Mark S. Blumberg
Greta Sokoloff
Kristen J. Kent
Program in Behavioral and Cognitive Neuroscience
Department of Psychology
University of Iowa
Iowa City, IA 52242

A Developmental Analysis of Clonidine's Effects on Cardiac Rate and Ultrasound Production in Infant Rats

Received 27 August 1999; accepted 24 November 1999

Abstract: *Under controlled conditions, infant rats emit ultrasonic vocalizations during extreme cold exposure and after administration of the α_2 adrenoceptor agonist, clonidine. Previous investigations have determined that, in response to clonidine, ultrasound production increases through the 2nd-week postpartum and decreases thereafter. Given that sympathetic neural dominance exhibits a similar developmental pattern, and given that clonidine induces sympathetic withdrawal and bradycardia, we hypothesized that clonidine's developmental effects on cardiac rate and ultrasound production would mirror each other. Therefore, in the present experiment, the effects of clonidine administration (0.5 mg/kg) on cardiac rate and ultrasound production were examined in 2-, 8-, 15-, and 20-day-old rats. Age-related changes in ultrasound production corresponded with changes in cardiovascular variables, including baseline cardiac rate and clonidine-induced bradycardia. This experiment is discussed with regard to the hypothesis that ultrasound production is the acoustic by-product of a physiological maneuver that compensates for clonidine's detrimental effects on cardiovascular function.*
© 2000 John Wiley & Sons, Inc. Dev Psychobiol 36: 186–193, 2000

Keywords: *ultrasonic vocalizations; clonidine; anxiety; separation distress; autonomic nervous system; cardiac rate; cardiovascular; development; rat; infant*

Infant rats emit ultrasonic vocalizations when separated from the nest (Hofer & Shair, 1978; Noirot, 1972). Emission of these vocalizations is viewed as a communicatory behavior by which the pup elicits maternal retrieval (Allin & Banks, 1972). In recent years, however, it is the promise of the vocalizing rat pup as an animal model of separation distress that has attracted the greatest attention (Miczek, Weerts, Vivian, & Barros, 1995; Winslow & Insel, 1991b). Indeed, numerous investigators have now examined the potential of various pharmacological agents to modulate ultrasound production by infants (Carden, Davachi, & Hofer, 1994; Kehoe & Blass, 1986; Vivian, Barros, Manitiu, & Miczek, 1997; Vivian & Miczek, 1991; Winslow & Insel, 1991a).

It has been known for many years that cold exposure, one of the consequences of nest separation, is perhaps the most effective stimulus for eliciting ultrasound production (Allin & Banks, 1971; Okon, 1971). In general, investigators interested in the pharmacological bases of ultrasound production have viewed cold exposure merely as a cue to the animal that it is no longer residing in the comfort of the nest. A cor-

Correspondence to: M. S. Blumberg
Contract grant sponsor: NIMH
Contract grant number: MH 50701

© 2000 John Wiley & Sons, Inc.

ollary to this view is that there exists no orderly, causal connection between the physiological consequences of cold exposure and emission of the vocalization. Recent work, however, has occasioned a reassessment of this perspective by delineating the physiological consequences of varying levels of cold exposure and their relationship to ultrasound production (Blumberg & Sokoloff, 1998). This work has shown that isolated infant rats exposed to moderately cold temperatures (i.e., 25–35°C for 1-week-olds) respond by producing heat using brown adipose tissue (BAT) and, by doing so, maintain cardiac rate and rarely emit ultrasonic vocalizations. In contrast, at extremely cold temperatures (i.e., at air temperatures below 25°C for 1-week-olds), BAT thermogenesis is overwhelmed, resulting in pronounced bradycardia and emission of high rates of ultrasound production (Blumberg, Sokoloff, & Kent, 1999).

Based on these and other results, we have hypothesized that cold exposure is more than simply a signal to the infant rat that it has been separated from its mother and littermates. Specifically, we have hypothesized that extreme cold exposure initiates a cascade of events, including decreased cardiac rate and cardiac output, that culminates in recruitment of a physiological maneuver, the abdominal compression reaction (ACR), that helps to propel venous blood back to the heart when such return has been compromised (Kirby & Blumberg, 1998). According to this hypothesis, the laryngeal constriction and increased intraabdominal pressure that accompany the ACR produce ultrasound as an acoustic by-product.

