

# Behavioral and Cardiac Responses to Mild Stress in Young and Aged Rats: Effects of Amphetamine and Vasopressin

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BUWALDA, B., J. M. KOOLHAAS AND B. BOHUS. *Behavioral and cardiac responses to mild stress in young and aged rats: Effects of amphetamine and vasopressin.* *PHYSIOL BEHAV* 51(2) 211–216, 1992.—Young (3-month-old) male Wistar rats showed a relative decrease in heart rate to a sudden silence superimposed on low intensity background noise. This bradycardia was accompanied by immobility behavior. In 26-month-old rats the magnitude of the heart rate response was reduced while immobility behavior remained in the same order of magnitude as in young controls. In the aged rats a shift in autonomic regulation of heart rate in the direction of increased sympathetic influence was indicated by the results obtained by blocking the autonomic input with atropine methyl-nitrate (0.5 mg/kg) or atenolol (1 mg/kg) given subcutaneously (SC) 30 min prior to testing. Pretest (30 min) administration of amphetamine (0.5 mg/kg SC) reinstated the bradycardiac response in aged rats to a level seen in young ones. Arginine-vasopressin (AVP, 10 µg/kg SC), administered 60 min before the experiment, markedly facilitated the cardiac response in young animals but failed to restore cardiac responses in aged ones. The immobility behavior in the peptide-treated aged rats was also absent. The present findings suggest that a diminished central aminergic drive in aged rats is causing a reduction of the parasympathetic cardiac response to stress of sudden silence. The results also indicate an age-related vasopressinergic modulation of behavioral and cardiac responses to mild stress.

Aging    Immobility behavior    Autonomic responses    Bradycardia    Stress    Amphetamine    Vasopressin

IN aging animals and humans, stress-related cardiac dysrhythmias and hypertension may involve autonomic dysfunctions (1, 18, 23, 24). Previous research in this laboratory was focussed on the age-related changes in the autonomic responses due to stressors inducing behavioral immobility. During this passive way of coping, young male Wistar rats appeared to react predominantly with a cardio-inhibitory response (3, 4, 13). The initial bradycardiac response to a conditioned emotional stressor of fear of inescapable footshock was absent in aged rats (18). Since this bradycardia can be abolished by atropine (Buwalda, unpublished results), one may consider it as a primarily vagally mediated response. Accordingly, an age-related attenuation of parasympathetic control of cardiac functioning during emotional stress situations was suggested (18).

The behavioral arousal induced by novelty stress is either delayed in onset in aged rats (5) or reduced in magnitude (26). Increasing the level of arousal by administration of the psychostimulant amphetamine (AMPH) appeared to reinstate the bradycardiac response to the conditioned stress of fear of inescapable footshock in aged rats (16). In a similar experimental design, the effect of the neurohypophyseal hormone arginine-8-vasopressin (AVP) was investigated. Experiments in freely behaving

rats in various stressful situations suggested that AVP serves as an important modulator of the vagally mediated cardiac response to conditioned emotional stressors (2,13). Peripheral administration of this neuropeptide also did restore the bradycardiac response in aged rats (17).

The question was raised as to whether the age-related diminution of the bradycardiac responses and its aminergic and peptidergic modulation can be generalized for both conditioned (predictable) and nonconditioned (unpredictable) stress stimuli. Bradycardia can also be elicited in a nonaversive, unconditioned situation. Orientation and attention towards stimulus changes is accompanied by a decrease in heart rate in the rat (12). Sudden silence superimposed on low intensity background noise is eliciting bradycardia and immediate behavioral arrest with orientational movements (13). Accordingly, the behavioral immobility is then a common factor of the unconditioned and conditioned stress response. The aim of this paper was to analyse behavioral and cardiac responses to the mild stress of sudden silence in young and aged rats, with special emphasis on accelerative sympathetic and decelerative parasympathetic influences on heart rate. In addition, the modulating effect of AMPH and AVP on cardiac and behavioral responses to sudden silence in

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young and aged rats was investigated.

