Research Report

DISTRESS VOCALIZATIONS IN INFANT RATS: What's All the Fuss About?

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Abstract-Ultrasonic vocalizations emitted by infant rodents are typically characterized as cries of distress. There are two contexts that are known to reliably elicit ultrasound production: extreme cold exposure and administration of clonidine, an α_2 adrenoceptor agonist. Noting that these two contexts both entail pronounced decreases in cardiac rate, we have hypothesized that the vocalizations are acoustic by-products of a physiological maneuver, the abdominal compression reaction (ACR), that increases venous return to the heart when return is compromised. As a critical test of this hypothesis, we measured venous pressure near the right atrium in 15-day-old rats after clonidine administration. Consistent with the ACR hypothesis, emission of ultrasound was accompanied by large and reliable increases in venous pressure and, therefore, venous return. These results provide strong, direct support for the ACR hypothesis and, by doing so, underscore the potential pitfalls of anthropomorphic interpretations of the vocalizations of infant rats.

Singing, crying, yelling. Sneezing, coughing, wheezing. Both groups of activities entail the production of sound, but the inferences we draw from these sounds are very different. Whereas the former group suggests emotional states such as joy, sorrow, or anger, the latter group suggests physical ailments such as a cold, an airway obstruction, or a respiratory infection. All these sounds may attract attention from other people, but they are not all emitted in order to attract attention from others. Thus, one might say that the former group of sounds represents voluntary communicatory acts, whereas the latter group represents involuntary physiological or biomechanical processes that produce sound as a by-product.

Since its discovery 45 years ago, the ultrasonic distress call emitted by infant rodents when separated from the nest has been interpreted as a cry for help to stimulate the mother to retrieve the pup to the warmth and comfort of the nest (Noirot, 1972; Sewell, 1970). But, how can we know if the call is more akin to a cry or a cough? We cannot read the pup's mind. Perhaps, however, we should look to its heart.

For example, when infant rats are exposed to extreme cold, cooling of the cardiac muscle cannot be prevented and cardiac rate plummets (Blumberg, Sokoloff, & Kirby, 1997); it is at this time that pups begin to vocalize (Blumberg & Sokoloff, 1998; Blumberg, Sokoloff, & Kent, in press). Moreover, administration of the α_2 adrenoceptor agonist clonidine, in addition to evoking ultrasound (Hård, Engel, & Lindh, 1988; Kehoe & Harris, 1989), causes large (~25%) reductions in cardiac rate (Blumberg, Sokoloff, & Kent, 1999; Sokoloff, Blumberg, Mendella, & Brown, 1997). For infant mammals, even precocial mammals, that are limited in their ability to increase stroke volume (Shaddy, Tyndall, Teitel, Li, & Rudolph, 1988), such substantial decreases in cardiac rate also diminish cardiac output and venous return.

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Might there be a causal connection between bradycardia and ultrasound production? We have hypothesized (Kirby & Blumberg, 1998) that the ultrasonic vocalization is the acoustic by-product of a maneuver, the abdominal compression reaction (ACR), that helps propel venous blood back to the heart through contraction of the abdominal muscles during expiration (Youmans, Tjioe, & Tong, 1974). The larynx, we suggest, is utilized as a brake during expiration, thus contributing to the buildup in intraabdominal pressure and resulting in the production of sound as a by-product. According to this perspective, an infant rat no more vocalizes in order to call its mother than a child sneezes in order to ask her parent for a tissue.

We have shown previously that a vocalizing infant rat generates large pressure pulses within the abdomen (Kirby & Blumberg, 1998). This observation alone, however, does not permit the conclusion that venous return is increasing. In fact, sustained straining against a constricted larynx, such as with the Valsalva maneuver during heavy lifting, actually decreases venous return (Berne & Levy, 1977). To resolve this issue, we conducted a critical test of the ACR hypothesis by measuring venous pressure in 15-day-old rats before and after injection with clonidine. We chose 15-day-olds for this study because they exhibit maximal ultrasonic responses to clonidine administration (Hård et al., 1988; Kehoe & Harris, 1989) and because their body size is sufficiently large to permit catheterization of the external jugular vein for measurement of venous pressure. Our results support the ACR hypothesis and provide strong evidence of a conjunction between the physiological causes and physiological consequences of ultrasound production.