In addition to cold exposure, administration of the α_2 adrenoceptor agonist, clonidine, is a reliable method for evoking ultrasound production, even at thermoneutral air temperatures (Hansen, 1993; Hård, Engel, & Lindh, 1988; Kehoe & Harris, 1989). On the one hand, it is tempting to suggest that this agent acts directly on central nervous mechanisms that mediate anxiety (Kehoe & Harris, 1989). It is difficult, however, to reconcile this anxiogenic view of clonidine with the observation that pups injected with clonidine are not quieted when exposed to maternal cues or when returned to the nest (Hansen, 1993; Kehoe & Harris, 1989).

Interestingly, clonidine, like extreme cold exposure, produces significant changes in cardiovascular function. For example, in adult animals, clonidine produces withdrawal of sympathetic tone that in turn causes significant decreases in cardiac rate (Gillis, Gatti, & Quest, 1985; Luft et al., 1986; van Zweiten, 1996). Similarly, in week-old rats, it has been reported that clonidine causes significant bradycardia and, moreover, this bradycardia was coincident with ultrasound production (Sokoloff, Blumberg, Mendella, & Brown, 1997). Thus, the possibility exists that clonidine's effect on ultrasound production, like that of extreme cold exposure, is mediated by its effects on the cardiovascular system.

Two separate groups of investigators have reported that the effect of clonidine on ultrasound production increases through the 2nd-week postpartum and declines to near-zero levels after 18 days of age (Hård et al., 1988; Kehoe & Harris, 1989). Noting that sympathetic neural dominance exhibits a similar inverted-U pattern of development (Slotkin & Seidler, 1988) and that clonidine exerts its cardiovascular effects through withdrawal of sympathetic tone, we hypothesized that clonidine's developmental effects on cardiac rate would mirror its effects on ultrasound production. Therefore, in the present experiment, 2- to 20-day-old rats were treated with clonidine (0.5 mg/kg) and cardiac rate and ultrasound production were monitored.

METHODS

Subjects

Sixty-four 2-, 8-, 15-, and 20-day-old Harlan Sprague-Dawley male and female rat pups from 27 litters were used ($n = 16$ at each age). At the time of testing, these pups weighed 6.2–8.9 g, 13.0–20.3 g, 32.2–40.6 g, and 44.6–58.8 g, respectively. All pups were born to females in the animal colony at the University of Iowa. The pups were raised in litters that were culled to 8 pups within 3 days after birth (day of birth = Day 0). Litters and mothers were raised in standard laboratory cages (48 × 20 × 26 cm) in which food and water were available ad libitum. All animals were maintained on a 12:12 hr light:dark schedule with lights on at 6:00 a.m.

Test Environment

Individual pups were tested inside a double-walled glass chamber, as described in detail elsewhere (Blumberg & Stolba, 1996). Water passed through the walls of the chamber and, by controlling the temperature of the water with a water circulator, air temperature inside the chamber could also be controlled. Compressed, humidified air passed through the chamber at the rate of 300 ml/min. A round platform constructed of polyethylene mesh was fitted inside the chamber. When placed on the platform, the pup could move freely on the platform's surface.

Electrocardiogram (ECG)

ECG data were acquired using three electrodes, two attached on either side of the thoracic cavity and a ground electrode attached near the base of the tail. Collodion was used to secure the electrodes and to improve the electrical connection. The ECG leads from the pup were connected to an impedance pneumograph (UFI, Morro Bay, CA) before being acquired by the computer. The ECG analog signal was digitized at a rate of 1,000 samples/s and interbeat intervals (IBI) were determined using a customized software program employing a peak threshold detector (this method provides results identical to those obtained through direct measurement of intervals between successive R-waves; Sokoloff, Kirby, & Blumberg, 1998). IBI data were acquired by the computer at a rate of 30 samples/min.

Ultrasonic Vocalizations

Ultrasonic vocalizations were made audible using a microphone sealed inside the lid of the metabolic chamber and connected to a detector (Model SM100, Ultra Sound Advice, London, England). Although the detector is sensitive to sounds emitted at the tuned frequency ± 5 kHz, pilot observations indicated that clonidine-treated pups of different ages emitted ultrasounds at significantly different frequencies. Therefore, based on these observations, the detector was tuned to approximately 47 kHz for 2-day-olds, 42 kHz for 8-day-olds, 35 kHz for 15-day-olds, and 25 kHz for 20-day-olds. Throughout each test, the detector was adjusted for maximum clarity and the final dominant frequency was noted.

