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Estimating sympathetic tone by recording subcutaneous nerve activity in ambulatory dogs

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Abstract

Introduction—We tested the hypothesis that subcutaneous nerve activity (SCNA) of the thorax correlates with the stellate ganglion nerve activity (SGNA) and can be used to estimate the sympathetic tone.

Methods and Results—We implanted radiotransmitters in 11 ambulatory dogs to record left SGNA, left thoracic vagal nerve activity (VNA) and left thoracic SCNA, including 3 with simultaneous video monitoring and nerve recording. Two additional dogs were studied under general anesthesia with apamin injected into the right stellate ganglion while the right SGNA and the right SCNA were recorded. There was a significant positive correlation between integrated SGNA (iSGNA) and integrated SCNA (iSCNA) in the first 7 ambulatory dogs, with correlation coefficient of 0.70 (95% confidence interval, CI, 0.61 to 0.84, $p < 0.05$ for each dog). Tachycardia episodes (heart rate exceeding 150 bpm for 3 s), were invariably preceded by SGNA and SCNA. There was circadian variation of both SCNA and SGNA. Crosstalk was ruled out because SGNA,

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VNA and SCNA bursts had different timing and activation patterns. In an 8th dog, closely spaced bipolar subcutaneous electrodes also recorded SCNA, but with reduced signal to noise ratio. Video monitoring in additional 3 dogs showed that movement was not a cause of high frequency SCNA. The right SGNA correlated strongly with right SCNA and heart rate in 2 anesthetized dogs after apamin injection into the right stellate ganglion.

Conclusions—SCNA recorded by bipolar subcutaneous electrodes correlates with the SGNA and can be used to estimate the sympathetic tone.

Keywords

autonomic nervous system; cardiac arrhythmia; sympathetic tone; tachycardia; autonomic ganglia; vagus

Sympathetic tone is important in cardiac arrhythmogenesis. Direct recording of the stellate ganglion nerve activity (SGNA) has shown that SGNA precedes the onset of spontaneous atrial and ventricular tachyarrhythmias in ambulatory canine models.¹⁻⁴ Because open chest surgery is necessary to access the stellate ganglion, it may be difficult to apply these invasive techniques to record human sympathetic nerve activities to improve the risk stratification and guide therapy for cardiac arrhythmias. An alternative method is microneurography, which can effectively record skin and muscle sympathetic nerve activity in humans and correlate the results with physiological changes such as hypertension.⁵ However, microneurography is also invasive and is difficult or impossible to record continuously for long periods of time. Heart rate variability is another alternative method to estimate cardiac autonomic nerve activities. However, that method requires proper sinus node response to autonomic stimulation. As sinus node function may be abnormal in heart failure or atrial fibrillation,^{6, 7} heart rate variability parameters may not reflect the sympathetic tone in those conditions.⁸ Because of the importance of sympathetic tone in cardiac arrhythmogenesis, it is highly desirable to develop less invasive methods to estimate sympathetic tone. Histological studies showed that abundant sympathetic nerves are present in subcutaneous space.⁹ Axonal tracer studies showed that the ipsilateral stellate ganglion is a major source of the thoracic subcutaneous sympathetic nerves.^{10,11} We hypothesize that (1) nerve activities can be recorded by electrodes implanted in the subcutaneous space and (2) the subcutaneous nerve activity (SCNA) correlates with the SGNA and can be used to estimate cardiac sympathetic tone. The purpose of the present study was to simultaneously recorded SGNA and SCNA in the thorax of ambulatory and anesthetized dogs to test these hypotheses.

Methods

The animal protocol was approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine and the Methodist Research Institute, Indianapolis, IN, and conforms to the Guide for Care and Use of Laboratory Animals. A total of 13 dogs were studied.