METHOD

Subjects

Five 15-day-old male and female rat pups from five litters were used. At the time of surgery, the pups weighed 32.3 to 37.8 g. They were born to Harlan Sprague-Dawley females in the animal colony at the University of Iowa and were raised in litters that were culled to 8 pups within 3 days after birth (day of birth = Day 0). Litters and mothers were housed in standard laboratory cages ($48 \times 20 \times 26$ cm) in which food and water were available *ad libitum*. All animals were maintained on a 12-hr/12-hr light/dark schedule with lights on at 6:00 a.m.

Test Environment

Pups were tested inside a double-walled glass chamber. Air temperature within the chamber was controlled by passing temperaturecontrolled water through the chamber walls. Pups were allowed to move freely inside the chamber on a platform constructed of polyethylene mesh.

Surgery and Venous-Pressure Measurements

For direct recording of venous pressure, the external jugular vein was catheterized on the day of testing. The catheter was constructed from Micro-Renathane tubing (MRE-040; Braintree Scientific, Inc., Braintree, Mass.) with a 1-cm tip hand-drawn under a stream of hot air. The catheter was filled with heparinized (20 IU/ml) isotonic saline. For catheter implantation, pups were anesthetized with ether, and the right external jugular vein exposed just anterior to its junction with the axillary vein. Using a stereomicroscope, we introduced the catheter into the vein through a small hole made with a 25-g needle and advanced the catheter 1 to 1.5 cm into the vein. This distance placed the catheter's tip within 1 mm of the entrance to the right atrium. After checking for adequate blood flow, we sutured the catheter in place and stabilized it with a drop of cyanoacrylate. The incisions were then closed and the animal was transferred to the chamber, where it was allowed to recover at an air temperature of 32 °C.

The implanted venous catheter was connected to a pressure transducer (Argon, Athens, Tex.), and the entire length of tubing from pup to transducer was filled with heparinized saline. The output of the transducer passed through an analog-to-digital converter, whose signal was then fed into a computerized data-acquisition system with custom-designed software (LabView, National Instruments, Austin, Tex.). Before or after each pup was tested, the system was calibrated using a sphygmomanometer with a resolution of 1 mm Hg.

Ultrasonic Vocalizations

Ultrasonic vocalizations were detected using a microphone sealed inside the test chamber. The microphone was connected to a bat detector (Model SM100, Ultra Sound Advice, London, England) tuned to a \pm 5-kHz range centered on 35 kHz (Blumberg et al., 1999). The output of the microphone was amplified and fed into the same data-acquisition computer that was used for acquiring venous pressure data.

Procedure

After surgery, the pup recovered in the chamber for at least 45 min, after which time the test began with acquisition of baseline venous pressure and ultrasound data for at least 1 min. The data-acquisition system was used to acquire venous pressure and ultrasonic vocalization data at the rate of 200/s. After this baseline period, the pup was injected subcutaneously with 0.5 mg/kg clonidine hydrochloride in a volume of 1 μ l/g body weight. Data recording continued for at least 30 min after the injection. After the test, the pup was euthanized and dissected to determine catheter placement.

