STABILITY OF VISCERAL BEHAVIOR IN THE AWAKE RAT DURING REST

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Brady and colleagues have championed the importance of careful delineation and control of a subject's behavioral state. In this paper we develop the concept of *visceral behavior* from a physiological perspective. Sprague-Dawley rats were instrumented to record arterial blood pressure, renal sympathetic nerve activity, and respiration. The rats were restrained in a conical cloth sock. Rats that were well adapted to the sock restraint showed a regular respiratory pattern and consistent pressure recordings; they rested quietly in the sock and moved only occasionally to adjust their position. Fourier analysis of blood pressure and nerve activity showed a concentration of power below 1 Hz. The coherence between the two signals was strong (0.83 ± 0.03) at 0.42 Hz. Conversely, during their initial adjustment to the sock restraint, the rats tended to show large fluctuations in blood pressure associated with episodic apneic breathing; 1 animal displayed this pattern of visceral behavior throughout most of the experiment. Despite this instability in pressure, the rats' overt behavior was stable: They rested quietly in the sock with only occasional position shifts. Spectral analysis and coherence computations showed large shifts in the distribution of power and frequency range over which arterial pressure and sympathetic activity were tightly coupled. These data are consistent with the view that an animal's circulatory adjustments, as well as adjustments in other aspects of its physiological state, constitute an important aspect of behavior, and that this behavior can influence the interpretation of biobehavioral data.

Key words: autonomic nervous system, blood pressure, respiration, power spectrum, rat

J. V. Brady's professional career has emphasized the importance of the control and stability of an experimental subject's behavior. In contrast, physiologists tend to focus their primary attention upon visceral, rather than somatic, function. With the advent of implantable transducers, many physiologists are now studying organ function in unanesthetized subjects. These subjects obviously interact with their environment and display many different somatic behavior patterns. This has required the physiologist to understand and appreciate the possible influence of the subjects' behavior upon the biological systems of interest at the moment.

This new appreciation has prompted novel ways to understand and classify behavior. For example, Engel and colleagues have pointed out that the cardiovascular responses of an organism may be considered to be an aspect of its overall behavior (Engel, 1986). More explicitly, they hold that "behavior is an inte-

grated set of responses to environmental stimuli, and that circulation is one of the response systems that constitute behavior" (Engel & Talan, 1991, p. II-9).

Physiologists are beginning to be receptive to the implications of this broader definition of behavior. It is apparent that skills from many disciplines will be required to understand how the nervous system is organized with respect to adapting an animal to its environment. In this regard, physiologists and behaviorists, as epitomized by Brady and colleagues, have professional armamentaria that must be applied in concert to solve effectively the most challenging problems facing our respective disciplines.

In this paper we use behavioral and physiological methodologies to develop one aspect of what we term *visceral behavior*. The work of Engel and colleagues has dealt largely with their subjects' cardiovascular adjustments *during* a specified activity such as exercise (Engel & Joseph, 1982). In this broader context, we now examine our subjects' behavior during periods prior to or between bouts of a specifically elicited behavior. We note that under certain circumstances, blood pressure or other physiological variables can fluctuate markedly, even though the subject's overt behavior is stable and apparently quiet.

This work was supported by grants from the Kentucky Affiliate, American Heart Association and the Kentucky Tobacco and Health Research Institute. Reprints may be obtained from the authors at Department of Physiology and Biophysics, College of Medicine, University of Kentucky, Lexington, Kentucky 40536-0084.

METHOD

Subjects

The experiments described here were performed using 9 Sprague-Dawley rats that weighed between 375 and 450 g. The animals were maintained on standard rat chow ad libitum throughout the experiment. The principles for care and use of animals specified by the American Physiological Society were followed throughout this experiment.