Ultrasonic vocalizations were scored by an experienced observer. To do this, the observer used an event recorder written in HyperCard for the Macintosh and pressed a computer key each time an ultrasonic pulse was detected. Each key press recorded the time at which the ultrasonic pulse occurred.

Procedure and Data Acquisition

On the day of testing, a pup was removed from its cage, lightly anesthetized with ether, and placed on a heating pad. After ECG leads were attached, the pup was then moved to the metabolic chamber. The air temperature in the chamber was regulated at 35.5°C for the 2-day-olds, 35°C for the 8-day-olds, and 32°C for the 15- and 20-day-olds; these temperatures are within the thermoneutral range of pups at these ages (Spiers & Adair, 1986). After a recovery and acclimation period of 45 min, baseline recording of cardiac data and monitoring of ultrasound production began for a period of 5 min. Cardiac data were recorded by computer on-line and ultrasound production was scored in real time; in addition, the ECG signal was recorded to an audio channel of the videotape for subsequent analysis and scoring, if necessary. After the 5-min baseline period, the experimenter, wearing latex gloves to prevent conductive cooling of the pup, gently picked up the pup and administered a subcutaneous injection of clonidine hydrochloride (0.5 mg/kg) or saline in a volume of 1 μl/g body weight; this dose is comparable to those used in other developmental studies (Hård et al., 1988; Kehoe & Harris, 1989; Reinstein & Isaacson, 1977). Recording of cardiac data and ultrasound production continued for 30 min, after which the test ended and the pup was returned to its home cage. Same-sex littermates were assigned to one of the two conditions at each age (with one exception where the 2 pups were from different litters), and no 2 littermates were tested in the same condition. Finally, order of testing was counterbalanced.

Statistical Analysis

IBI and ultrasound data were imported into StatView 4.5 for the Macintosh. IBI data were converted to cardiac rate data in beats per min (bpm) before analysis. Values at the end of the 5-min baseline period and at three postinjection time points (i.e., 5, 15, and 30 min) were then compared. A repeated measures analysis of variance (ANOVA) was used to test for differences in cardiac rate at each time point. Post hoc ANOVAs and Fisher's PLSD were also used to examine significant main effects and interactions. For all tests, α was set at 0.05.

Cumulative amounts of ultrasound production were calculated during the 5-min baseline period and for 30 min postinjection. The Mann-Whitney *U* test was used to test for differences between groups at the end of baseline and at the end of the 30-min test. For these one-tailed tests, α was set at 0.05 and was adjusted for multiple comparisons using the Bonferroni procedure.

All means are presented with their standard errors.

RESULTS

The top row of Figure 1 presents the ultrasound data for the infant rats treated with saline or clonidine at 2, 8, 15, and 20 days of age. As expected from the work of others (Hård et al., 1988; Kehoe & Harris, 1989), 8- and 15-day-old rats treated with clonidine vocalized significantly more over the 30-min period than did those injected with saline, 8-day-olds: $U = 0$, $p <$

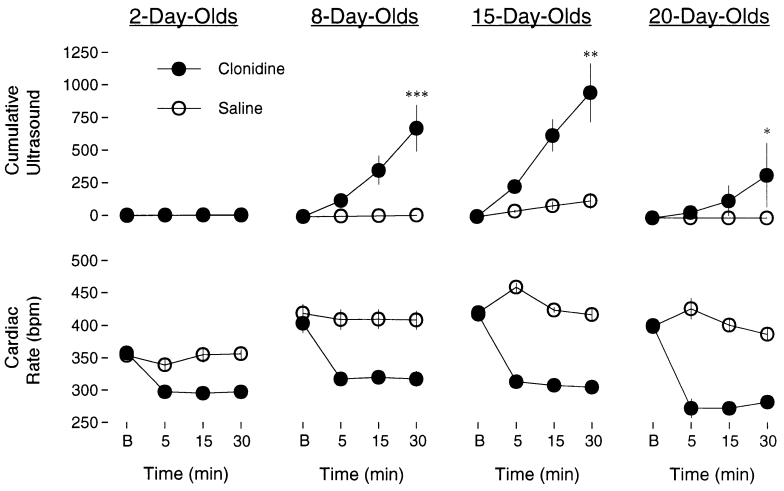

FIGURE 1 Cumulative ultrasound production and cardiac rate (bpm) during the 5-min baseline period and at 5, 15, and 30 min after clonidine adminstration. Pups were injected with saline (open circles) or with the α_2 adrenoceptor agonist, clonidine (0.5 mg/kg; filled circles); $n = 7–8$ per group. Mean ± *SEM*. Asterisks denote significant differences, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, in ultrasound production from saline over the entire 30-min test.