#### METHOD

##### *Animals and Housing*

Male Wistar rats of 3–4 and 26 months of age were used. The animals originated from the Winkelman substrain and were generously donated by Troponwerke, Cologne, Germany. They were housed 6 to a cage (40 × 60 × 15 cm), with food and water ad lib, in a temperature-controlled environment of  $21 \pm 2^\circ\text{C}$ ; the lights were on from 0730 to 1930 h. All experiments were performed between 0900 to 1300 h.

##### *Surgery*

In order to record the electrocardiogram (ECG), transcutaneous stainless steel electrodes made of standard paperclips were implanted under light ether anesthesia. One was placed between the scapulae and the other in the midback, according to the method described previously (4). At least three days were allowed for recovery before the start of the experiment.

##### *Procedure*

The rats' behavioral and cardiac responses to a sudden drop in background noise were measured in a rectangular clear Plexiglas cage (85 × 60 × 60 cm), which we will designate as an "open field" in this paper. This open field was located in an acoustically isolated experimental room. The floor was covered with wood shavings. A noise generator produced a constant "mixed" background noise (65 dB, 2–8 kHz) in the experimental room. This noise was on from the time of entering the experimental room. Upon entering, a miniature FM-transmitter for the ECG recordings was fixed on the rat and subsequently the animal was placed in the open field for 5 min on Day 1. The time interval from entrance to exposure to the open field lasted about 1 min. On Day 2, the test day, the animals were exposed again to the open field for 5 min. After the first 2 min the background noise was then switched off, leaving the animal in almost total silence for the final 3 min. Heart rate and behavior recordings were taken 3 times for periods (P) of 60 s. Second-min recordings (P1) were considered to be prestimulus measurements. Third- (P2) and fifth-min (P3) recordings were regarded as response measurements. As a behavioral measure the time spent on "immobility" was determined by an observer during the three periods. Immobility was defined as almost motionless scanning of the environment with only minor head movements.

##### *Recording and Analysis of the ECG*

The ECG of freely moving rats was monitored telemetrically by means of a miniature FM transmitter (model SNR 102F, Dynamic Electronics Ltd., London, England) as described earlier (4). The transmitter was attached to a velcro strap secured around the chest of the rat and connected to the transcutaneous electrodes. The transmitted signals were received on a commercial FM receiver, amplified (Narco Bio-System Inc., Mod. FM-1100-7) and stored on tape by a commercial tape recorder. During recording and analysis, the quality of the ECG signal was continuously monitored on an oscilloscope.

Recorded ECG samples were played back through a cardiota-chometer pulsegenerator (Schmitt-trigger) that generated a square wave pulse at each R wave. The interbeat interval (IBI), which is the time between onset of the two consecutive pulses, was measured using a personal computer (Olivetti M24). The mean IBIs were computed for periods of 55 s. IBIs shorter than 100

and longer than 220 ms were discarded because these were likely to be due to artifacts. For the analysis of recordings after AVP treatment, the upper limit was set on 250 ms because of the excessive bradycardia in some of the young animals.

##### *Autonomic Blockade*

**Substances.** To examine the degree of vagal contributions to the cardiac responses, cholinergic muscarinic receptors at peripheral level were blocked with atropine methyl-nitrate (0.5 mg/kg b.wt.). The  $\beta$ 1-adrenergic antagonist atenolol (1 mg/kg b.wt.) was used to block the sympathetic input to the heart. Atenolol was used because of its relative selectivity for cardiac  $\beta$ 1 receptors and its minimal central nervous system actions (8). Saline (SAL) injections served as vehicle. All substances were administered in a volume of 1 ml saline/kg b.wt. and applied subcutaneously (SC) 30 min prior to testing.

