
<td>0.98</td>
<td>0.92</td>
<td>0.98</td>
<td>0.96</td>
<td>0.99</td>
<td>0.99</td>
<td>0.88</td>
<td>0.96</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>$r^2$(sBP)</td>
<td>0.96</td>
<td>0.03</td>
<td>0.49</td>
<td>0.46</td>
<td>0.19</td>
<td>0.18</td>
<td>0.42</td>
<td>0.45</td>
<td>0.47</td>
<td>0.69</td>
<td>0.48</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full range</td>
<td>10–100%</td>
<td>10–100%</td>
<td>10–100%</td>
<td>10–100%</td>
<td>10–100%</td>
<td>10–100%</td>
<td>10–100%</td>
<td>10–100%</td>
<td>10–100%</td>
<td>10–100%</td>
<td>10–100%</td>
<td>10–100%</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>$r^2$(HTL ERs)</td>
<td>0.98</td>
<td>0.32</td>
<td>0.86</td>
<td>0.25</td>
<td>0.53</td>
<td>0.85</td>
<td>0.10</td>
<td>0.02</td>
<td>0.69</td>
<td>0.96</td>
<td>0.26</td>
<td>0.35</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>$r^2$(sBP)</td>
<td>0.96</td>
<td>0.03</td>
<td>0.49</td>
<td>0.46</td>
<td>0.19</td>
<td>0.18</td>
<td>0.42</td>
<td>0.45</td>
<td>0.47</td>
<td>0.69</td>
<td>0.48</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$r^2$ measures the strength of the relationship of each response magnitude to stimulus amplitude. The full stimulus range is 10–100%; “Fit Range” is the threshold-to-saturation stimulus amplitude ranges for the A or HTL complexes to which the linear fit was applied; thus, the reliability of the ER and sBP to stimulus amplitude relationships can be compared for each rat, over the linear stimulus ranges for the ERs. The mean and SD are over the 12 rats. The bottom row gives $r^2$ for the sBP-stimulus amplitude relationship over the full stimulus range. Note that, in comparison to the neuronal ERs, the sBP effects are much less reliable. For each rat, the data sets were identical, and the number of values of ER and sBP averaged for each point of the transduction curve was the same.

![Fig. 9. The average stimulus amplitude-ER latency relationships, for the cases, where, A and HTL complexes are discrete (n = 7 rats) (A) and contiguous (n = 5 rats) (B). The stimulus amplitudes are given as the percentage of the current that fully saturated the HTL response complex. Each point is a mean ± SE of the RHL and LHL. The outer (A + HTL) envelope of A is similar to that of B; the principal difference is the absence of evoked activity in the range of 17–25 ms for the rats in A.](http://ajpregu.physiology.org/)
have used 20 ms to define the outer latency limit for A and inner limit for the HTL complex.

Recordings are From Second- or Higher-Order NTS Neurons, not From Fibers of the Solitary Tract

Blocking glutamatergic transmission should block ERs from second- or higher-order neurons but not from fibers. Using a prepositioned brain stem cannula, we injected the broad-spectrum EAA antagonist, KYN, into the fourth ventricle. Because of diffusion and the high flow rate of the cerebral fluid, we found that a relatively large dose was required (for 16.8 μg in IG produced 40% and 450 μg in IZ produced 65% ER attenuation). That intrathecal KYN significantly reduced the magnitudes of the ERs confirms that at least the major component of the ER depended on a glutamatergic synapse.

Pathway of Cardiac Synchronism at the Recording Site

Thirty to seventy percent of NTS barosensory neurons are reported to have activity that is synchronized to the cardiac cycle (18, 19); ~50% of our recording sites showed EKG synchronism. To determine whether the cardiac synchronous activity was transmitted through the stimulated (ipsilateral) ADN, we lesioned the nerve with a reversed DC current. The lesion completely eliminated ADN-elicited ERs but failed to attenuate the cardiac synchronism at the recording site (Fig. 10), indicating that cardiac synchronous input reached the recording area via other pathways. Mapping studies using horseradish peroxidase conjugates of cholera toxin, which are consistent with this observation, have shown that ipsilateral axons from the carotid sinus nerve overlap those of the ADN and that axons from both carotid sinus nerve and ADN cross the midline (2); see Fig. 1.

Stimulus Amplitude was Better Related to the NTS ER Than ΔsBP

Table 1 shows that the evoked NTS neuronal response was much more reliable and robust than the evoked systolic blood pressure change (ΔsBP). There are two likely reasons: 1) Although some fibers of carotid and contralateral aortic inputs reach the ER recording side, the variance contribution that these trigger-random inputs make to the ER is proportionally less than to the net depressor response, where the four inputs are equally represented. 2) The rostral ventral lateral medulla (RVLM) is the basis of the sympathetic tone (17), and
the activity in the RVLM has substantial variability (15). The baroreflex acts through inhibition of the RVLM; thus, a blood pressure response is the convolution of an inverted baroreceptor input, with noisy RVLM activity (21). In contrast, when an ADN-elicited, stimulus-locked response is measured at the NTS, because RVLM variability is not involved, the variance is less, and the correlation is augmented. Changes in magnitude of the long-term NTS ER is, thus, a more sensitive and reliable measure of changes in the baroreceptor input pathway than evoked changes in blood pressure, and we exploit this advantage in the companion article.

**Perspectives and Significance**

The NMB rat preparation was developed to study autonomic processes, separate from respiratory and skeletal behavior, with precision and over extended time. The general goal was to understand the role of autonomic learning in regulation (4, 6, 7). The earlier papers in this series used a chronically prepared aortic nerve of a NMB rat to analyze, in the frequency and time domain, the cardiac and vascular mechanisms of the baroreflex, and to determine the influence of sleep and arousal on these mechanisms (5, 8, 9). The present study has extended the analysis from the peripheral mechanisms to the neural responses in the dorsal-medial NTS. We analyzed population-evoked responses rather than the activity of individual cells because we needed a repeatable quantitative measure of the responses of NTS neurons to myelinated aortic nerve inputs. The companion article (22) explains the regulatory hypothesis that we sought to test and gives the experimental result.

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