Exaggerated Response to Alerting Stimuli in Spontaneously Hypertensive Rats

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The startle response, consisting of behavioral and cardiovascular components, was used to study the reaction of the cardiovascular system to a mild environmental stressor. We used tactile air puff startle to study responses in adult Wistar-Kyoto and spontaneously hypertensive rats. In both strains, air puff elicits a transient motor response with rapid habituation over the test session of 30 trials. Spontaneously hypertensive rats exhibit exaggerated motor responses compared with Wistar-Kyoto rats. Similarly, a 2–3-second duration pressor response was significantly greater in spontaneously hypertensive rats than in Wistar-Kyoto rats (47.7±2.0 versus 37.1±1.5 mm Hg, respectively). However, spontaneously hypertensive rats and Wistar-Kyoto rats exhibited strikingly dissimilar heart rate responses. Wistar-Kyoto rats exhibited a transient bradycardia (—42±7 beats/min) on early trials yielding to tachycardia on later trials (35±11 beats/min). In contrast, spontaneously hypertensive rats exhibited only tachycardia to all stimuli with an absence of bradycardia. Adrenal medullary secretions chronically modulate cardiac responses in both strains. Sinoaortic denervation did not alter the magnitude or profile of the heart rate responses. Spontaneously hypertensive–Wistar-Kyoto rat differences were not secondary to hypertension because renovascular hypertensive Wistar-Kyoto rats show normal responses to air puff. Four-week-old spontaneously hypertensive rats exhibit enhanced pressor and suppressed bradycardia responses relative to age-matched Wistar-Kyoto rats, indicating chronotropic differences precede development of established hypertension. Our results indicate parasympathetic activation by the mild startle stimuli rather than sympathetic withdrawal allows bradycardia to mask a latent tachycardia in Wistar-Kyoto rats. Spontaneously hypertensive rats exhibit a parasympathetic insufficiency in the startle response to novel alerting stimuli. Thus, mild air puff startle identifies a unique and discriminatory phenotypic difference between inbred normotensive and hypertensive rats. (Hypertension 1990;16:290–300)

Spontaneously hypertensive rats (SHR) have been reported to react to a variety of stressful or alerting stimuli with exaggerated behavioral or autonomic responses. A wide range of behavioral experimental paradigms have been used to explore this hyperreactivity as reactivity to psychosocial and environmental stimuli may reflect a genetic trait of this rat that, at an early age, may be a component of the ultimate blood pressure elevation. Most types of environmental stimuli elicit a complex pattern of behavioral, cardiovascular, and other autonomic responses. Repeated episodes of increased sympathetic discharge from either direct stimulation of the central "defense area" or environmental stimuli have been hypothesized by Folkow to trigger a series of events resulting in chronically elevated arterial pressure. Further, the Folkow hypothesis presumed a subset of humans (or animals) with a genetic predisposition to develop hypertension as a result of an environmental stressor. Thus, the response to experimental environmental stressors may reveal an early predisposition to the development of sustained hypertension.

In a previous report, we described pressor and tachycardic responses in both Wistar-Kyoto (WKY) rats and SHR to tactile or acoustic startle stimuli. The pressor response, greater in the SHR compared with the WKY rat, habituated with repeated stimulation in both strains, whereas the tachycardic response did not habituate. More recently, we described an early bradycardic component of the cardiac response to tactile (air puff) startle in WKY rats, which habituates to extinction after 5–10 successive stimuli. This component of the heart rate response to tactile startle is absent in SHR. We reported cosegregation of this component of the...
startle response with hypertension in crosses between SHR and WKY rats, which demonstrates genetic linkage of the absence of early trial bradycardia to hypertension. The linkage of the absence of startle-induced bradycardia to elevated arterial pressure in this model of hypertension serves not only as a genetic marker but also may indicate a pathophysiological role in the etiology of hypertension as a maladaptive response to acute stressors. Thus, the cardiac response to air puff startle stimuli in rats naive to the stimulus was more complex than we originally reported. In the present report, we have extended these observations and quantified the behavioral (motor) component and mechanistically examined the differences in the complex cardiovascular response between hypertensive SHR and normotensive WKY rats. In addition, we compared the responses in the adult with those in 4-week-old rats, which is the age before significant elevation of blood pressure occurs in the SHR.