Protocol 1: Subcutaneous Nerve Activity and Heart Rate in Ambulatory Dogs

Eight dogs were intubated and anesthetized with isoflurane. A left thoracotomy was performed for the implantation of a radiotransmitter (D70EEE, Data Sciences International, St. Paul, MN) to record SGNA and VNA according to methods reported previously.^{1–4} A third pair of bipolar electrodes was placed in the subcutaneous space through the same thoracotomy wound. In the first 7 dogs, one electrode was inserted approximately 5–10 cm cranially into the subcutaneous tissues of the upper thorax, while the other was inserted caudally for the same distance into the subcutaneous tissues of the left abdomen. The inserted device and leads were fixed into the subcutaneous pocket with multiple interrupted sutures to minimize the movements of the electrodes. In an 8th dog, we used the second pair of electrodes to record closely spaced (approximately 4 cm apart) SCNA instead of VNA 10 days after left thoracotomy. After 2 weeks of recovery, the radiotransmitter was turned on to continuously record from all 3 electrodes at a sampling rate of 1,000/s.

Data Analysis—The nerve signals were high-pass filtered at 150 Hz and simultaneously displayed with the low pass (100 Hz) filtered ECG from the subcutaneous recording. In the first 7 dogs (Dogs A–G in Table 1), the data were first manually analyzed between 12:00 am and 2:00 am on a baseline recording day. We identified episodes with heart rate exceeding 150 bpm and duration exceeding 3 seconds as supraventricular tachycardia. The first 10 tachycardia episodes in which the recordings showed no evidence of noise or motion artifacts were selected for analysis. In addition, we performed quantitative analyses by integrating SGNA, VNA, and SCNA minute by minute over a 24-hour period during baseline recording after high-pass filtering at 150 Hz and by using frequency domain analysis to identify periods of increased nerve activity. For the frequency domain analysis, the filtered electrograms were detrended and multiplied by a Hamming window. A fast Fourier transform (FFT) was then calculated over a sliding 2-second window every 1 second. The area under the spectrum between 150 – 250 Hz was calculated and normalized to a baseline (no nerve activity) level.

Pearson correlation coefficients between heart rate, integrated SCNA (iSCNA), SGNA (iSGNA), and VNA (iVNA) were calculated for each dog and 95% confidence intervals were calculated for the average correlation coefficient using all dogs. A paired t-test was used to compare the average correlation between heart rate and SCNA with heart rate and SGNA between all dogs. A cubic smoothing spline was used to fit nerve activity measurement as a function of time over the 24-hour period for each dog. A generalized additive mixed-effects model was used to fit the same data of all dogs. All analyses were performed in SPSS and R software. A two-sided p value of 0.05 is considered statistically significant.

Protocol 2: Subcutaneous Nerve Activity and Movement

Three additional dogs underwent video monitoring along with simultaneous nerve activity recordings. We used a video camera (Axis Communications, Chelmsford, MA) to record the movement of the dogs when there was light in the kennel (7 AM to 7 PM). The first 5 minutes of every hour in one day were manually analyzed. We divided the times into 30-second windows, and determined if movement was observed in that window. We then

compared the movement with the recordings on all channels to determine if movement is a source of the high frequency electrical activities recorded on the SGNA, VNA and SCNA channels.

Protocol 3: Effects of apamin on right stellate ganglion and subcutaneous nerve activity

Two male dogs with body weight 29.5 Kg and 34.3 Kg, respectively, were used in this protocol. The dogs underwent isoflurane general anesthesia for thoracotomy using the right intercostal space. A pair of bipolar electrodes was inserted under the fascia of the right stellate ganglion. Two additional pairs of bipolar electrodes were inserted into the subcutaneous tissues of the 3rd intercostal space cranial to the incision. One pair has interelectrode distance of 18 cm, with one pole located 4 cm lateral to the right sternal border. A second pair has an interelectrode distance of 4 cm, with the first and second poles located at 6 and 10 cm, respectively, lateral to the right sternal border. These electrodes were connected to a World Precision Instrument (Sarasota, Florida) Iso-Damm-8 amplifier, with a noise level of $< \pm 2.5 \mu\text{V}$. The signals were digitized by Digidata 1400a using AxoScope software (Sunnyvale, Calif) at 10,000 times per second per channel. After all surgical procedures were performed, the anesthetic agents were switched from isoflurane to alpha-chloralose (up to 100 mg/kg) and morphine. We then injected 1 ml apamin (concentration 0.2 ng/ μL) directly into the right stellate ganglion. The same dose was repeated 4–9 minutes later. Apamin is a western honey bee toxin that inhibits the small conductance calcium activated K (SK) currents to increase nerve activity.^{12, 13} We used the apamin in this study to transiently increase the right SGNA and observe the changes of heart rate and SCNA. ECG and nerve activities were simultaneously acquired for up to 20 min after the first injection. The integrated nerve activity and the heart rate after apamin injections were analyzed. The dogs were then euthanized.