**Experimental design.** Fourteen young and fifteen aged rats were divided in 2 groups. Rats in group I were tested for their cardiac responses to stress of sudden silence after SAL administration. Three days later the animals were injected with atropine methyl-nitrate and tested again. Rats in group II were tested for cardiac responses to placement in the open field only in order to test the sympathetic activation. The animals in group II were injected with SAL followed 3 days later by atenolol.

##### *Aminergic and Peptidergic Modulation*

**Substances.** d-Amphetamine sulfate (AMPH, OPG, Utrecht, The Netherlands) was administered SC (0.5 mg/kg b.wt.) 30 min prior to testing. Arginine-vasopressin (AVP) was injected SC (10  $\mu\text{g/kg}$  b.wt.) 60 min prior to the exposure to the open field. SAL was used as a vehicle. The substances were applied in a volume of 1 ml saline/kg b.wt. The selection of the single doses of the peptide and the drug was based on the results of the previous studies of young and aged rats' cardiac response to the conditioned stress paradigm (16,17). Nyakas et al. (16) showed that an AMPH dose of 0.5 mg/kg significantly enhanced behavioral activity of young and aged rats in a small open field to a same level without inducing stereotypic behavior. The AVP dose of 10  $\mu\text{g/kg}$  was the most potent dose to reinstate the bradycardia in aged rats in the conditioned stress paradigm (17).

**Experimental design.** Nineteen young and eighteen aged rats were divided in two groups (groups III and IV). Rats in these groups were tested for their cardiac and behavioral responses to stress of sudden silence. Animals in group III were injected in a cross-over design with SAL and AMPH, each animal serving as its own control. Rats in group IV were injected in a cross-over design with SAL and AVP. Between administrations were 7 days to minimize interaction of treatments.

##### *Statistics*

Results are presented as means  $\pm$  SEM. Cardiac data were analyzed using a multivariate analysis of variance with repeated measures (MANOVA-STATS program), a two-tailed Student's *t*-test and a paired *t*-test. Behavioral data were evaluated for significance using the Mann-Whitney U-test and the Wilcoxon matched-pairs ranked-signs test. A probability level of  $p < 0.05$  was taken as statistical significance for all tests.