Methods

Animals

All experiments were carried out in SHR or WKY rats. Rats were originally obtained from Charles River Laboratories, Inc., Wilmington, Mass., and have been bred and housed in our facility for over 12 generations. Rats were housed in groups of three in clear plastic cages with wood shavings covering the floor of the cage. The facility was temperature-controlled (20±1° C) and maintained on a 12-hour light/dark cycle. After any surgical procedure or assignment to a specified treatment group, rats were moved to individual cages. Rats routinely received standard laboratory chow and tap water ad libitum. All experiments were conducted between 8:00 AM and noon.

Cardiovascular Measurement

Mean arterial pressure and heart rate were recorded from an indwelling catheter, constructed by fusing a 4.5 cm length of PE-10 polyethylene tubing to a 15 cm length of PE-50 tubing. Under sodium pentobarbital (40 mg/kg i.p.) anesthesia, the femoral artery was isolated at the ventral junction of the right hind limb with the abdomen. At this time, the artery was partially transected, and the PE-10 portion of the catheter was inserted into the vessel and was advanced into the abdominal aorta. The catheter, containing heparinized 0.09% saline, was securely sutured in place, plugged, and tunneled under the skin to exit through a small incision at the dorsal aspect of the neck. After recovery from anesthesia, rats were returned to their home cage for 3 days before experimentation. Rats that displayed abnormal grooming or consummatory behavior during the recovery period as well as rats displaying compromised hind limb function were eliminated from the study.

For measurement of cardiovascular parameters during the startle experiments, the catheter was connected to an external PE-50 catheter, extended through the chamber, and connected to a Statham P23Db strain gauge transducer (Gould Statham, Oxnard, Calif.) placed at the level of the animal’s heart. The transducer connected to a Gould 2400S recorder, and the signal was fed to a pressure processor for determination of pulsatile and mean arterial pressure. The signal was then fed to an EKG/Biotach (Gould, Inc., Cleveland, Ohio) for determination of beat-to-beat heart rate and was displayed on the recording oscillograph. The transducer was positioned outside the startle chamber, which allowed flushing of the catheter without disturbing the animal or interfering with the motor activity recording.

Behavioral Response Measurement

Magnitude of the motor response to startle was measured by use of a calibrated stabilimeter, which consisted of a 10 cm acrylic cylinder held at top and bottom within a rigid frame by large rubber spacers (San Diego Instruments, San Diego, Calif.). The size of the chamber allowed the animal relatively free movement, including reversal of direction, but prevented avoidance of the air puff stimulus. Movement of the rat, reflected by displacement of the cylinder, was detected with a ceramic transducer mounted to the outer frame as described by Geyer et al. A rectified signal representing motion of the rat was fed to the recording oscillograph. The startle response was quantified using a temporarily attached electric solenoid. The solenoid delivers an easily repeatable force to the tube. Daily variation in the response to the electric solenoid was corrected by mechanically adjusting the tension on the rubber spacers. Fine adjustments were performed with a potentiometer, which is part of the commercial startle unit. Motor response units represent displacement of the tube by the rat in response to the startle stimulus as a percentage of the displacement produced by the electric solenoid. The significance of this measurement is strictly the displacement of the tube. As displacement takes place against resistance placed on the chamber by the rubber spacers, displacement is proportional to the force produced by the rat. Motor response to the tactile startle stimulus (in percent) was expressed as displacement of the chamber within 300 msec of the startle stimulus relative to the calibrating solenoid and represents a linear scale. The stabilimeter chamber permitted simultaneous measurement of cardiovascular parameters. The entire chamber was insulated from room light, motion, and sound by an isolation cabinet. The cabinet contained an internal light source and a fan for continuous circulation of fresh air.

Air puff stimulus presentation occurred from a tube 1 cm in diameter suspended 2 cm above the lumbosacral region of the rat. Transient puffs of air were delivered from a compressed air cylinder. Timing, strength, and duration of the stimuli were controlled by an electrical solenoid and a pressure
regulator located at a distance from the site of delivery to minimize acoustic components. The tactile stimulus used for studies reported herein consisted of a 12.5 psi puff of air with a 100 msec duration. Successive stimuli were delivered at 30-second intervals. This interval between stimuli permitted cardiovascular parameters to return to baseline values before delivery of subsequent stimuli. Unless otherwise specified, only one test session consisting of 30 stimuli was given to each rat. Testing was begun when continuously measured cardiovascular parameters reached a stable baseline but in no case earlier than 30 minutes after a rat entered the startle chamber.