Physiological Preparation

The rats were anesthetized with pentobarbital (35 mg/kg, i.p.) in preparation for implantation of an arterial catheter. Surgery was conducted using "clean" techniques; infection was not a problem. A rat was positioned on its back and an incision made on the ventral surface at the base of the tail to expose the caudal artery. A Teflon® catheter (#30 LW, 0.012 in. inside diameter) was inserted 2 to 4 cm into the vessel and then tied. The animal was rotated to its side and the left kidney exposed. A renal sympathetic nerve was identified, and a small section was freed by dissection; it was placed on fine, closely spaced bipolar gold electrodes (A-M Systems Inc.). The exposed nerve and electrode were encased in silicon gel. A pair of twisted wires previously soldered to the free ends of the gold wire were exited from the body. The end of the catheter and the electrode leads were tunneled under the skin, exited at the nape of the neck, and led through a flexible wire tether. The distal end of this catheter was glued to a short segment of PE 50 tubing to facilitate coupling to a pressure transducer at a later time. The animal was returned to its home cage, where it was free to roam subject to the nominal restrictions imposed by the tether.

Experimental Procedures

The animals were habituated to handling and restraint in a soft, conical terry-cloth sock. The rats were shown the large opening of the sock and gently encouraged to enter. The end of the cone had been cut from the sock so the rat's nose protruded into room air. Details of sock habituation are given elsewhere (Randall et al., 1993). The rat's respiration was recorded by encircling its chest with a mercury-filled silastic tube (Whitney gauge); when connected to a bridge circuit, changes in the

resistance of the gauge are proportional to the amplitude of chest excursions as the animal breathes.

Data Acquisition and Analysis

The arterial pressure signal from a transducer (Cobe Model CDX-III) attached to the caudal artery catheter was recorded on a Grass Model-7 polygraph. The electrical signal from the renal sympathetic nerve was amplified (50 K) and band pass filtered between 300 and 3 kHz by a Grass P511 differential amplifier and displayed on a Tektronix 511 oscilloscope.

Renal sympathetic nerve activity (SNA), arterial pressure, and respiration were recorded for 10 min and were initially digitized at 10,000 samples per second using a Cache 486 microprocessor and Data Translation DT2821-F analog to digital converter and then reduced to 1,000 samples per second before storage to disk (see Brown, Brown, Patwardhan, & Randall, in press, for details). The digitized SNA signal was full-wave rectified and integrated every 0.005 s. Pulse intervals were determined from the stored data by finding the end diastole from the pulsatile blood pressure signal.

RESULTS

Figure 1 shows a digital reconstruction of a segment of a longer recording of respiration, heart rate, renal sympathetic nerve activity, and mean arterial pressure (MAP) in a sock-restrained rat. The animal typically snuggled into the sock as far as possible, its nose protruding at the small opening at the apex of the cone. The rat whose data are shown here was not struggling and, other than occasional shifts in position, rested quietly during most of the 10-min recording. Although there was some moment-to-moment variability in respiratory frequency, the rhythm and amplitude of the chest excursions occurred within a fairly narrow range of values. Although the respiratory variation in heart rate in the awake rat in our experience is not large, there is some evidence of a sinus arrhythmia. As we report elsewhere (Brown et al., in press), SNA occurred in bursts; this periodicity was loosely linked to the arterial pressure pulse, but nerve activity was often quiet during individual pressure pulses. Finally, MAP showed only small fluctuations that seldom exceeded 10 mmHg.