#### RESULTS

##### *Autonomic Contributions to Cardiac and Behavioral Stress Responses*

Figure 1A shows young and aged rats' cardiac responses to

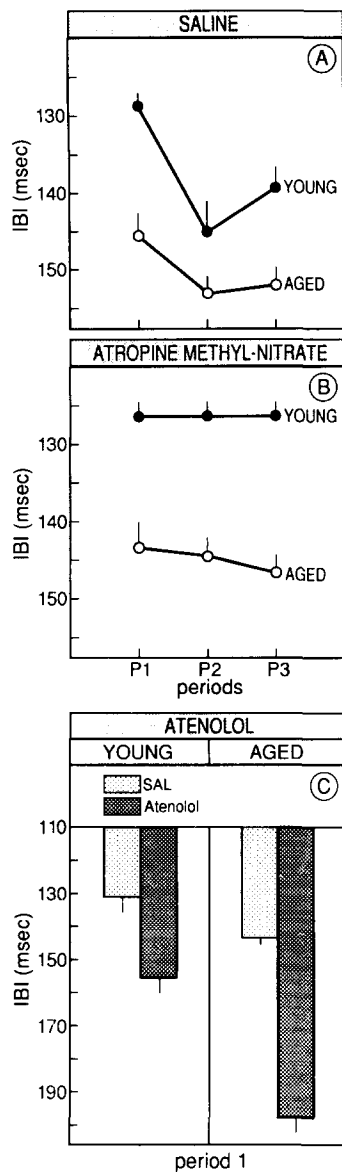


FIG. 1. Heart rate expressed as interbeat interval (IBI) during a 5-min exposure to an open field before and after sudden reduction of nonaversive background "mixed" noise, measured in young (3-month-old)  $\bullet$ — $\bullet$  and aged (26-month-old)  $\circ$ — $\circ$  male Wistar rats. One-min recordings were made during the second min of exposure to the field with noise on (P1); during the third min immediately after the noise was switched off (P2) and during the fifth min (P5). Young and aged rats' IBIs are presented after subcutaneous (SC) administration of 0.5 ml saline (A) or atropine methyl-nitrate (0.5 mg/kg b.wt.) (B) 30 min prior to open field exposure. The lower panel (C) shows IBIs only during the first recording in the open field (P1) 30 min after intraperitoneal (IP) administration of saline or atenolol (1 mg/kg b.wt.). Means  $\pm$  s.e. from 7–11 rats per group are shown.

stress of sudden silence. MANOVA revealed a significant effect of age: lower heart rate was observed during all 3 periods in aged rats,  $F(1,13)=11.61$ ,  $p<0.01$ . The interaction between age and periods was almost significant,  $F(2,26)=3.07$ ,  $p=0.06$ . Young rats responded to sudden silence with a significant increase in IBI, i.e., a decrease in heart rate ( $p<0.01$ ). Aged rats also showed a bradycardiac response to switching off the noise

TABLE 1

EFFECT OF AMPHETAMINE ON BEHAVIORAL AND CARDIAC RESPONSES TO STRESS OF SUDDEN SILENCE

	3 Months (n = 11)		25 Months (n = 11)	
	IBI ms	Immob. s	IBI ms	Immob. s
Saline				
period 1	127 $\pm$ 2*	15 $\pm$ 4	143 $\pm$ 3	20 $\pm$ 4
period 2	138 $\pm$ 2	55 $\pm$ 3	147 $\pm$ 4	48 $\pm$ 6
period 3	131 $\pm$ 2	39 $\pm$ 8	147 $\pm$ 3	37 $\pm$ 7
Amphetamine				
period 1	128 $\pm$ 2	13 $\pm$ 3	142 $\pm$ 2	25 $\pm$ 6
period 2	139 $\pm$ 2	53 $\pm$ 3	150 $\pm$ 3	43 $\pm$ 6
period 3	137 $\pm$ 3	40 $\pm$ 4	154 $\pm$ 3	27 $\pm$ 6

\*Mean  $\pm$  s.e.

Cardiac rate, expressed as interbeat interval (IBI) in ms, in saline- or amphetamine-treated young and old rats tested in an open field. Duration of immobility during each period is given in s. Period 1 represents a 60-s recording just before the sudden reduction of background noise. Period 2 and 3 were recorded, respectively, immediately and two min after sudden silence.

( $p<0.01$ ). The magnitude of the bradycardia in aged rats, however, was smaller than the one seen in young ones ( $p<0.05$ ).

Administration of atropine methyl-nitrate blocked the bradycardiac response to sudden silence in both age groups (Fig. 1B). Heart rate after handling, transport and placement in the open field (P1), however, was not affected by atropine as revealed by a comparison with the P1 values of the SAL-treated controls (Fig. 1A). The effect of age as observed in controls (Fig. 1A) was also preserved: lower heart rate appeared in the aged animals,  $F(1,12)=26.32$ ,  $p<0.001$ . Figure 1C shows the mean heart rate values after handling, transport and placement in the open field (P1) in rats receiving atenolol. The difference in IBI between young and aged saline-treated groups ( $p<0.05$ ) was the same as in the former experiments (Fig. 1A and 1B). Atenolol administration significantly increased IBIs both in young ( $p<0.01$ ) and aged rats ( $p<0.001$ ). The difference in mean IBI between the two age groups after atenolol treatment, however, was larger ( $43.6 \pm 5.7$  ms) than after saline administration ( $11.4 \pm 5.5$  ms) ( $p<0.001$ ).