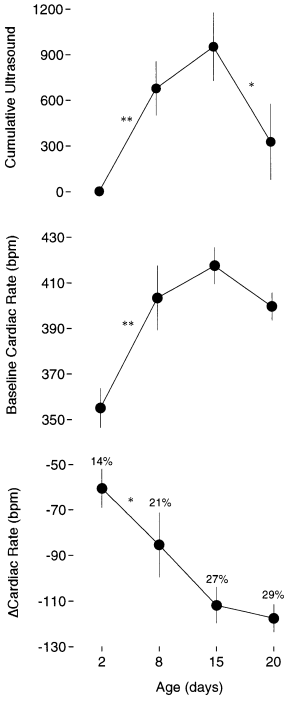

FIGURE 2 Top panel: cumulative ultrasound production for the 30-min period after injection of clonidine; $n = 7–8$ per group. Middle panel: cardiac rate (bpm) at the end of the baseline period for the clonidine-treated pups; $n = 8$ per group. Bottom panel: change in cardiac rate (ΔCardiac Rate; bpm) over the course of the 30-min period after clonidine administration; data expressed as percentage reduction in cardiac rate from baseline are also indicated; $n = 7–8$ per group. Mean ± *SEM*. Asterisks denote significant differences, $*p < .05$, $**p < .01$, between adjacent points.

0.001; 15-day-olds: $U = 2$, $p < 0.01$, but clonidine did not have this effect on 2-day-olds, $U = 66$, n.s. Contrary to these earlier reports, however, 20-day-olds did vocalize significantly more after clonidine than after saline, $U = 10$, $p < 0.05$; indeed, although 2 pups did not vocalize at all after clonidine injection, 6 pups emitted more than 50 pulses during the 30-min test, 3 of which emitted more than 100 pulses, including that emitted more than 2,000 pulses.

The bottom row of Figure 1 presents the cardiac rate data at the end of baseline and at each of the post-injection time points for the clonidine- and saline-treated pups. It is clear from these plots that clonidine elicited pronounced, rapid, and sustained decreases in cardiac rate. (One 2-day-old pup did not exhibit bradycardia after clonidine administration, most likely the result of unsuccessful drug delivery; because these data fell two standard deviations beyond the mean for that group, they are not included in the analyses below.) Repeated measures ANOVA indicated that all main effects and interactions were significant, including age, $F(3, 53) = 12.5$, $p < 0.0001$, condition, $F(1, 53) = 136.2$, $p < 0.0001$, Age × Condition, $F(3, 53) = 4.2$, $p < 0.01$, and Age × Condition × Time, $F(9, 159) = 6.9$, $p < 0.0001$.

Follow-up analyses were conducted to explore further how infant rats differ with respect to cardiac rate at baseline and after clonidine injection. First, the top panel in Figure 2 presents cumulative ultrasound production after clonidine administration for the different-aged pups. These data exhibit an age-related inverted-

U pattern of ultrasound production that is consistent with previous findings (Hård et al., 1988; Kehoe & Harris, 1989). Second, the middle panel in Figure 2 presents baseline cardiac rate data for the pups injected with clonidine. One-factor ANOVA revealed a significant effect of age on baseline cardiac rate, $F(3, 28) = 7.9$, $p < 0.001$, and post hoc tests revealed that cardiac rate increased significantly between 2 and 8 days of age but did not change significantly thereafter. This finding of increasing baseline cardiac rate during the first 2 weeks postpartum is well established (Hayne, Richardson, & Campbell, 1991; Tucker, 1985) and reflects increasing sympathetic neural activity over this time of development (Slotkin & Seidler, 1988; Tucker, 1985). Third, the bottom panel in Figure 2 presents the change in cardiac rate over the course of the 30-min test for the clonidine-treated pups. One-factor ANOVA revealed a significant effect of age on the magnitude of bradycardia produced, $F(3, 28) = 8.4$, $p < 0.0005$, and post hoc tests indicated that pups exhibited significantly smaller bradycardias at 2 days of age than at the other three ages. (As indicated on this panel, a similar developmental pattern is seen when the bradycardia is expressed as a percentage of baseline cardiac rate.) Finally, as suggested in Figure 2, there was a significant linear relationship between baseline cardiac rate and the magnitude of bradycardia produced by clonidine, $r^2 = 0.45$, $F(1, 29) = 23.7$, $p < 0.0001$, consistent with the notion that as sympathetic neural tone increases during development, sympathetic withdrawal by clonidine has a proportionately larger effect.