**Cardiovascular and Behavioral Response in Adult Spontaneously Hypertensive Rats versus Wistar-Kyoto Rats**

Adult WKY rats (230–270 g) and SHR (240–270 g) were instrumented with indwelling arterial catheters and, after a 3-day recovery period, were subjected to the above-described tactile startle protocol. Each animal was submitted to only one test session consisting of 30 stimuli.

**Startle in Two-Kidney, One Clip Renovascular Hypertensive Rats**

Eighteen male WKY rats weighing 120–140 g received unilateral constriction of the left renal artery using a fine silver clip (0.2 mm i.d.) as described by Leenen and De Jong. Briefly, the rat was anesthetized with pentobarbital sodium (40–50 mg/kg i.p.), and the left kidney was exposed from a dorsolateral approach. Using sterile technique, the renal artery was freed from connective tissue and the silver clip was placed midway between the aorta and junction with the kidney. Blood flow distal to the clip was visibly confirmed as flow to the kidney should be restricted but not obstructed. The right kidney was untouched. Sham treatment (n=8) consisted of surgical isolation of the left renal artery without placement of the clip. The wound was closed in layers with 4-0 silk suture. Rats were allowed to recover from anesthesia and were returned to their home cages. Systolic blood pressure was then recorded on alternating days by tail-cuff plethysmography (IITC, Woodland Hills, Calif.) to monitor the development of hypertension in these animals. Development of sustained hypertension with this method occurred within 3 weeks, at which time the rats were tested in the startle paradigm. Elevated arterial pressure failed to develop in four animals from the renal clip group (more than 2 SDs from group mean), and they were removed from the study. Three days before startle testing, rats were subjected to a short secondary surgery during which each subject received an indwelling arterial catheter. After completion of startle testing, one half of the rats were killed, and kidney weight and morphology were determined postmortem. The remaining rats were maintained until 3 months after clipping and then were retested in the startle paradigm to determine the effects of chronic renal hypertension on the response patterns to tactile startle stimuli.

**Startle Responsivity of Prehypertensive Spontaneously Hypertensive Rats**

Four-week-old intact WKY rats (n=8) and SHR (n=8) were tested in the standard startle paradigm to determine the characteristics of prehypertensive SHR during the development phase of hypertension. Rats received indwelling arterial catheters and were allowed to recover from surgery for 3 days, during which period food and water consumption were monitored before the rats were subjected to the startle test paradigm.

**Effect of Sinoaortic Denervation on the Cardiovascular Response to Startle**

To determine the role of baroreceptor reflex mediation of heart rate changes related to the blood pressure effects of tactile startle stimuli, a group of WKY rats (n=12) received sinoaortic denervation of baroreceptor afferents as described by Krieger. Unilateral denervation was performed, and after a 1-week recovery period, the contralateral side received similar treatment. Briefly, aortic denervation was accomplished under microscopic guidance by cutting the sympathetic trunk including the aortic nerve at the level of the bifurcation of the common carotid artery. Next, the recurrent and superior laryngeal nerves were isolated and transected. Sinus denervation was accomplished by stripping fibers and connective tissue from the carotid bifurcation. After the vessels were stripped, they were carefully painted with 10% phenol in ethanol. Rats were allowed to recover from the secondary surgery for 1 week when indwelling arterial and venous catheters were implanted to allow construction of baroreceptor reflex function curves and cardiovascular monitoring during startle tests. Three days after implantation of catheters, the efficacy of baroreceptor denervation was tested by administration of five graded bolus doses (0.25–5 µg/kg i.v.) of phenylephrine administered with 15 minutes allowed between doses. Baroreceptor reflex function curves were constructed by determining peak arterial pressure versus peak bradycardia within a 3-minute period subsequent to phenylephrine administration. Two days subsequent to baroreceptor reflex assessment, rats were tested with the startle paradigm.

