Chapter 5

NEUROENDOCRINE AND CARDIOVASCULAR RESPONSES TO MILD STRESS IN YOUNG AND AGED RATS

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ABSTRACT

Under resting conditions, mean arterial pressure (MAP) and heart rate (HR) were not significantly different in free moving, naive 24 mo old male Wistar rats when compared to young adults (3 mo old). Basal levels of plasma norepinephrine (NE) and corticosterone (CORT) were elevated in the aged animals, while epinephrine (E) levels were the same order of magnitude in both age groups. Mild stress, consisting of transportation to an open field where a sudden silence was imposed on background noise, caused similar cardiovascular responses, -i.e. increase in MAP and HR - in young and aged rats. Plasma NE and CORT responses to stress were slightly, but not significantly blunted in aged rats. Plasma E responses were in the same order of magnitude in both ages. Post-stress recovery rates of MAP and HR were similar in young and aged animals, while recovery of plasma NE response was delayed in aged rats.

The present findings indicate that tonic sympathetic and adrenocortical activity is elevated in aged rats, while post-stress recovery rate of plasma NE appears to be delayed in aged rats. The cardiovascular and endocrine responsiveness to mild stress, however, is little affected by age.

INTRODUCTION

It is well recognized that stimuli which provoke changes in emotionality have a strong impact on the neuroendocrine and autonomic nervous system (3,13,25). The character of the stress response is the result of interactions between the environment (controllability/ predictability), the coping strategy , the properties of the stressor and bodily factors (4). Aging represents an important variable in stress responsiveness. Previous studies in this laboratory have focussed on the age-related changes in the autonomic stress-responses due to stressors inducing behavioral immobility. During such immobility - i.e. passive way of coping, young male Wistar rats react predominantly with a parasympathetic, cardio-inhibitory response (2,3,19,28). This stress-induced bradycardia, which is a relative change in heart rate in comparison to non-punished free moving male rats, appears to be absent in aged rats (28). It was suggested that an age-related reduction of vagal responses might be associated with a deficit in the immediate evolvement of neuronal activity underlying behavioral and autonomic arousal (28). Arousing or stressful events are well known to activate both the pituitary adrenocortical axis and the sympatho-adrenomedullary system resulting in raised plasma concentrations of the adrenal corticosterone and of the catecholamines norepinephrine and epinephrine (1,13). Since sympathetic output is modulated prejunctionally by vagal acetylcholine (27), the diminished vagal stress responsiveness may lead to a disturbed autonomic balance in aged rats. Although the relation between aging and physiological stress responses is receiving increasing attention, only a few studies have examined autonomic and endocrine responses to emotional stress in freely moving aged rodents (9,26). Accordingly, it is of interest to investigate these stress responses, in particular to less demanding situations, in order to find out whether the age-related reduction of vagal

stress responses (28) is also reflected in sympathetic as well as endocrine responses.

In this paper young and aged rats' cardiovascular, neuroendocrine and autonomic stress responses to a short mild emotional stressor are presented and evaluated. Stress consisted of transportation to a different environment where a sudden silence was imposed on background noise. Former observations showed that this type of stressor causes pronounced cardiac and behavioral responses in young male Wistar rats (8,19).

METHODS

Animals

Young (3 mo old) and aged (24 mo old) male Wistar rats (weighing resp. 298 ± 3 and 514 ± 53; originating from CpB TNO, Zeist, The Netherlands and bred in this laboratory) were housed individually in clear Plexiglas cages (25x25x30 cm) on a 12h light-dark regime (light on between 07.30h - 19.30h) at a room temperature of 21 ±2°C. All animals had free access to standard food (Hope Farms rat chow) and water.

Surgery

All surgery was performed under complete ether anesthesia at least one week prior to testing.

Venous catheter. The animals were provided with a permanent silicon catheter (0.95 mm OD., 0.50 mm ID.) in the right atrium inserted via the right jugular vein and externalized on the top of the skull according to the techniques described earlier (35). The rats were provided with these catheters to allow frequent blood sampling in unrestrained and undisturbed freely moving rats (37).