Young as well as aged rats responded with a significant increase in time spent on immobility behavior (resp.  $25 \pm 7$  s and  $24 \pm 9$  s) ( $p<0.05$ ). Administration of atropine and atenolol did not affect the behavioral responses (data are not shown).

#### Modulation of Cardiac and Behavioral Stress Responses by Amphetamine and Vasopressin

**Amphetamine.** The mean heart rate of young and old animals following vehicle injection was the same as in the former experiment, and both young ( $p<0.001$ ) and aged rats ( $p<0.05$ ) responded to sudden silence with a bradycardia. The response in young rats was, again, significantly larger than the one seen in aged animals ( $p<0.01$ ). AMPH administration did not cause a general treatment effect on IBI in young and aged rats. In the aged group, however, it did result in an interaction between treatment and periods,  $F(2,40)=4.8$ ,  $p<0.01$  (see Table 1). Figure 2 and Table 1 show that an increased bradycardiac response to sudden silence occurred in aged rats after administra-

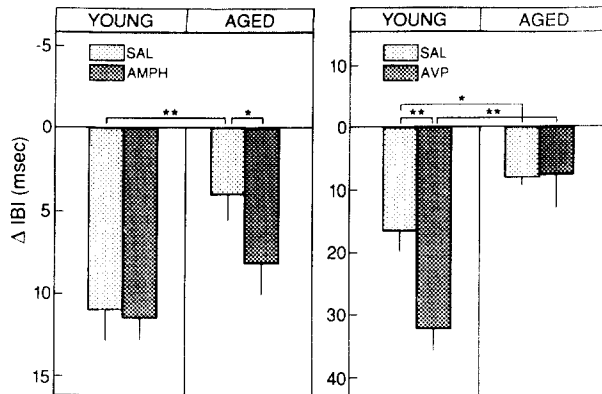


FIG. 2. Effect of Amph (0.5 mg/kg b.wt.; SC 30 min prior to testing) and AVP (10  $\mu$ g/kg b.wt.; SC 60 min prior to testing) on heart rate responses in IBI (P2-P1) to sudden silence in young (3-4-month-old) and aged (26-month-old) rats. Means  $\pm$  s.e. from 7-10 animals per group are shown. \* $p$ <0.05, \*\* $p$ <0.01 young vs. aged (two-tailed  $t$ -test).

tion of AMPH. The magnitude of the response was the same as in young animals. The cardiac responses to sudden silence in young rats were not affected by AMPH. Figure 3 shows that both young and aged rats treated with SAL responded behaviorally with similar increases in immobility ( $p$ <0.01). AMPH failed to influence significantly behavioral reactivity in young and aged rats, but increased the difference that was present between vehicle injected groups ( $p$ <0.001).

**Arginine-vasopressin.** Table 2 shows that administration of AVP resulted in a general increase in mean IBI when compared to SAL treatment in both young,  $F(1,14)=39.95$ ,  $p$ <0.0001, and aged rats,  $F(1,12)=10.25$ ,  $p$ <0.01. AVP also caused a significant interaction between treatment and periods in young,  $F(2,28)=8.5$ ,  $p$ <0.005, but not in aged rats (Table 2). Like in the former experiments, SAL-treated animals showed a significant bradycardiac response after sudden silence (Fig. 2) with an age-dependent difference in the magnitude of the response ( $p$ <0.05). After AVP administration, cardiac inhibition to sudden silence in young rats was much stronger when compared to SAL treatment ( $p$ <0.01). In aged rats AVP failed to enhance the magnitude of the bradycardiac response. The behavioral ef-