**Adrenal Enucleation in Spontaneously Hypertensive Rats**

In a separate group (n=11) of adult SHR, the adrenal medulla was bilaterally removed at the time of catheter implantation via a single midline abdominal incision. Medullary tissue was mechanically removed through a small puncture in the cortical layer of the adrenal gland. Cortical tissue was otherwise left intact. Rats were allowed to recover from surgery for 3 days and were then tested in the startle
paradigm as described above to assess possible contribution of adrenal catecholamine secretion to either the behavioral or cardiovascular response to startle. Sham-operated rats (n=7) received similar surgical intervention except that the adrenal gland was merely exposed bilaterally. All rats exhibited normal weight gain and consummatory and grooming behavior after the adrenal medulla was bilaterally removed. After completion of the startle paradigm, rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and transcardially perfused with 10% buffered formalin for histological verification of adrenal enucleation. Adrenal glands were removed, 10 mm serial sections were mounted and stained with hematoxylin and eosin.

Effect of Cholinergic Receptor Blockade on Response to Startle
To explore the role of parasympathetic cholinergic systems in the heart rate responses observed in WKY rats (n=8), the quartenary cholinergic receptor antagonist atropine methyl nitrate (Sigma Chemical Co., St. Louis, Mo.) was administered as a 1 mg/kg i.v. bolus followed by a 0.1 mg/kg i.v. infusion during the startle testing. A control group (n=6) received an equimolar infusion of the phosphate-buffered saline solution, which served as vehicle for the atropine. Efficacy of this dose was verified in a separate group of rats by monitoring reflex bradycardia to 1 and 2.5 μg/kg i.v. bolus of phenylephrine.

Data Management and Statistics
Analog signals from the Gould signal conditioner were fed online to the PC-based computer acquisition system DASA (Gould) at a rate of 400 samples/sec for subsequent analysis. Motor activity after a startle stimulus was determined by measuring peak amplitude, latency to peak, and latency to response. Cardiovascular data were stored for time-averaged statistical analysis (Systat, Evanston, Ill.). Maximum habituation of all responses occurred between the first and 10th trials. Therefore, for trials 10–30, only every fifth trial was reported and statistically analyzed. Cardiovascular and behavioral responses were assessed for group-by-trial interaction in a repeated-measures analysis of variance (ANOVA) with level of significance set at 0.05. When resting arterial pressure was significantly different between groups, ANOVA was performed with control pressure as a covariate. Pairwise comparisons were performed using Tukey’s test only when ANOVA was passed. Data are expressed as mean±SEM.

Results
Startle Responses in Spontaneously Hypertensive Rats Versus Wistar-Kyoto Rats
Figure 1 illustrates the magnitude of motor and pressor responses within the two groups to the repeated tactile startle stimulus. Analysis of variance revealed that behavioral responses were significantly exaggerated in SHR compared with WKY rats [83±4 versus 62±4, initial trial; F(9,261)=4.357; p<0.001] for the overall strain by trials analysis. Post hoc examination by Newman-Keuls revealed significantly (p<0.05) greater motor responses in SHR for the first five trials, which then habituated to a level not different from the normotensive WKY rats. There did not appear to be any significant differences in latency to the motor response between the strains. Despite starting with elevated resting arterial pressure (SHR, 148.7±3.4 mm Hg versus WKY, 104.5±2.0 mm Hg), SHR also responded to the tactile startle stimulus with elevated pressor responses [F(9,261)=2.308; p<0.016]. The greatest pressor response occurred on the initial stimulus in both
groups of rats (SHR, 47.7±2.0 mm Hg versus WKY, 37.1±1.5 mm Hg). The pressor episode was brief; the maximum response occurred between 1.5 and 2.0 seconds after stimulus for all trials. Pressor responses were significantly (p<0.05) greater in SHR throughout the initial 15 trials, at which time they habituated to a level not different from WKY rats. As previously reported, the pattern of heart rate responses was dependent on trial number in WKY rats with a significant bradycardia occurring that, within the group, was not observed subsequent to the fifth trial. The time course of the bradycardia was closely related to the pressor episode; however, as discussed below, the bradycardia appears not to be reflexly generated by the elevated arterial pressure. At trial 5, a significant tachycardia became evident that was temporally delayed relative to the bradycardia and pressor episodes. Tachycardia in WKY rats had a latency to peak of 3.2±0.1 seconds for all trials. In contrast to the bradycardia of WKY rats, SHR demonstrated a significant tachycardia in responses at all trials; however, the time course of tachycardia in SHR was not different from that seen in WKY rats. The average of tachycardic responses for the 30 trials in SHR was 34±2 beats/min and did not show significant habituation during the test session, as shown in Figure 1.