Arterial catheter. For direct recording of arterial blood pressure and heart rate the rats were also provided with a catheter in the descending aorta according to slight modifications of techniques described earlier (7,36). The aorta was exposed via a midline incision in the abdomen. Aortic blood flow was briefly stopped by application of a small artery clip rostral to the level of the iliolumbar vessels. A silicon catheter (0.95 mm OD., 0.50 mm ID.) with a "J" shaped teflon tip (TW30, Talas, Ommen, The Netherlands), oriented in an upstream direction, was inserted through a 23 gauge needle puncture into the abdominal aorta. The puncture was made approximately 0.3 cm rostral to the bifurcation of the aorta. The length of the teflon tubing in the aorta was ±2 cm. After insertion the catheter was anchored to the left psoas muscle, just lateral to the aorta. No leakage occurred at the point of insertion, the elasticity of the aortic wall being sufficient to close the wound around the catheter. Like the venous catheter the arterial catheter was externalized to the top of the skull and filled with a 50 percent heparinized polyvinylpyrrolidonum (M = ±25000) (PVP) solution (35). This PVP solution was refreshed daily.

Experimental procedure

All experiments were performed between 09.00 and 13.30 hr, -i.e. in the period of stable and low plasma levels of E, NE and CORT (12). To expose the animals to a mild stress of environmental change the rats were transferred to an open field of clear Plexiglas measuring 85x60x60 cm, for a period of 5 min on Day 1. The floor was covered with wood shavings. A constant background "white" noise (65 dB, 2-8 kHz) produced by a noise generator was also provided in the field. The open field was located in an experimental room acoustically isolated from the animal housing. On Day 2, i.e. the test day, the animals were connected to polyethylene tubes (±0.4 m length, 1.45 mm OD. and 0.75 mm ID.) for blood sampling and cardiovascular monitoring at least 45 min prior to testing. At t=0 min the rats were exposed again to the open field. During the first 2 min the background noise was on but was then switched off, leaving the chamber in silence for the final 3 min. After the fifth min the rats were transferred back to their home cages.

Blood sampling and cardiovascular monitoring

Blood samples of 0.5 ml were taken in the home cage (at 15 min before transportation to the open field (baseline) and at t=20 min) and in the open field (at t=1.5, 2.5 and 4.5 min). After each sample the same quantity of heparinized donor blood (25 units per ml) was given in order to minimize the changes in blood volume with related changes in hemodynamics (35). Donor blood was obtained from unstressed rats with permanent heart catheters.

Before the onset of the experiments mean arterial pressure (MAP, mm Hg) levels were calibrated by applying water pressures to the transducer. The connecting tube was filled with heparinized saline (10% heparin of 500 IU/ml). MAP and heart rate (HR, beats/min) were measured in the home cage (30 sec recordings) immediately prior to blood sampling at t=-15 and t=20 min. In the open field measurements were taken at t=1, 2 and 4 min. All recordings in the open field were continuous. The data were calculated from 30 sec samples except of the t=2 min. This 10 sec period represented the immediate stimulus change, -i.e. switching off the noise.

Cardiovascular data acquisition

Arterial blood pressure was recorded via a pressure transducer (Honeywell 130 PC) with an amplifier (Electronics Service, Biological Center, Haren, The Netherlands) and an analog-to-digital converter (RTI-800, Analog Devices, Inc.). The pressure transducer was placed at the level of the heart. Interbeat intervals were measured from the pulse wave. The signal was fed into a microcomputer (Olivetti M24) for data processing and display. The blood pressure was analog-to-digital converted (12 bits) at a rate of over 1.0 kHz. Data processing and display was performed by the CARDIA software package (F.W.Maes, in preparation). HR in beats per min was calculated from interbeat intervals. Following each heart beat, high and low peak values for arterial blood pressure (systolic and diastolic pressures, in mm Hg) were determined. MAP was calculated as (systolic pressure + 2x diastolic pressure)/3. The data acquisition loop of the main program had a cycle time of not more than 1.0 millisecond. This included an on-line graphical representation of data on screen and resulted in a numerical dump of data on floppy disk for subsequent numerical and graphical analysis.