As described earlier and as shown in Figure 2, the inverted-U response of ultrasound production to clonidine administration, with a peak response around 15 days of age, is consistent with previous findings. There is, however, one important discrepancy. Specifically, Kehoe and Harris (1989) reported negligible rates of ultrasound production to 0.5 mg/kg of clonidine by 18 days of age, and the 20-day-old subjects of Hård and colleagues (1988), injected with 0.4 mg/kg of clonidine, were virtually silent. Although our methods differ somewhat from these earlier studies with respect to duration of observations and handling of animals after injection, one possible explanation involves the frequency at which ultrasound was monitored. In our pilot observations, with the detector tuned to 42 ± 5 kHz, we failed to detect clonidine-induced ultrasound production from 20-day-olds. Observation of the pups' breathing pattern, however, led us to tune the detector to a lower frequency, and at 25 kHz we discovered that loud ultrasonic emissions were indeed occurring. As shown in Figure 3, there is a highly significant linear relationship between body weight and the dominant frequency of ultrasound production, $r^2 = 0.90$, $F(1,23) = 208.3$, $p < 0.0001$. This relationship derives from the physics of sound production within tubes of different sizes. Thus, depending on the frequency selectivity of the detectors used by other investigators, and the frequency to which these detectors were tuned, it is possible that many instances of ultrasound production in older pups went undetected in earlier studies.

Finally, there were clear changes in behavioral activity after clonidine injection across the ages examined here, and our observations are generally consistent with those reported previously (Reinstein & Isaacson, 1977). Specifically, 2-day-old pups responded to clonidine by laying on their side and engaging in repetitive and persistent stepping responses. Eight-day-olds responded similarly to 2-day-olds, except the stepping responses now resulted in actual movement in the chamber; these pups also exhibited a lordosislike response while laying on their sides, that is, a pronounced arching of the back. By 15 days of age, pups typically exhibited wall climbing and other signs of hyperactivity. Finally, by 20 days of age, pups responded to clonidine with apparent catalepsy, piloerection, and pronounced bulging of the eyes.

DISCUSSION

Numerous investigators have examined clonidine's ability to modulate autonomic nervous system activity and cardiovascular function in adults (Gillis et al., 1985) and evoke ultrasound production in infants (Hansen, 1993; Hård et al., 1988; Kehoe & Harris, 1989). There had been no suggestion of the possibility that these two effects of clonidine are causally related until it was shown that clonidine simultaneously evokes bradycardia and ultrasound production in week-old rats (Sokoloff et al., 1997). The present results provide additional support for this causal hypothesis by indicating that clonidine's effects on cardiac rate and ultrasound production exhibit similar developmental profiles. Specifically, age-related changes in baseline cardiac rate and the magnitude of clonidine-induced bradycardia and ultrasound production were characterized by large and progressive changes occurring between 2 and 15 days of age and a plateau or reversal occurring between 15 and 20 days of age (see Figure 2). Both the developmental changes in baseline cardiac rate (Hayne et al., 1991; Tucker, 1985) and the inverted-U pattern of ultrasound production (Hård et al., 1988; Kehoe & Harris, 1989) are consistent with previous reports.

Clearly, the observation of similar developmental

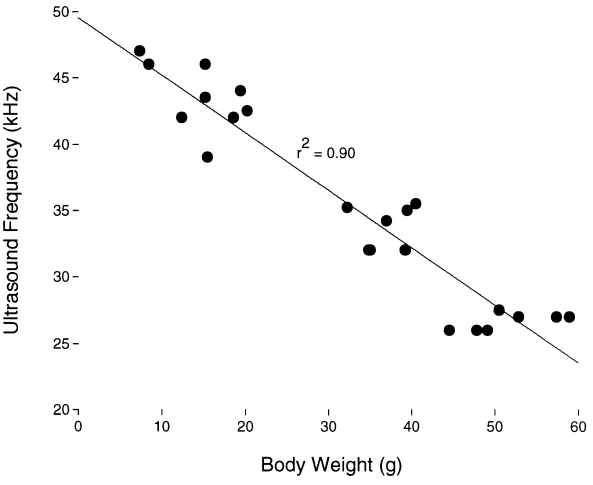

FIGURE 3 Linear regression for the frequency of ultrasonic vocalizations (kHz) versus body weight (g) for the infant rats treated with clonidine. Vocalization frequency was assessed by tuning the detector to the frequency that produced the clearest tone. The four clusters of data correspond to the 2- ($n = 2$), 8- ($n = 7$), 15- ($n = 8$), and 20-day-old ($n = 7$) subjects for whom clear determinations of vocalization frequency were possible.