Data Analysis

Blood pressure and ultrasound data were imported into DataDesk 6.0 for the Macintosh. For each of the 5 subjects, scatter plots of the data were produced for both variables, and each point in the scatter plots was linked to a row number that represented the passage of 1/200 s. Then, a 10-min period of contiguous postinjection data was selected for statistical analysis. Because all subjects emitted ultrasonic vocalizations at high rates throughout the test, the primary criterion for selection of these periods was stable venous-pressure records that

were relatively free of noise (e.g., movement artifact). Such a 10-min period was found for each subject, beginning from 5 to 23 min postinjection; in all other respects, these periods were representative of each subject's complete data set. For each 10-min period, venous-pressure data (i.e., all 120,000 points) were categorized as either ultrasound-associated or non-ultrasound-associated. Finally, for each subject, the mean venous pressures for these two categories were determined, and a paired t test was used to test for significant differences between them.

RESULTS

In all 5 subjects, administration of clonidine elicited ultrasonic vocalizations that were associated with large increases in venous pressure. Data for 1 subject are shown in Figure 1, and the interrelations between venous pressure and ultrasound emission are evident. The upper plot presents the data over a 38-s period, and it is clear that bouts of ultrasound emission are associated with pronounced increases in venous pressure. A subset of these data is expanded for the lower plot in Figure 1, where it can be seen that sound production occurs immediately after the increase in venous pressure begins and ceases immediately prior to the subsequent fall in pressure.

Large pressure pulses were not always accompanied by ultrasound production. Figure 2 presents a particularly striking example. This subject often emitted bouts of ultrasound production that began with very loud vocalizations that decreased in intensity over time, as represented by the decreasing amplitude of the ultrasound signal within each bout. Interestingly, the amplitude of the venous-pressure pulses also decreased slightly over the course of each bout. In addition, there were occasions when pressure pulses were not accompanied by detectable ultrasonic emissions. This observation is consistent with our previous report of intraabdominal pressure pulses that sometimes were not associated with the emission of ultrasound (Kirby & Blumberg, 1998).

In order to provide an objective measure of the relationship between ultrasound production and venous pressure, we analyzed 10min periods of contiguous data for each subject by determining for each venous-pressure data point whether it was ultrasound-associated or non-ultrasound-associated. For each infant, mean venous pressure for each category was calculated. As expected, the ultrasoundassociated pressures were significantly greater than the nonultrasound-associated pressures, t(4) = 3.9, p < .02, thus providing objective support for the conclusions drawn from the representative data presented in Figures 1 and 2.

DISCUSSION

Ultrasound emission is reliably evoked during extreme cold exposure and after clonidine administration (Kehoe & Harris, 1989; Okon, 1971). As we have recently shown, ultrasound emission in both these contexts is accompanied by significant decreases in cardiac rate (Blumberg et al., 1999, in press). Furthermore, because cardiac output (i.e., the volume of blood pumped by the heart per unit time) is a function of cardiac rate and stroke volume, clonidine-induced reductions in cardiac rate will necessarily result in decreased cardiac output if the animal is unable to compensate by increasing stroke volume. Such compensatory increases in stroke volume are indeed unlikely for

Ultrasound in Infant Rats



Fig. 1. Venous pressure (mm Hg) and emission of ultrasonic vocalizations (arbitrary units) in a 15-day-old rat after clonidine administration (0.5 mg/kg). Data at two time scales are presented. The upper plot shows that bouts of ultrasound production are accompanied by significant increases in venous pressure. The lower plot, which expands upon a subset of the data in the upper plot, shows that the initiation of each ultrasonic pulse coincides with a rapid increase in venous pressure.

two reasons: First, infant mammals are limited in their ability to increase stroke volume above basal levels (Teitel et al., 1985), and, second, withdrawal of sympathetic activity by clonidine likely results in decreased stroke volume. Therefore, as cardiac output falls, right atrial pressure will increase and, as a result, there will be increased resistance to venous return (Guyton & Hall, 1996). Furthermore, clonidine's negative effect on venous return will be exacerbated if withdrawal of sympathetic activity also results in venous dilation: Venodilation causes pooling of venous blood and decreased venous return (Guyton & Hall, 1996).