Chemical determinations

Blood samples of 0.45 ml were withdrawn for determination of plasma epinephrine (E), norepinephrine (NE) and corticosterone (CORT). The samples were immediately transferred to chilled (0°C) centrifuge tubes containing 0.01 % EDTA as antioxidant and 10 μl heparin solution (500 IU/ml) as anticoagulant. Blood was centrifuged at 4°C for 10 min at 5000 rpm, and 100 μl of the supernatant were stored at -20°C for corticosterone and at -80°C for the catecholamine measurements. Plasma corticosterone (CORT, μg/dl) was measured by means of reversed phase high performance liquid chromatography, as described earlier (11). Determination of plasma catecholamine concentrations was performed by HPLC in combination with electrochemical detection (ECD) as described earlier (33), with minor modifications.

Statistics

Results are presented as means ± SEM. For statistical analysis of data Multivariate Analysis of Variance (MANOVA-Stats program) with repeated measures was used (5 levels). MANOVA was followed by two-tailed t-tests. The paired t-test was used for comparisons within individuals. A probability level of $p<0.05$ was taken as statistical significance.

RESULTS

Arterial pressure and heart rate

Fig.1 shows MAP before, during and after mild stress of stimulus change. Basal MAP in the aged rats was 123.5±4.5 mm Hg. Although this value was higher than the MAP in young rats (114.8±3.4 mm Hg), the difference was not significant. Placement in the open field significantly increased MAP in the young rats (9.9 ± 3.5 mm Hg). The increase of MAP in the aged animals (7.9 ± 3.5 mm Hg) was the same order of magnitude. Switching off the background noise did not cause a further elevation in MAP neither in young nor in aged rats. Home cage measurements 20 min after the stress showed that post-stress recovery was similar in young and aged animals. Both groups regained pre-stress basal MAP levels.

Fig.1 also depicts measures of HR before, during and after the stress of stimulus change. Basal HR was slightly but not significantly higher in young rats (345±34 beats/min) compared to aged rats (329±43 beats/min). HR increased to transfer to the open-field were the same order of magnitude in young (75 ± 12 beats/min) and aged (68 ± 19 beats/min) rats. A different pattern of changes was seen after auditory stimulus change: a slight, but significant ($p<0.05$) decline was seen in the aged rats but not in the young ones. Home cage measurements of HR 20 min after the stress in young and aged rats were not different from pre-stress basal levels.

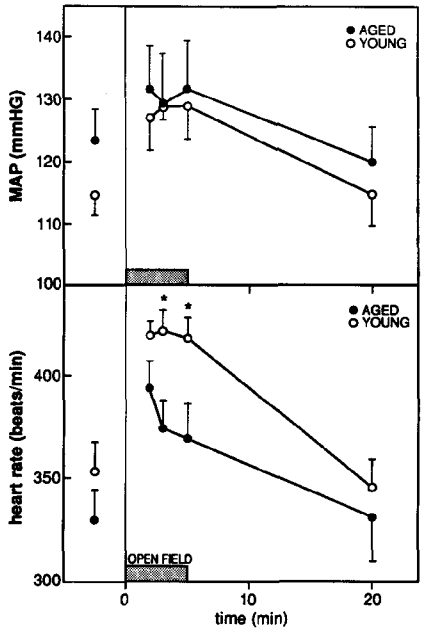

Fig.1.
*Mild stress and changes in mean arterial pressure (MAP, upper panel) and heart rate (lower panel) of young -o- (3 mo old) and aged -●- (24 mo old) rats. A 5 min exposure to an open field is indicated by the shaded area on the horizontal axis. The background noise was switched off immediately before the second sampling in the open field. The values before 0 min, and at 20 min were taken in the home cage. Means ± SEM are plotted from 8 animals in both groups. * $p<0.05$ (two-tailed t-test).*