Figure 2 shows a power spectral analysis of 9.56 min of blood pressure (top) and sympa-

Fig. 1. Digital reconstruction of a 20-s segment from a 10-min recording of respiration (RESP, arbitrary units), heart rate (HR, beats per minute), sympathetic nerve activity (SNA, arbitrary units), and mean arterial pressure (MAP, mmHg) in a sock-restrained awake rat. Amplitude and frequency of chest excursions are regular. Nerve traffic occurs in bursts, although these do not show any obvious pattern with respect to other variables. Mean arterial pressure shows small fluctuations, generally less than 10 mmHg.

thetic nerve activity (middle) from this same animal. Spectral analysis objectively quantifies the variability of any given physiological signal, provided that care is given to the mathematical requirements of the process and the behavioral status of the subject (Brown, Randall, Knapp, Lee, & Yingling, 1989). Both spectra show large concentrations of power at frequencies below 1 Hz. The spectral peaks seen here indicate that both MAP and SNA oscillated (i.e., are periodic), with maxima and minima occurring at a rate of one per second or less. The peak frequency for SNA of 0.34 Hz exceeded the peak frequency for MAP of 0.15 Hz.

Arterial blood pressure and sympathetic activity should be tightly coupled by physiolog-

ical control mechanisms such as the baroreflex. The tightness of this coupling can be measured across frequency by computing the coherence between the two signals. That is, two variables that are tightly coupled and whose signals contain no noise will have a coherence approaching 1.0; the computation of coherence will approach zero for two variables that fluctuate independently, or whose relationship is obscured by a poor signal-to-noise ratio. The bottom panel in Figure 2 shows the coherence between SNA and MAP. Note that the computed value is high, .86, at 0.34 Hz. This maximum coherence occurred at a frequency near the peak in SNA spectral power at ≈ 0.4 Hz; conversely, the peak in MAP spectral power occurred at a much lower frequency,

Fig. 2. Power spectral analysis of sympathetic nerve activity (top) and arterial pressure (middle) based upon entire 10-min recording from the rat in Figure 1. Both spectra show concentrations of power below 1 Hz, although the peak in spectrum for nerve traffic occurs at higher frequency than for blood pressure. Bottom panel shows coherence between nerve traffic and arterial pressure. Coherence is high at ≈ 0.4 Hz, dropping for higher and lower frequencies.

although there was a modest secondary peak in the pressure power spectrum at or about 0.4 Hz. For 7 rats, the average peak coherence was $.83 \pm .03$ at a frequency of 0.42 ± 0.03 Hz.

Figure 3 shows a digital reconstruction of a 40-s segment of data in another rat. This rat's overt behavior was similar to that of the previous subject: It was snuggled into the sock and appeared to be resting comfortably. Its physiological "behavior," however, was markedly different. First, this rat showed alternating periods of apnea, with occasional gasps and deep but fairly regular breathing. Second, there were pronounced oscillations in both SNA and MAP that appeared to be tightly coupled with the respiratory variations. Note especially that the amplitudes of the arterial pressure oscillations were several times larger than those in the 1st animal. Figure 4 shows the spectral analysis of 9.56 min of data from the 2nd rat. In contrast to the previous analysis, there was a strong coupling between the two signals (i.e., a high coherence), not only at 0.4 Hz but at much lower frequencies as well.

Most of the rats showed at least short periods of instability in their physiological be-

havior. These episodes occurred most frequently following the subjects' initial introduction to the sock restraint or, in another set of experiments (Randall et al., 1993), after delivery of tail shock as part of a Pavlovian conditioning paradigm. The rat whose data are shown in Figures 3 and 4 experienced the longest episode of physiologically unstable behavior. However, when this rat was returned to its home cage, the large oscillations in blood pressure and heart rate ceased.

DISCUSSION

One of us (D.C.R.), while working in the Division of Behavioral Biology at The Johns Hopkins University in collaboration with Brady and colleagues, began to develop a Pavlovian conditioning procedure to study the autonomic control of cardiac function (e.g., Randall, Kaye, Randall, Brady, & Martin, 1976). The conditional stimulus (CS+) activated the cardiac sympathetic and parasympathetic cardioregulatory pathways, effectively mimicking the physiologist's stimulation of nerve centers in the anesthetized animal. The behavioral paradigm was advantageous with

Fig. 3. Digital reconstruction of recordings from a sock-restrained rat showing periods of apnea. Details as in Figure 1. Note corresponding pattern in renal sympathetic nerve activity and large oscillations in arterial pressure. The rat was not struggling in the sock, giving the appearance of being at rest despite the instability in physiological function.

respect to our overall goals because one can repeatedly elicit reproducible, neurally mediated changes in heart rate and blood pressure with a high degree of experimental control.