Immunocytochemical Studies—Three Pieces of subcutaneous tissue were randomly sampled near the subcutaneous recording sites of 2 dogs and fixed in 4% formalin for 45 to 60 minutes, followed by storage in 70% alcohol.¹⁴ The tissues were processed routinely, paraffin embedded, and cut into 5 μm –thick sections. Immunohistochemical staining was performed with antibodies against tyrosine hydroxylase using mouse monoclonal anti-tyrosine hydroxylase antibody (Accurate Chemical, Westbury, NY).

Results

Protocol 1: Subcutaneous Nerve Activity and Heart Rate

There were electrical signals resembling nerve activities in the subcutaneous tissues of all dogs studied. Figure 1A represents recordings from Dog A, showing initial heart rate variations that most likely represent baseline respiratory heart rate responses (RHR) commonly observed in dogs.¹⁵ The RHR was followed by a sustained increase of heart rate exceeding 150 bpm (upward arrow) and simultaneous SGNA and SCNA (downward arrows). The RHR resumed after the bursts of nerve activities terminated. Figure 1B shows similar activities 25 s later in the same dog. Again, the SGNA was associated with sustained heart rate elevation that exceeded 150 bpm. We analyzed 10 episodes per dog in which heart rate exceeded 150 bpm. All episodes had regular narrow QRS complexes preceded by

distinct p waves, with morphologies that were either the same or very similar to baseline sinus p waves. These findings are consistent with supraventricular tachycardia (including sinus tachycardia). No atrial flutter or fibrillation episodes were observed. SGNA and SCNA invariably preceded these supraventricular tachycardia episodes.

Figure 1C shows recordings from Dog D. Initial RHR was followed by the onset of SCNA (first downward arrow) and heart rate acceleration (upward arrow). The SGNA occurred slightly later and resulted in further heart rate acceleration exceeding 150 bpm and persisted for over 20 seconds. VNA did not blunt the heart rate response. The latter findings could be explained by the activation of the sympathetic component within the thoracic vagal nerve¹⁶. Figure 1D shows an example from Dog C. There was simultaneous onset of SGNA, VNA and SCNA (downward arrows), along with sustained increase of heart rate that exceeded 150 bpm. The patterns of activities in SGNA and SCNA were different. The latter findings were inconsistent with cross talk between the recording channels. Note that high amplitude spike discharge activity⁴ of SGNA was associated with burst discharges of SCNA, but there were less apparent separations between the spikes of SCNA than of SGNA.

In only one dog did we record high frequency nerve or muscle activities associated with RHR. In the remaining 6 dogs, the high frequency activities associated with RHR were either completely absent or were very small (mostly < 0.01 mV). These activities contributed minimally to the integrated nerve activity.

Closely spaced electrodes—In the 8th dog, we identified SGNA episodes with signal to noise ratio exceeding 2. We then selected the first episode of SGNA beginning at each half hour for 24 hours at the 10th postoperative day, resulting in a total of 48 episodes of SGNA. The signal to noise ratio of these SGNA episodes averaged 26 ± 26 (range 2–125). Closely spaced electrodes simultaneously registered nerve discharges 12.5% of the time with a mean signal to noise ratio of 4.3 ± 2.3 (range 2–8.5). Widely spaced electrodes registered nerve discharges 75% of the time with a signal to noise ratio of 12.2 ± 10.4 (range 2–50). Figure 2A shows a typical example of simultaneous SGNA, closely spaced SCNA and widely spaced SCNA. These data show that widely spaced electrodes had better signal to noise ratio than closely spaced electrodes. Figure 2B shows that each signal has frequency content between 150 – 250 Hz which increases with an increase in nerve activity. When this frequency content is quantified, a large increase is seen at the point of increased nerve activity as the FFT window slides along the signal.