Startle in Two-Kidney, One Clip Renovascular Hypertensive Rats

The onset of significantly elevated arterial pressure in the two-kidney, one clip (2K1C) group occurred within 9 days after clipping. As shown in Figure 2, resting systolic pressure reached a plateau within 2 weeks and remained elevated (208.8±8.6 mm Hg, systolic) until the day of startle testing. Further, the systolic pressure of sham-clipped rats did not vary significantly over the duration of the study period (136.6±7.6 mm Hg). The presence of hypertension in the clipped rats was reflected both in the tail-cuff systolic values 21 days after clipping and in the mean arterial pressure determined from indwelling catheters (Figure 2) 3 days later on the day of startle testing. The pattern of exaggerated motor and pressor responses as well as differential heart rate responses observed in WKY rats versus SHR was present in 2K1C renovascular hypertensive rats. In addition, the magnitude of motor and pressor responses of the 2K1C group was not significantly different from either the sham-treated group or untreated WKY rats (data not shown). As shown in Figure 2, the heart rate response of the renovascular hypertensive rats was equivalent to that of sham-operated controls with both exhibiting typical WKY-like bradycardia to initial startle stimuli, which habituated to extinction with repeated stimuli. There were also no differences, relative to sham-operated or the 2K1C rats tested above, in the startle response of 2K1C rats tested 3 months after implantation of the renal clip. Postmortem examination of the kidneys from the sham and renal hypertensive groups revealed the expected hypertrophy of the right (unclipped) kidney compared with the left kidney in the 2K1C group (1.43±0.09 g versus 1.00±0.03 g, p<0.01) or either kidney from the sham group (1.15±0.04 g).

Startle Responsivity of Prehypertensive Spontaneously Hypertensive Rats

Resting arterial pressure was not different at this age (WKY, 111.4±4.0 mm Hg versus SHR, 106.1±3.9 mm Hg) nor was resting heart rate (WKY, 466±23 beats/min versus SHR, 461±16 beats/min). Four-week-old WKY rats and SHR had similar startle response patterns to adult animals; however, the differences between strains were less evident. Motor reactivity to startle was not different over the course of the 30-trial session (Figure 3). Pressor responses to tactile startle were still significantly exaggerated in SHR for the first three trials.

Figure 2. Panel A: Line graph showing development of sustained arterial pressure in two-kidney, one clip (2K-1C) renovascular hypertensive Wistar-Kyoto (WKY) rats. Rats were 4–6 weeks old when a 0.2 mm diameter silver clip was placed on left renal artery. Solid symbols represent averages of three values per animal, determined by tail-cuff plethysmography. Open symbols are mean arterial pressure on day of startle testing from an indwelling arterial catheter. Panel B: Bar graph showing heart rate responses for sham-operated (n=8). Panel C: Bar graph showing renovascular hypertensive (n=14) rats in a repeated tactile startle paradigm. Values are mean±SEM for maximum heart rate change from a 5-second prestimulus control period. *Significant (p<0.05) difference between groups by multiple comparison after analysis of variance.
but habituated rapidly to a level not different from WKY rats \( F(9,63) = 3.587, p < 0.001 \).

Interestingly, heart rate responses of the young WKY rats were somewhat different from those of adults. The first stimulus produced bradycardia of similar duration and magnitude as found previously for adults\(^{13}\) (results described above). However, in contrast to adult WKY rats, the bradycardic response was less subject to habituation and remained significant throughout the duration of the study (Figure 3). Tachycardia, not evident in adult WKY rats until trial 5 or beyond, was similar in duration and magnitude in young WKY rats but was evident at earlier trials. In contrast to adult SHR, 4-week-old SHR demonstrated bradycardia to the initial trials in the startle tests. However, relative to the bradycardia evident in young WKY rats, the response was significantly smaller and more subject to habituation.

**Effect of Sinoaortic Denervation on Cardiovascular Response to Startle**

Twelve adult rats that were subjected to sinoaortic denervation exhibited no reflex bradycardia to graded doses of phenylephrine indicative of an absent baroreceptor reflex. Sham sinoaortic denervated rats demonstrated normal baroreceptor reflex function curves constructed from systolic pressure (mm Hg) versus pulse interval (msec) responses to phenylephrine (slope=0.61, \( r^2=0.976 \)), whereas sinoaortic denervated rats lacked significant reflex bradycardia to all doses of phenylephrine (slope=-0.08, \( r^2=0.392 \)). Baroreceptor denervation did not alter motor responses to air puff startle (Figure 4). The pressor response to tactile startle tended to be decreased in sinoaortic denervated rats during later trials. Pretest mean pressure of the sinoaortic denervated group (130.2 ± 4.2 mm Hg) was elevated relative to the sham rats (113.1 ± 4.4 mm Hg); however, ANOVA indicated that, as a group, the sinoaortic denervated and sham rats did not exhibit statistically different blood pressure responses. Heart rate responses of the sinoaortic denervated group were not different from the sham rats, indicating that the bradycardic response of intact WKY rats is not reflex to the pressor episode provoked by tactile startle.