Our original experiments were performed in primates (e.g., Randall et al., 1976) and dogs (e.g., Billman & Randall, 1980). These animals tend to rest quietly during intertrial periods, so the conditional response patterns emerged clearly above stable "resting" levels of heart rate and arterial blood pressure. We recently developed techniques for recording renal sympathetic nerve activity in the unanesthetized rat (Brown, 1991; Brown & Randall, 1989). Concurrently, we developed a Pavlovian conditioning paradigm similar to our canine model and very recently described the conditional blood-pressure response pattern (Randall et al., 1993).

In the conditioning experiments with rats, we restrained the animals in a soft cloth pouch; this seemed to satisfy the rat's tendency to conceal itself during stress. To all appearances, the rats' gross overt (i.e., somatic) movements were minimal between presentations of the conditional stimuli. Despite the apparent stability of this overt behavior, we recorded instances of instability in blood pressure and heart rate during the rest periods (Randall et al., 1993). The importance of maintaining stable visceral as well as somatic behavior quickly emerged.

We made very little progress in understanding the nature of the instability in the rats' cardiovascular status until we started to record their respiratory patterns. That is, we could not understand why blood pressure oscillated so markedly even though the animal appeared

Fig. 4. Power spectra and coherence for the full set of data shown in Figure 3. Note that peaks in power spectra and coherence spectrum occur at lower frequencies than in the previous example. Instability in visceral behavior, despite apparent stability in overt behavior, is associated with a striking change in the relationship between sympathetic nerve activity and blood pressure.

to be resting quietly. Although we believe that the concepts we present here are relevant to any mammalian species, our previous experience with dogs and primates did not prepare us for this situation. This was myopic on our part, because our dogs undoubtedly had episodes of panting that must have been reflected in multiple aspects of the cardiovascular function, even if we did not fully appreciate the impact on their visceral behavior.

The phenomenon we report here is reminiscent of earlier observations by W. H. Gantt, who worked in the Division of Behavioral Biology at Johns Hopkins University during the early years of Brady's tenure there. Gantt championed the concept of *schizokinesis* (reviewed in Gantt, 1980). According to Reese's (1982) summary of Gantt's concept, "schizokinesis specifically involves (1) a lack of parallel between general autonomic patterns, such as respiratory and cardiac, and the more voluntary motor patterns, and (2) persistence of maladaptive reactions to the environment" (p. 113). Gantt felt that schizokinesis and another phenomenon, which he called *autokinesis*

(Gantt, 1953), were important concepts relative to the development of psychological disorders. Visceral instability in our rats clearly occurred when the animals were introduced to a novel environment (initial stages of habituation to sock restraint) or after acute stress (tail shock); both of these seem to be consonant with the contexts within which Gantt described schizokinesis and autokinesis.

We are impressed by the marked change, described above, in the coherence between MAP and SNA as a function of frequency. Regulation of blood pressure is among the most vital of physiological functions, and changes in this regulation occurring in hypertensive individuals are clinically important. The broadening of the range of tight coupling between pressure and nerve activity, especially if prolonged, must eventually have important implications for the overall function and health of the organism. It might be possible, with methodologies used in the rat experiment, to quantify important changes in the interaction of an individual with his or her environment; such interactions have been difficult to measure

reliably in many previous experimental and clinical settings. It seems likely, for example, that people who appear overtly calm during periods of stress would reveal clear changes in visceral behavior, much as Gantt postulated many years ago (e.g., Gantt, 1953). If so, quantification of these changes by power spectral and coherence analysis might help to clarify the critical underlying biobehavioral relationships.

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