patterns in clonidine's effects on ultrasound production and cardiac rate does not demonstrate a causal connection. Such a demonstration requires experiments in which inhibition of clonidine's cardiovascular effects also inhibits ultrasound production. In one such experiment, week-old rats were pretreated with prenalterol, a selective β_1 adrenoceptor agonist that stimulates cardiac rate peripherally, followed by clonidine administration (Blumberg, Kreber, Sokoloff, & Kent, in press-a). It was found that prenalterol pretreatment inhibited both clonidine-induced bradycardia and ultrasound production. Although additional experiments are necessary, this experiment provides the first direct support for the hypothesis that clonidine's effects on ultrasound production are mediated by its peripheral effects on the cardiovascular system.

Through a series of investigations (Blumberg et al., in press-a; Blumberg et al., 1999; Blumberg, Sokoloff, Kirby, & Kent, 2000; Kirby & Blumberg, 1998; Sokoloff & Blumberg, 1997), we have sought to identify the physiological causes and consequences of ultrasound production in infant rats. As described earlier, our hypothesis is that ultrasound production is the acoustic by-product of the ACR and is triggered by decreased venous return or a related variable. Although the exact relationship between cardiac rate and venous return has never been established in infants, reasonable inferences can be drawn regarding the impact of bradycardia, whether induced by cold or clonidine, on venous return. Drawing such inferences, however, requires consideration of cardiovascular function in infants, to which we now turn.

Cardiac output (i.e., the volume of blood pumped by the heart per unit time) is the product of cardiac rate and stroke volume, both of which are regulated by sympathetic nervous system activity (Guyton & Hall, 1996). Given that clonidine withdraws sympathetic tone (Luft et al., 1986; van Zweiten, 1996), it is likely that clonidine administration causes a reduction in cardiac output via its effects on both cardiac rate and stroke volume. When cardiac output decreases, right atrial pressure increases relative to mean circulatory filling pressure, resulting in diminished venous return to the heart (Guyton & Hall, 1996). Finally, because one of the primary factors in determining resistance to venous return is sympathetic tone to the vasculature (Goslinga, 1984), it is likely that administration of clonidine also causes venodilation and venous pooling, thus intensifying the infant's inability to maintain venous return to the heart.

If clonidine administration does indeed reduce venous return, then recruitment of the ACR is an appropriate response (Youmans et al., 1963; Youmans, Tjioe, & Tong, 1974). The ACR consists of contraction of the abdominal muscles during or after expiration, leading to increased intraabdominal pressure and the propulsion of blood back to the heart. Laryngeal constriction provides resistance to airflow and thus amplifies this increased intraabdominal pressure. Importantly, ultrasound production in infant rats is produced by a unique "bird whistle" mechanism characterized by the constriction of the laryngeal folds and the movement of air under high pressure (Roberts, 1975). Furthermore, ultrasound production is accompanied by sizeable increases in intraabdominal pressure (Kirby & Blumberg, 1998). Finally, in direct support of the ACR hypothesis, we have recently shown that ultrasonic emissions in infant rats are accompanied by pronounced pulsatile increases in venous pressure, indicative of increased venous return (Blumberg et al., 2000).

Given that clonidine produces significant decreases in cardiac rate in 2- and 20-day-olds, why is ultrasound production absent or diminished at those ages? With regard to the ACR hypothesis, we would predict that clonidine affects venous return differently at different ages. Is there any independent evidence that might support this prediction?

As we discussed earlier, clonidine acts centrally to cause withdrawal of sympathetic outflow (Gillis et al., 1985; Luft et al., 1986; van Zweiten, 1996). Based on developmental studies of the autonomic control of the heart, cardiac control in newborn rats depends in part on the contributions of circulating catecholamines released from the adrenal medulla and organ of Zuck-

erkandl (Tucker, 1985). Thus, in the 2-day-olds in the present experiment, the low baseline cardiac rate and the relatively modest bradycardia induced by clonidine (see Figure 2) may result from relatively diminished sympathetic neural influence at this age. This influence increases over the first 2 weeks postpartum, when the sympathetic nervous system exhibits a period of "intense hyperactivity" (Slotkin & Seidler, 1988), and then wanes as parasympathetic influences develop (Tucker, 1985). Therefore, age-related changes in clonidine's influence on cardiac rate and ultrasound production may reflect age-related changes in neural sympathetic dominance.