**Adrenal Enucleation**

In a previous study\(^{13}\) we demonstrated that adrenal enucleation significantly exaggerated bradycardia
in normotensive rats (−89±13 beats/min versus sham control −40±6 beats/min). In addition, we reported that bradycardia persisted throughout the test session, whereas in sham rats, bradycardia was not evident after the fifth trial. However, adrenal enucleation did not change delayed tachycardia to air puff startle either in magnitude or time course.

In adrenal enucleated SHR, resting mean arterial pressure and heart rate were significantly different than sham SHR either before initiation of the test session or at the time of delivery of each stimulus. Motor reactivity tended to be reduced during initial trials but was not significantly different. There was no significant difference in the magnitude or time course of the pressor component of the response.

The heart rate responses of SHR were significantly altered by removal of adrenal medullary tissue. In contrast to intact SHR, which show tachycardia to repeated tactile startle, adrenal enucleated SHR failed to exhibit any significant heart rate response to the first stimulus (Figure 5). In addition, the heart rate response to subsequent stimuli exhibited greater variation than intact SHR. Although the magnitude of tachycardia appears greater than sham-operated SHR (Figure 5), the increased lability of heart rate in response to the air puff stimulus resulted in a failure to reach statistical significance when compared with sham-operated WKY rats. Histological examination of the remaining adrenal tissue from the enucleated group revealed an absence of medullary cells with cortical tissue intact. Sham-operated rats demonstrated normal staining of medullary secretory cells and cortical tissue in the adrenal.

Effect of Cholinergic Receptor Blockade on Response to Startle

Atropine methyl nitrate administration to intact WKY rats significantly elevated resting heart rate and arterial pressure compared with control WKY rats (374±7 versus 346±12 beats/min; 128.2±4.5 versus 105.2±2.3 mm Hg; *p*<0.05 by unpaired *t* test). The extent of muscarinic receptor blockade was demonstrated by the abolition of reflex bradycardia to intravenous doses of phenylephrine (Figure 6, inset). Atropine methyl nitrate abolished the bradycardic response to air puff stimuli in the WKY rat (Figure 6). Strikingly, the normal bradycardia anticipated for the first stimulus (−31±6 beats/min; control group) was supplanted...
by a tachycardia (61±11 beats/min) similar to that observed in SHR. However, motor and blood pressure responses to air puff startle were not altered by cholinergic muscarinic blockade.

**Discussion**

The SHR has been studied extensively as a model of a genetic form of hypertension and for its potential relevancy to human essential hypertension. The present study extends the reports from this laboratory and others that this rat strain also exhibits an exaggerated motor and cardiovascular response to tactile (air puff) startle stimuli. The startle reaction has long been recognized as a complex behavioral response. Temporally, after application of the startle stimulus the first event was a motor response 100 msec in duration. Visual observation of the animal indicates that this consists of a forelimb contraction and extension of the hind limbs, essentially identical to that described by Davis. Within a second after stimulus presentation, all rats, both SHR and WKY, exhibited a pressor response that peaks within 2 seconds after stimulus for all trials. A complex heart rate response was observed that differs between SHR and WKY rats. In the first five trials, normotensive WKY rats exhibited a transient bradycardia that peaked within 3 seconds and exhibited a trial-dependent extinction that was replaced between trials 5 and 10 with a delayed tachycardia that remained evident for all remaining trials. In the present study, we documented that the SHR is strikingly different from the WKY rat in not exhibiting any bradycardia response in the early trials, only the delayed tachycardia. In a separate study, we presented evidence that this difference in heart rate response in the early trials segregates with high blood pressure in crossbreeding and backbreeding studies between SHR and WKY rats. Thus, in response to air puff startle stimuli WKY rats exhibited the following temporal sequence: motor response, transient bradycardia and pressor response, and delayed tachycardia, whereas SHR exhibit all but the transient, early-trial bradycardia.