Given these considerations, it is likely that clonidine administration results in substantial decreases in venous return in infant rats. We have hypothesized that infant rats respond to this problem by recruiting the ACR to increase venous return (Kirby & Blumberg, 1998). This maneuver is a reflexive response, perhaps elicited by decreased atrial filling and subsequent activation of low-pressure atrial stretch receptors (Youmans et al., 1963). According to our hypothesis, the ACR, which comprises abdominal contractions during expiration against a constricted larynx, propels blood back to the heart and, additionally, produces the ultrasonic vocalization as a by-product. There are, however, two key features of the ACR that must be demonstrated for our hypothesis to remain viable, that is, increased intraabdominal pressure and increased venous return. The first feature has previously been documented in 8-day-olds during cold exposure



Fig. 2. Venous pressure (mm Hg) and emission of ultrasonic vocalizations (arbitrary units) in a second 15-day-old rat after clonidine administration (0.5 mg/kg). As in Figure 1, bouts of ultrasound production are accompanied by significant increases in venous pressure. There are, however, several pressure pulses that are not accompanied by ultrasound production.

(Kirby & Blumberg, 1998); the second is documented here in 15-dayolds after clonidine administration.

The present results constitute an existence proof that the maneuver that produces ultrasound is compatible with increases in venous pressure and, therefore, venous return (Fig. 1). In general, emission of ultrasound was highly predictive of pulsatile increases in venous pressure. However, there were instances of pulsatile increases in venous pressure that were not accompanied by ultrasound production (Fig. 2). This finding is consistent with previous observations that increases in intraabdominal pressure in 8-day-olds during cold exposure were sometimes unaccompanied by ultrasound production (Kirby & Blumberg, 1998), and is also consistent with the interpretation of ultrasound production as an incidental by-product of the ACR.

This report began by distinguishing between vocalizations emitted for their communicatory effects and vocalizations emitted as incidental by-products. The particular vocalization at the heart of this article, the "distress call" of infant rodents, was first discovered 45 years ago and is still the subject of numerous psychological, behavioral, acoustic, physiological, and pharmacological investigations (Blumberg & Stolba, 1996; Hofer, Masmela, Brunelli, & Shair, 1998; Kehoe & Blass, 1986; Kehoe & Harris, 1989; Sokoloff & Blumberg, 1997; Winslow & Insel, 1991a, 1991b). Currently, for many investigators, it is the fascination with vocalizations as potential signals of emotional states that fuels enthusiasm for this research and shapes the experimental questions that are asked. Specifically, these investigators view the vocalizing infant as a useful model for exploring the neurochemical bases of emotional states such as fear, distress, and anxiety (Miczek, Weerts, Vivian, & Barros, 1995; Winslow & Insel, 1991b).

The present experiment arises from an alternative perspective of ultrasound production, one that assumes neither that the infant's vocalization reflects a particular emotional state nor that an emotional state is the motivating force underlying its emission. Rather, we have explored the physiological causes and consequences of ultrasound production. As a result, we can now identify a causal chain linking the physiological events that precede production of the infant's vocalization-decreased cardiac output and venous return-and the physiological events that accompany the vocalization-recruitment of the ACR and enhanced venous return. The sound emitted by the infant, although produced as a by-product of the ACR, attracts the mother's attention and elicits retrieval to the nest (Allin & Banks, 1972). It is important to emphasize that incidental emission of the vocalization does not preclude the development of a communicatory relationship between infant and mother; indeed, such relationships often arise when a receiver responds reliably to a signal in the environment, regardless of the proximate cause of the signal's emission (Blumberg & Alberts, 1997).

In conclusion, although we cannot rule out the possibility that a distinct emotional state causes, motivates, or co-occurs with emission of the infant's vocalization, it appears that the designation of the vocalization as a distress call or as crying assumes the existence of an emotional component to the behavior that is not necessary to explain its occurrence. Therefore, the present experiment serves as a reminder that "our penchant for anthropomorphic interpretations of animal behaviour is a drag on the scientific study of the causal mechanisms for it" (Kennedy, 1992, p. 5).

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