An additional factor that may be relevant here is the development of humoral factors that modulate vasomotor mechanisms and that therefore play a role in regulating venous return. For example, plasma renin activity (PRA), which is used as an index of the renin-angiotensin system, develops rapidly beginning around 15 days of age (Kirby & Johnson, 1990). High PRA reflects the functional capacity to produce angiotensin II. Furthermore, angiotensin II stimulates the release of vasopressin, and both of these hormones are important direct-acting regulators of vasomotor tone. Therefore, 20-day-olds may vocalize less than 15-day-olds in response to clonidine as a result of waning sympathetic dominance and increased release of humoral factors such as angiotensin II and vasopressin. Significantly, it has been reported that vasopressin decreases the expression of the ACR in adult dogs (Youmans et al., 1963).

Although the experiment described here was not designed as a direct test of the ACR hypothesis, it was designed to explore the developmental relation between clonidine's two well-known effects on ultrasound production and cardiac rate. Accordingly, this experiment adds an important developmental dimension to a body of literature that is increasingly suggesting a causal connection between cardiovascular physiology and emission of ultrasonic vocalizations in infant rats. Further experiments are needed to determine whether other pharmacological agents implicated in the modulation of ultrasound production exert their effects by altering cardiovascular function.

REFERENCES

Allin, J. T., & Banks, E. M. (1971). Effects of temperature on ultrasound production by infant albino rats. Developmental Psychobiology, 4, 149–156.

Allin, J. T., & Banks, E. M. (1972). Functional aspects of ultrasound production by infant albino rats (Rattus norvegicus). Animal Behaviour, 20, 175–185.

Blumberg, M. S., Kreber, L. A., Sokoloff, G., & Kent, K. J. (in press-a). Cardiovascular mediation of clonidine-induced ultrasound production in infant rats. Behavioral Neuroscience.

Blumberg, M. S., & Sokoloff, G. (1998). Thermoregulatory competence and behavioral expression in the young of altricial species—Revisited. Developmental Psychobiology, 33, 107–123.

Blumberg, M. S., Sokoloff, G., & Kent, K. J. (1999). Cardiovascular concomitants of ultrasound production during cold exposure in infant rats. Behavioral Neuroscience, 113, 1274–1282.

Blumberg, M. S., Sokoloff, G., Kirby, R. F., & Kent, K. J. (2000). Distress vocalizations in infant rats: What's all the fuss about? Psychological Science, 11, 78–81.

Blumberg, M. S., & Stolba, M. A. (1996). Thermogenesis, myoclonic twitching, and ultrasonic vocalization in neonatal rats during moderate and extreme cold exposure. Behavioral Neuroscience, 110, 305–314.

Carden, S. E., Davachi, L., & Hofer, M. A. (1994). U50,488 increases ultrasonic vocalizations in 3-, 10-, and 18-day-old rat pups in isolation and the home cage. Developmental Psychobiology, 27, 65–83.

Gillis, R. A., Gatti, P. J., & Quest, J. A. (1985). Mechanism of the antihypertensive effect of alpha$_2$-agonists. Journal of Cardiovascular Pharmacology, 7(Suppl. 8), S38–S44.

Goslinga, H. (1984). Blood viscosity and shock: The role of hemodilution, hemoconcentration, and defibrination. New York: Springer-Verlag.

Guyton, A. C., & Hall, J. E. (1996). Medical physiology (9th ed.). Philadelphia: W. B. Saunders.

Hansen, S. (1993). Effect of clonidine on the responsiveness of infant rats to maternal stimuli. Psychopharmacology, 111, 78–84.

Hård, E., Engel, J., & Lindh, A. (1988). Effect of clonidine on ultrasonic vocalization in preweaning rats. Journal of Neural Transmission, 73, 217–237.

Hayne, H., Richardson, R., & Campbell, B. (1991). Developmental constraints on the expression of behavioral and heart rate orienting responses: II. The role of ambient temperature. Developmental Psychobiology, 25, 51–65.

Hofer, M. A., & Shair, H. (1978). Ultrasonic vocalization during social interaction and isolation in 2-week-old rats. Developmental Psychobiology, 11, 495–504.