Integrated nerve activities and heart rate—iSCNA correlated positively with iSGNA in all dogs. The plots in Figure 3 show the results from Dog B in Table 1. Figure 3A shows iSGNA plotted against iSCNA. The average correlation coefficient for all dogs studied was 0.70 (95% CI 0.61 to 0.84). The correlation between iSGNA and iSCNA was positive for all dogs (Table 1). The iSCNA and iVNA correlation showed an L-shaped pattern (Figure 3B), as did the correlation between iSGNA and iVNA (Figure 3C). The L-shaped correlation¹⁷ was observed in all 7 dogs. A second set of comparisons was performed to determine the relationship between heart rate and nerve activities. As shown in Figures 3D and 3E, there was a significant positive correlation between heart rate and iSGNA, and between heart rate and iSCNA, respectively. There was an L-shaped correlation between heart rate and iVNA

(Figure 3F). Table 1 demonstrates that the correlation between heart rate and iSCNA (average $r=0.74$, 95% CI 0.68 to 0.80, $N=7$) was significantly better than the correlation between heart rate and iSGNA (average $r=0.56$, 95% CI 0.45 to 0.67, $N=7$, $p=0.0135$) in all dogs.

Circadian Variation of iSGNA and iSCNA—We plotted the hourly iSGNA, iSCNA and iVNA over a 24-hour period (Figures 4A–G). iSCNA showed circadian variation in all dogs. The iSGNA showed circadian variation in all dogs except dog E. In contrast, only 3 dogs (C, D and G) showed circadian variation of iVNA. Figure 4H shows aggregated data of all 7 dogs. Both iSCNA and iSGNA had circadian variation, while iVNA showcased a linear pattern.

Protocol 2: Subcutaneous Nerve Activity and Movement

We identified a total of 120 episodes of movement, including 64 episodes (53.3%) from dog 1, 26 episodes (21.7%) from dog 2 and 30 episodes (25%) from dog 3. These movements included standing up, walking around or sitting down. In addition, we recorded 15 episodes of dogs climbing on the cage. In the analysis of one dog, 95.8% of the time the presence of SCNA was directly proportional to the movement of the dog. The results among the 3 dogs analyzed were very consistent. Figure 5 shows that when the dog stood up and walked around, SGNA was activated. However, SCNA was observed only during transient tachycardia (arrow). Increased heart rate, SCNA and SGNA were also observed while the dogs changed from sitting to standing or from standing to sitting. There was usually no SCNA when the dog was lying on the floor. There was increased SCNA when the dog was barking, moving, jumping and climbing the cage consistent with increase in sympathetic tone. We noted that cage climbing and barking (which should have produced large muscle movements) were not a cause of the high frequency activity typical for SCNA.

Protocol 3: Effects of apamin on right stellate ganglion and subcutaneous nerve activity

Apamin injection into the right stellate ganglion increased right SGNA, SCNA and heart rate in both dogs studied (Figure 6A). There is a strong correlation between the right SCNA and right SGNA (Figures 6B, 6C, 6E and 6F) and between heart rate and right SGNA (Figures 6D and 6G) in both dogs studied. The correlation coefficients between heart rate and 4-cm SCNA and 18-cm SCNA were, respectively, 0.867 ($p=0.002$) and 0.917 ($p=0.001$) for dog 1 and 0.868 ($p=0.002$) and 0.900 ($p=0.001$) for dog 2.