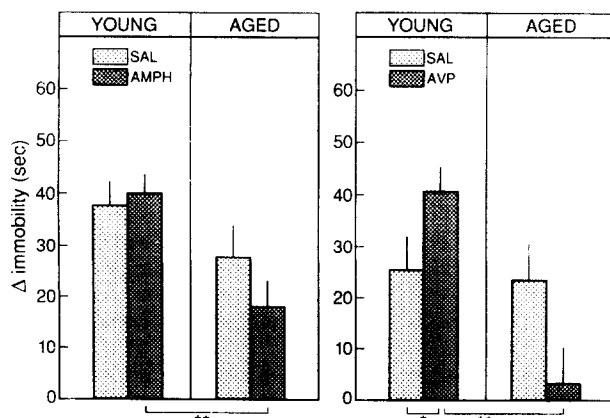


FIG. 3. Effect of Amph and AVP administration on behavioral responses to sudden silence in young and aged rats, measured in increase in time spent on immobility behavior (P2-P1). \* $p$ <0.05, \*\* $p$ <0.01 young vs. aged (two-tailed  $t$ -test). For further information see Fig. 2.

TABLE 2

EFFECT OF VASOPRESSIN ON BEHAVIORAL AND CARDIAC RESPONSES TO STRESS OF SUDDEN SILENCE

	3 Months (n = 8)		25 Months (n = 7)	
	IBI ms	Immob. s	IBI ms	Immob. s
Saline				
period 1	129 $\pm$ 2*	27 $\pm$ 5	145 $\pm$ 3	30 $\pm$ 6
period 2	146 $\pm$ 4	53 $\pm$ 6	153 $\pm$ 2	53 $\pm$ 3
period 3	139 $\pm$ 3	36 $\pm$ 8	152 $\pm$ 3	36 $\pm$ 5
Vasopressin				
period 1	168 $\pm$ 8	19 $\pm$ 5	172 $\pm$ 9	41 $\pm$ 5
period 2	201 $\pm$ 7	60 $\pm$ 0	179 $\pm$ 6	44 $\pm$ 8
period 3	197 $\pm$ 8	43 $\pm$ 6	176 $\pm$ 9	32 $\pm$ 10

\*Mean  $\pm$  s.e.

Cardiac rate and immobility behavior in saline- and vasopressin-treated young and old rats in an open field. For further information see Table 1.

fect of AVP was also differential. An increase in immobility was observed in young rats, while an almost significant decrease in immobility response was found in aged rats ( $p$ <0.06).

#### DISCUSSION

In the presented experiments the occurrence of sudden silence was used as a model for mild unexpected stress. The results show that this mild stressor caused a marked bradycardia in young rats. This bradycardiac response was diminished in aged rats. The results after blockade of parasympathetic or sympathetic input to the heart indicated a shift in autonomic regulation of the heart rate in aged rats. Finally, it was demonstrated that AMPH, and not AVP administration, increased bradycardiac responses in the aged animals to a level that was seen in young ones. The diminished bradycardiac response to mild unexpected stress in aged rats confirms the earlier observations on the effects of an aversive, conditioned emotional stressor (18). Since aged and young rats showed similar behavioral reactions to sudden silence, the lower bradycardiac response in aged rats is not attributed to a behavioral deficit but to an autonomic dysregulation of the heart in response to stress. The results after atropine administration indicate that the reduced bradycardiac response to sudden silence in aged rats is caused by a reduction of stress-induced parasympathetic responsivity in old animals. In accordance with this finding, age-related decreases in parasympathetically mediated cardiovascular responses were reported in the magnitude of the baroreceptor reflex-induced bradycardia (22) and reduced heart rate variability (7).

Overall heart rate after transport, handling and exposure to the open field was lower in the saline-treated aged rats when compared to young ones. This age difference was similar to the one found during resting conditions in another study (6), suggesting an age-related change in autonomic tone. Muscarinic blockade failed to affect the difference in heart rate. Since administration of atropine neither caused a shift in general heart rate towards acceleration during P1 in young or aged rats, tonic parasympathetic activation of the heart appears to be absent during this period. The lower heart rate in old animals is, therefore, not caused by an elevated parasympathetic activation. Blocking the  $\beta_1$ -adrenoceptors on the heart with atenolol increased the existing age-related difference in the heart rate of