The temporal association between the transient bradycardia and pressor response might suggest a direct, baroreceptor reflex origin. Because the bradycardia was absent in SHR and was extinguished in WKY rats after trial 5, it could be argued that an independent tachycardic response could mask a baroreceptor reflex-mediated bradycardia. Potentially therefore, the difference in heart rate responses between SHR and WKY rats may reflect an absence of tachycardia masking in WKY rats. However, as shown above in WKY rats that were sinoaortic denervated, the initial bradycardia is still present. Rather than a tachycardia masking, we believe that the bradycardia reflects enhanced parasympathetic activity cued by the central nervous system in response to the initial startle stimulus. This enhanced parasympathetic activity overcomes a sympathetic cardioaccelerator stimulation that is somewhat temporally delayed. As stated above, our data suggest the bradycardia reflects direct parasympathetic activity in normotensive WKY rats. Because there is much documentation of a blunted high pressure barorecep-

![Table 1](https://hyper.ahajournals.org/doi/abs/10.1161/HYPERTENSIONAHA.117.136204)
tor reflex in SHR,\textsuperscript{20,21} we hypothesize a parasympathetic insufficiency in the acute response to novel alerting stimuli in SHR. In support of this interpretation is our demonstration that peripheral muscarinic receptor blockade in WKY rats abolished the bradycardia and unmasked the delayed tachycardic response in the early trials. In addition, the difference in the first trial heart rate responses between SHR and WKY rats was probably due to failure by the SHR to enhance parasympathetic stimulation and not an exaggerated increase in sympathetic tone to the heart. This is evidenced by the observation that the magnitude of the tachycardia is not different between SHR and WKY rats in later trials and that peripheral muscarinic blockade unmasked a tachycardia in WKY rats, which is at least as large as that seen in early trials in the SHR. We conclude that air puff startle stimuli elicit both a transient parasympathetic activation as well as a sustained (over trials) sympathetic activation.

What of a possible relation between the magnitude of the motor and pressor responses? Because SHR exhibit exaggerated motor and pressor responses, is there a causal relation? This is a legitimate question as statistical differences in the blood pressure and motor response between SHR and WKY rats were not evident in later trials. However, our analyses argue against such a linkage. Although the rate of apparent habituation between the blood pressure and motor responses to repeated stimuli was similar, a significant correlation between the two responses was not observed when single-animal responses were analyzed. This argues against the pressor response being determined by the magnitude of the motor response. We would further conclude that habituation of the response to startle occurs distal to the site of divergence of the efferent outflow to the skeletal muscle and to the cardiovascular systems suggesting independent feedback systems that allow for individual habituation patterns.

The observation that both SHR and WKY rats exhibited persistent tachycardia throughout the 30 trials suggested that this cardiovascular response is an essential component of the overall response to air puff startle stimuli. The temporal delay in the tachycardia could reflect a humoral component to the response. That activation of the adrenal medulla influences the cardiovascular responses to startle is documented both in this paper and our earlier publication\textsuperscript{13} in which we showed that adrenal demedullation intensified the extent of the early trial bradycardia in WKY rats. In the present study, we showed that adrenal demedullation of SHR results in increased heart rate lability and a failure to exhibit tachycardia but only on the first trial. Chronic catecholamine levels may contribute to setting cardiac responsiveness to parasympathetic versus sympathetic stimulation. Support for this interpretation comes from the adrenal demedullation results in normotensive animals where removal resulted in an exaggerated bradycardia and prevented habituation\textsuperscript{13} as well as in SHR, where tachycardia to the initial stimulus was completely abolished. Although bradycardia was not observed in the adrenal demedullated SHR, abolition of first trial tachycardia suggests that the transient influence of parasympathetic over sympathetic tone to the heart was altered by adrenal enucleation.

Studies of the response of prehypertensive, young SHR and normotensive WKY rats demonstrate that the difference in heart rate response to air puff startle between these rat strains exists before the development of overt hypertension. Interestingly, there is a small but significant bradycardia evident in trials 1 to 3 in young (4-week-old) SHR as well as a striking persistence of the bradycardia response in young (4-week-old) WKY rats through all trials despite the evidently delayed tachycardia response. These observations indicate a greater parasympathetic response in young rats compared with adults and an apparent loss of this heightened parasympathetic response with development and maturation. Together, these differences between young and adult WKY rats parallel the age-dependent development of baroreceptor function\textsuperscript{22} and central nervous control of cardiac activity. Further, young SHR exhibit accelerated development of cardiac sympathetic responses\textsuperscript{23} compared with age-matched WKY rats.