Immunocytochemical Studies—Tyrosine hydroxylase-positive structures were identified in 6 of the 6 samples examined. Figure 7 shows an example of tyrosine hydroxylase staining of subcutaneous nerves near the 4-cm bipolar electrodes of dog 2 in Protocol 3. Arrows point to the positively stained sympathetic component within that subcutaneous nerve. Similar to that found in human skin,⁹ these sympathetic nerves were located close to the subcutaneous blood vessels.

Discussion

Sympathetic innervation to the heart and the chest wall

Cardiac sympathetic innervation comes from the paravertebral cervical and thoracic ganglia.¹⁸ Among them, the stellate (cervicothoracic) ganglion is a major source of sympathetic innervation. It connects constantly with phrenic nerves and almost as often to the vagal nerves.¹⁸ The paravertebral ganglia also directly connect with spinal nerves,¹⁹ which connect with the intercostal nerves.²⁰ These intercostal nerves split into ramus cutaneous lateralis and a deep branch to the musculus rectus abdominis.²¹ Histological studies of human skin biopsy confirmed the presence of abundant sympathetic nerves in arteriovenous anastomoses, arrector pilorum muscles, and arterioles.⁹ Using horseradish peroxidase as tracer, Baron et al.¹¹ found that all skin sensory and sympathetic neurons are located ipsilaterally. Nearly all sympathetic somata are located in the middle cervical and stellate ganglia. Because of the direct and extensive connections among various nerve structures, it is possible for the sympathetic nerves in the various structures to activate simultaneously. Using bipolar electrodes located in the chest wall, we aimed to obtain good ECG signals for heart rate analyses and, in the meantime, record nerve signals over a wide area in the left lateral thorax. We documented the simultaneous or nearly simultaneous onset and offset of nerve activities in the thoracic subcutaneous space and the stellate ganglion, and that these nerve activities correlate with the heart rate. These observations made it possible to directly assess cardiac sympathetic tone by electrodes embedded in the thoracic subcutaneous space of ambulatory dogs.

Sources of electrical signals from the subcutaneous space

Electrical signals from the subcutaneous space of the thorax may come from multiple different sources, including low frequency motion artifacts, electrocardiogram generated by the heart, respiratory muscle activity and nerve activities. The nerve activities may include electrical activities from motor, sensory and autonomic nerves. High-pass filtering can eliminate the low frequency electrical activities including the electrocardiogram, leaving mostly the high frequency electrical activities for analyses. Manual analyses of the recordings showed that the high amplitude electrical signals in the subcutaneous space occurred simultaneously or nearly simultaneously with the SGNA, leading to heart rate acceleration. The frequency content of the nerve signals increased in parallel with nerve activation. The supraventricular tachycardia episodes were invariably preceded by SCNA. While the timing might be similar, the patterns of SGNA, VNA and SCNA were sufficiently different to rule out significant cross talk among the channels. Therefore, a major component of electrical activity on the SCNA recording appeared to be sympathetic.

Correlation between iSCNA and heart rate

We unexpectedly found a better correlation between the left iSCNA and heart rate than between left iSGNA and heart rate. One possible explanation was that we recorded the left SGNA. Had we recorded the right SGNA that controls the sinus node, then the correlation between SGNA and heart rate might have been better than that observed in the present study. The Protocol 3 of the study was used to test that hypothesis. We found that there was a strong correlation between heart rate and right iSGNA, a finding that supports the latter

hypothesis. A second possibility is that the stellate ganglion contains tyrosine hydroxylase-negative and cholineacetyltransferase-positive ganglion cells.^{22, 23} Activation of these cholinergic ganglion cells may be recorded as part of the SGNA. A third possibility was incomplete elimination of the ECG by high-pass filtering. Elevated heart rate increases the ECG contamination, which might artificially improve the correlation between iSCNA with heart rate. This third hypothesis was also tested in Protocol 3, which uses equipment with a higher sampling rate and wider frequency band width. By high pass filtering at 500 Hz, we were able to more completely eliminate the surface ECG signals and other low frequency artifacts. The correlation between iSCNA and heart rate in both dogs were strong. A fourth possibility is that due to the limitation of the recording equipment, not all nerve signals are recorded. The DSI D70EEE transmitter is designed to record low frequency signals, such as ECG. There is significant amplitude and phase modulation of the signals at higher frequencies. When compared with recording equipment that has wider bandwidth, we found that the timing of the nerve discharges on D70EEE was accurate, but the morphology of the signal was distorted.¹ Because of the missing signals in the nerve recordings, the correlation between either iSCNA or iSGNA with heart rate may also be distorted in Protocol 1.