free moving rats. Accordingly, the lower heart rate cannot be ascribed to a reduced sympathetic drive in aged rats. Since both  $\beta$ -adrenergic and muscarinic blockade fail to diminish the existing difference in heart rate, possibly a decreased intrinsic cardiac rate in aged rats is involved (19). Although it is generally accepted that  $\beta$ -adrenergic stimulation of rat cardiac contraction is reduced with increasing age (21,25), the increased difference seen in heart rate between young and aged rats after  $\beta$ -blockade seems to reflect an increased accelerative impact of the existing sympathetic tone on cardiac rate in aged Wistar rats.

AMPH administration, like in a conditioned emotional stress paradigm (16), restored the bradycardiac response to mild unexpected stress in aged rats to levels seen at young age. The normalized cardiac responses in aged rats cannot be ascribed to an improvement of behavioral responses: immobility behavior was even diminished in aged rats after AMPH treatment. Similar attenuation of immobility was observed in the conditioned stress paradigm (16). These findings suggest a decoupling of the organization of behavioral and cardiac stress responses. Since AMPH caused a slight decrease in systolic blood pressure 30 min after administration in both resting and stress conditions (16), it is unlikely that the facilitation of the bradycardiac response in aged rats did result from a simple baroreflex activation. AMPH administration did not influence cardiac and behavioral responses to stress in young rats. This probably indicates that the central drive of these particular responses in the SAL-treated young group were already maximal under these stress conditions. It is also possible that the sensitivity to AMPH is lower in the young animals, but our former dose-response study in a conditioned stress paradigm (16) does not support such a view. The drug action on cardiac response is probably due to an increased parasympathetic activation in aged rats. Atropine treatment appeared to abolish the cardiac effect of AMPH in old animals during conditioned aversive stress situations (Buwalda, in preparation). Since AMPH facilitates the release of biogenic amines through presynaptic mechanisms (14), a direct peripheral effect on heart rate would be of an acceleratory nature. Therefore, AMPH probably modulates autonomic stress responses through central mechanisms. This action is not simply due to aminergic activation in the brain, since AMPH failed to induce comparable effects in resting conditions (16). Rather, a modulation of stress-induced arousal is likely. The nature of the mechanism is now under study.

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AVP caused a reduction in heart rate both in young and in aged rats. Nyakas et al. (17) reported a moderate bradycardia in resting conditions and a slightly decreased mean blood pressure 60 min after the subcutaneous administration of 10  $\mu$ g/kg AVP in both resting and stress conditions. It is, therefore, unlikely that the general reduction in heart rate as observed in the present experiment is the result of baroreceptor reflex-induced vagal activation. Vasopressin-induced enhancement of cardiac responses to unexpected stress was very strong in young, while absent in aged rats. Furthermore, the behavioral response to sudden silence was absent in aged rats after AVP administration. The responses seen in the young rats confirm earlier observations seen in mild stress (13). During conditioned stress situations bradycardiac responses in aged rats were enhanced by AVP (17). The aged rats used in that experiment, however, were 14 months old. The age difference in the two experiments may explain the lack of peptidergic reinstatement of the bradycardia in the present experiments. To what extent central and peripheral mechanisms are involved in the modulating properties of peripherally administered AVP on stress-related behavior and autonomic functioning is still a major issue of discussion (9, 10, 15). A decline in brain AVP innervation in aged rats (11,20) was reported, but data on central receptor states are not yet available. It is possible that the effects of AVP on behavioral and cardiac responses are caused by viscerally induced signals (15). To elucidate the exact mechanisms of action of peripherally applied AVP, further investigation is needed.

In conclusion, the present study provides evidence for a general decrease in parasympathetic cardiac inhibition to stressors evoking immobility behavior in aged rats. The results also indicate that vasopressinergic and central aminergic systems are involved in the stress-related parasympathetic drive.

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