Kehoe, P., & Blass, E. M. (1986). Opioid-mediation of separation distress in 10-day-old rats: Reversal of stress with maternal stimuli. Developmental Psychobiology, 19, 385–398.

Kehoe, P., & Harris, J. C. (1989). Ontogeny of noradrenergic effects on ultrasonic vocalizations in rat pups. Behavioral Neuroscience, 103, 1099–1107.

Kirby, R. F., & Blumberg, M. S. (1998). Maintenance of arterial pressure in infant rats during moderate and extreme thermal challenge. Developmental Psychobiology, 32, 169–176.

Kirby, R. F., & Johnson, A. K. (1990). Effects of sympathetic activation on plasma renin activity in the developing rat. Journal of Pharmacology and Experimental Therapeutics, 253, 152–157.

Luft, F. C., Veelken, R., Becker, H., Ganten, D., Lang, R. E., & Unger, T. (1986). Effect of urapidil, clonidine, and pra-

zosin on sympathetic tone in conscious rats. Hypertension, 8, 303–311.

Miczek, K. A., Weerts, E. M., Vivian, J. A., & Barros, H. M. (1995). Aggression, anxiety, and vocalizations in animals: $GABA_A$ and 5-HT anxiolytics. Psychopharmacology, 121, 38–56.

Noirot, E. (1972). Ultrasounds and maternal behavior in small rodents. Developmental Psychobiology, 5, 371–387.

Okon, E. E. (1971). The temperature relations of vocalization in infant Golden hamsters and Wistar rats. Journal of Zoology, London, 164, 227–237.

Reinstein, D. K., & Isaacson, R. L. (1977). Clonidine sensitivity in the developing rat. Brain Research, 135, 378–382.

Roberts, L. H. (1975). The rodent ultrasound production mechanism. Ultrasonics, 13, 83–85.

Slotkin, T. A., & Seidler, F. J. (1988). Adrenomedullary catecholamine release in the fetus and newborn: Secretory mechanisms and their role in stress and survival. Journal of Developmental Physiology, 10, 1–16.

Sokoloff, G., & Blumberg, M. S. (1997). Thermogenic, respiratory, and ultrasonic responses of week-old rats across the transition from moderate to extreme cold exposure. Developmental Psychobiology, 30, 181–194.

Sokoloff, G., Blumberg, M. S., Mendella, P., & Brown, R. E. (1997). Clonidine- and separation-induced ultrasound production in infant rats: Cardiovascular interactions. Developmental Psychobiology, 30, 265.

Sokoloff, G., Kirby, R. F., & Blumberg, M. S. (1998). Further evidence that BAT thermogenesis modulates cardiac rate in infant rats. American Journal of Physiology, 274, R1712–R1717.

Spiers, D. E., & Adair, E. R. (1986). Ontogeny of homeothermy in the immature rat: Metabolic and thermal responses. Journal of Applied Physiology, 60, 1190–1197.

Tucker, D. C. (1985). Components of functional sympathetic control of heart rate in neonatal rats. American Journal of Physiology, 248, R601–R610.

van Zweiten, P. A. (1996). From α to β to I_1: An overview of sympathetic receptors involved in blood pressure control targets for drug treatment. Journal of Cardiovascular Pharmacology, 27(Suppl. 3), S5–S10.

Vivian, J. A., Barros, H. M. T., Manitiu, A., & Miczek, K. A. (1997). Ultrasonic vocalizations in rat pups: Modulation at the γ-aminobutyric receptor complex and the neurosteroid recognition site. Journal of Pharmacology and Experimental Therapeutics, 282, 318–325.

Vivian, J. A., & Miczek, K. A. (1991). Ultrasound during morphine withdrawal in rats. Psychopharmacology, 104, 187–193.

Winslow, J. T., & Insel, T. R. (1991a). Endogenous opioids: Do they modulate the rat pup's response to social isolation? Behavioral Neuroscience, 105, 253–263.

Winslow, J. T., & Insel, T. R. (1991b). The infant rat separation paradigm: A novel test for novel anxiolytics. TIPS, 12, 402–404.

Youmans, W. B., Murphy, Q. R., Turner, J. K., Davis, L. D., Briggs, D. I., & Hoye, A. S. (1963). Activity of abdominal muscles elicited from the circulatory system. American Journal of Physical Medicine, 42, 1–70.

Youmans, W. B., Tjioe, D. T., & Tong, E. Y. (1974). Control of involuntary activity of abdominal muscles. American Journal of Physical Medicine, 53, 57–74.