Clinical implications

The present study showed that it was possible to record sympathetic nerve activities from subcutaneous tissues of ambulatory dogs. This method may be useful in risk stratification using implanted devices.

Limitations

As compared with the widely spaced electrodes, the closely (4-cm) spaced electrodes had reduced signal to noise ratio as compared with electrodes with 18-cm spacing. This problem has reduced the ability of narrowly spaced electrodes to record nerve signals. However, when the recording equipment is capable of recording with wide band width and high sampling rates (such as that used in Protocol 3), we were able to high pass filter at 500 Hz to improve the signal to noise ratio and improve the correlations. These findings suggest that with better recording equipment, it may be possible to accurately assess the sympathetic tone by direct subcutaneous nerve recordings using implanted devices.

Conclusions

We conclude that nerve activities can be recorded by electrodes implanted in the subcutaneous space. The SCNA correlates with the SGNA and can be used to estimate cardiac sympathetic tone in ambulatory dogs.

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Figure 1.

SCNA and SGNA are associated with heart rate acceleration. This example is from Dog A. The first portion of Panel A shows rhythmic heart rate (HR) variations consistent with respiratory heart rate responses (RHR). SGNA and SCNA (downward arrows) activated simultaneously, resulting in heart rate acceleration (upward arrow). There were no obvious changes of VNA in this recording. Simultaneous cessation of the SGNA and SCNA was associated with a reduction of the heart rate and the resumption of RHR. B shows simultaneous activation of SGNA, SCNA (downward arrows) in the same dog 25 s after Panel A. Downward arrows point to simultaneous nerve activities in SGNA and SCNA. Upward arrow indicates the onset of tachycardia. Panel C is from Dog D. It shows the onset of SCNA (first downward arrow) initiated persistent heart rate acceleration for over 20 s. In this episode, the SGNA and VNA occurred after SCNA. Reduction of these nerve activities was followed by heart rate deceleration. Panel D is from Dog C. SGNA, SCNA and VNA had nearly identical times of onset and offset. However, the morphology of SCNA differed considerably from that of SGNA and VNA. The latter findings were inconsistent with cross talk among channels. HASDA, high amplitude spike discharge activity. ECG, electrocardiogram.

Figure 2.

Narrowly spaced versus widely spaced subcutaneous electrodes for SCNA recording. The SCNA1 was recorded with a pair of electrodes separated by 4 cm interelectrode distance. The SCNA2 was recorded with widely spaced subcutaneous electrodes, one in the upper left thorax and the other in the abdomen. Panel A shows that simultaneous onset of SGNA, SCNA1 and SCNA2 was associated with tachycardia. The SCNA2 has a better signal to noise ratio than SCNA1. Different time windows in each recording are labeled as (a) – baseline; (b) – nerve activity; (c) – post increase in nerve activity. Corresponding FFTs for those time windows are shown in Panel B, indicating an increase in frequency content as the nerve activity increased. A quantification of this increased frequency content is shown in the far right of Panel B as an integration of the area in the resulting FFT between 150–250 Hz normalized to the baseline level. Due to the limitation of the DSI-D70EEE bandwidth, no data are available above 250 Hz.

Figure 3.

Relationships between integrated nerve activities and heart rate over a 24-hr period are plotted in a representative dog (dog B). Each dot represents the integrated nerve activity (mV-s) or heart rate (bpm) over one min. A shows a positive correlation between integrated SGNA (iSGNA) and integrated SCNA (iSCNA). B shows an L-shaped correlation between iSCNA and iVNA. C shows an L-shaped correlation between iSGNA and iVNA. D shows heart rate correlates positively with iSGNA. E shows heart rate correlates positively with iSCNA. Note that the correlation coefficient in E is better than that in D. F shows a poor correlation between heart rate and iVNA.