Because pharmacological blockade of bradycardia in WKY rats did not alter the magnitude of the pressor episode, the brief slowing of the heart during this phase of the response apparently did not compromise cardiac output. This suggests that the magnitude of the pressor episode was somewhat independent of the heart rate response. In fact, one potential functional significance of the transient bradycardia elicited by a simple alerting stimulus may be to permit increased coronary circulation with increased cardiac perfusion in preparation for a possible prolonged secondary avoidance behavior.

The exaggerated magnitude of pressor episodes observed in SHR may not be explained by a lack of bradycardia accompanying the response. Alternatively, the origin of bradycardia may be a cardiopulmonary reflex eliciting direct enhancement of vagal tone\textsuperscript{24,25} subsequent to increased contractility, cardiac output, or venous return. This would explain preservation of the response after sinoaortic denervation and again demonstrate dissimilarities between SHR and WKY rats. Current studies focusing on regional blood flow and acute contractility changes to startle are examining these possibilities.

In a much broader sense, the characteristic startle response pattern is not a consequence of hypertension or changes secondary to hypertension as the 2K1C model of hypertension demonstrated normal behavioral as well as cardiovascular startle responses. In addition, the development of cardiovascular dissimilarities between SHR and WKY rats precede detection of elevated arterial pressure in young SHR. Enhancement of behavioral responses to startle did not exist in the 4-week-old SHR relative to age-matched WKY rats.
providing evidence for separation of the behavioral component from the blood pressure response.

In summary, the SHR demonstrates unique behavioral and cardiovascular responses to air puff startle stimuli. Exaggeration of the motor and blood pressure responses to startle appear to be a primary characteristic of this strain of rat and does not appear secondary to hypertension or secondary to altered cardiovascular regulation secondary to hypertension. These conclusions agree with hyperactivity studies of SHR and WKY rats conducted by Hendley et al.26,27 which showed a dissociation between hyperactivity and hypertension. Air puff startle stimuli initiate synchronous sympathetic and parasympathetic stimulation of the heart with the resulting change in rate dependent on the balance between parasympathetic and sympathetic stimulation. Domination by one component will dictate the cardiac response profile. In light of our cosegregation studies, the unique cardiac profile of the SHR may provide a useful marker for the genetic predisposition for hypertension as well as a tool for studying cardiovascular function in the SHR itself.

Our results do not directly prove or disprove the Folkow hypothesis9—11 that repeated exposures to environmental stressors, which result in sympathetic activation, ultimately translate into hypertension in susceptible individuals. Certainly, we do not believe that hypertension can result from a single session of 30 repetitive startle stimuli. However, our results raise interesting questions about the nature of cardiovascularly important stressors. The original Folkow thesis emphasized a defense-type reaction as the implied culprit in a stressor that is linked to the etiology of hypertension. More recently, Folkow has argued that mild stressful stimuli, even those that raise awareness, would activate sympathetic responses in a mild defense reaction.11 A classic defense reaction would include heightened blood pressure and heart rate.28 Our mild air puff startle stimuli elicits a cardiovascular response that, in a limited way, mirrors the sympathetic activation of the defense reaction. However, the stimulus also elicits a transient, trial-dependent parasympathetic activation. We believe this reflects the described "orienting" response18,29 and will present the arguments in support of this thesis in detail elsewhere. The results presented in this study, together with our report on the segregation of this parasympathetic activation with hypertension,14 raise the possibility that this bradycardia component is a cardiovascularly important element of the stress response. Interestingly, this conclusion would also agree with results obtained by other investigators on the importance of parasympathetic activation to cardiovascular disorders in general. Further, an important cardioprotective effect of parasympathetic activation in reducing myocardial electrical instability in response to biobehavioral stimuli has been shown by several investigators and summarized recently by Verrier.30 Thus, we do believe that our air puff paradigm more closely reflects possible everyday life environmental stressors that may have detrimental effects on humans and animals. As such, the ability of this paradigm to dissect differential response patterns in SHR versus normotensive controls may inherently be very informative.

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