Figure 4.

Circadian variation of iSCNA, iSGNA, and iVNA. A 24-hour period was measured for all seven dogs (A–G). The aggregate data are shown in Panel H. The curves are fitted using cubic smoothing spline (A–G) and generalized additive mixed-effects model (H). The iSCNA showed strong evidence of circadian variation in all 7 dogs, while iSGNA showed circadian variation in 6 dogs (all but dog E). Only 3 dogs (dogs C, D and G) showed circadian variation in iVNA. From the aggregate data, it can be shown that both iSCNA and iSGNA have a circadian pattern, while iVNA does not have circadian variation.

Figure 5.

Nerve activity while standing. The dog was standing and walking during this 30 s episode. The times of the video images 1, 2, 3, 4 were labeled on the SCNA channel. Note that standing was associated with activation of the SGNA. Abrupt onset of heart rate acceleration (arrow) was associated with SGNA withdrawal and SCNA activation. There were heart rate variations at the beginning and the end of the episodes, consistent with RHR. However, the SCNA channel did not record activities of the respiratory muscle.

Figure 6.

Effects of apamin on right SGNA, right SCNA and heart rate. A shows that apamin injection into the stellate ganglion (arrows) increased right SGNA, SCNA1 (4-cm interelectrode spacing) and SCNA-2 (18-cm interelectrode spacing). Note that the maximum amplitude recorded with the 4-cm spaced electrodes is lower than the amplitude recorded with the widely spaced electrodes. B–D demonstrate a good positive correlation between iSGNA and iSCNA as well as good positive correlation between iSGNA and HR in dog 1. As some of the data points were not normally distributed, Spearman's rho coefficients were reported in dog 1. E–G show similar findings in dog 2. bpm=beats per minute. Units for SGNA ECG and SCNA are listed in mV (A), units for iSGNA and iSCNA are listed in mV-s (B–G). All nerve activities are displayed after 500 Hz high pass filter.

Figure 7.

Tyrosine hydroxylase stain of the subcutaneous tissues near the recording site. The tissue was harvested from the second dog in Protocol 3. Arrows point to the positively stained sympathetic nerves. These nerves are located next to a small artery. Objective lens magnification is 20X.

Table 1

Pearson Correlation Coefficients Between Nerve Activities and Heart Rate for Each Dog

Dog #	ISGNA vs. iSCNA	iSCNA vs. iVNA	iSCNA vs. iVNA	HR vs. iSCNA	HR vs. iSGNA	HR vs. iVNA
A	r = 0.78 p < 0.0005	0.18 < 0.0005	0.23 < 0.0005	0.66 < 0.0005	0.55 < 0.0005	0.10 < 0.0005
B	r = 0.78 p < 0.0005	0.09 0.001	0.004 0.843	0.80 < 0.0005	0.59 < 0.0005	0.26 < 0.0005
C	r = 0.77 p < 0.0005	0.68 < 0.0005	0.80 < 0.0005	0.79 < 0.0005	0.71 < 0.0005	0.63 < 0.0005
D	r = 0.71 p < 0.0005	0.85 < 0.0005	0.68 < 0.0005	0.74 < 0.0005	0.48 < 0.0005	0.66 < 0.0005
E	r = 0.54 p < 0.0005	0.11 < 0.0005	0.54 < 0.0005	0.80 < 0.0005	0.36 < 0.0005	0.12 < 0.0005
F	r = 0.59 p < 0.0005	0.58 < 0.0005	0.68 < 0.0005	0.63 < 0.0005	0.55 < 0.0005	0.43 < 0.0005
G	r = 0.69 p < 0.0005	0.56 < 0.0005	0.53 < 0.0005	0.76 < 0.0005	0.69 < 0.0005	0.49 < 0.0005

HR, heart rate