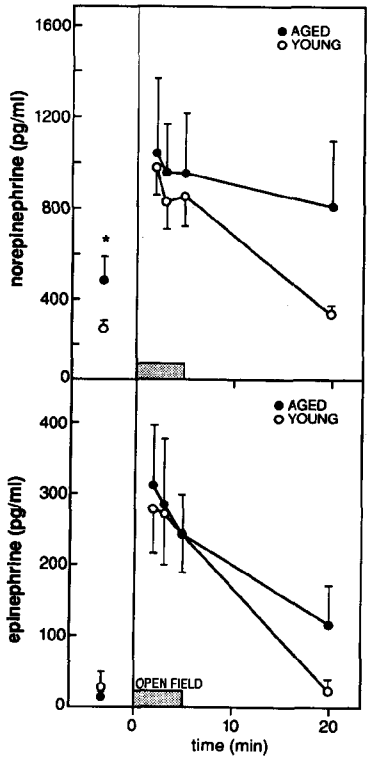

Fig.2.
Mild stress and changes in plasma norepinephrine (NE) and epinephrine (E) in young -o- (3 mo old) and aged -●- (24 mo old) rats. Means ± SEM from 9 animals in the young and from 7 in the aged group are shown.
** $p<0.05$ (two-tailed t-test).*
For further explanation see Fig.1.

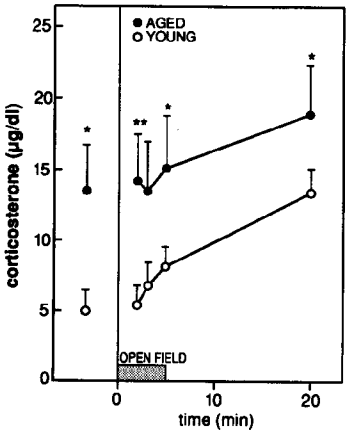

Fig.3.
*Mild stress and changes in plasma corticosterone (CORT) in young -o- (3 mo old) and aged -●-(24 mo old) rats. Means ± SEM from 9 animals in the young and 7 in the aged group are shown. * $p<0.05$, ** $p<0.01$ (two-tailed t-test).*
For further explanation see Fig.1.

Norepinephrine

Fig.2 shows plasma levels of NE before, during and after the stress experience in the open field. Basal levels of NE were significantly higher ($p<0.05$) in aged rats (491±99 pg/ml) compared to young ones (253±35 pg/ml). MANOVA for the repeated measures showed a significant increase in plasma NE levels to the stress situation in young ($F(4,5)=9.5$; $p=0.0003$) as well as in aged rats ($F(4,4)=3.1$; $p=0.04$). There was no significant effect of age, nor was an interaction seen between age and sampling time. Switching off the noise did not cause a further change in NE levels. The rate of recovery of the NE response to stress was measured by comparing peak values at t=1.5 min with home cage values at t=20 min. In young rats NE levels rapidly fell 658 ± 111 pg/ml which was significantly higher ($p<0.01$) than the fall in aged animals (236 ± 41 pg/ml). This difference indicates a delayed recovery in the aged group.

Epinephrine

Fig.2 also shows plasma levels of E before, during and after stress. Basal levels of E were the same in young (29.8±19.4 pg/ml) and aged rats (13.3±8.6 pg/ml). MANOVA for the repeated measurements showed a significant increase in plasma E levels both in young ($F(4,6)=7.2$; $p=0.0008$) and aged rats ($F(4,3)=5.6$; $p=0.009$). There was no significant effect of age, nor was an interaction seen between age and sampling time. Switching off the background noise did not cause further change in E levels. At t=20 min E levels in aged rats were slightly higher than in young rats. This difference was close to significance ($p<0.08$). Recovery rates, however, were not significantly different between young and aged rats.

Corticosterone

Fig.3 shows plasma CORT levels before, during and after stress. Basal level of CORT in aged rats (13.5±3.2 μg/dl) was significantly higher ($p<0.05$) than the one seen in young adults (5.1±1.4 μg/dl). While both the young ($F(4,8)=19.6$; $p<0.000$) and aged ($F(4,4)=4.1$; $p=0.02$) group showed a significant increase in time of plasma CORT levels, MANOVA indicated a significant group difference ($F(1,12)=7.4$, $p=0.02$) and a significant interaction between age and sampling time ($F(4,48)=3.8$; $p=0.01$). This interaction probably is caused by a delayed and diminished CORT response in aged rats. However, the maximal plasma CORT responses which were reached in the home cage at t=20 min in both age groups were not significantly different between young (8.7 ± 1.9 μg/dl) and aged (5.6 ± 2 μg/dl) rats.

DISCUSSION

In the present study differences between young and aged rats' baseline sympathetic and adrenocortical activity were observed, while cardiovascular and endocrine responsiveness to a mild stress situation were not significantly different in aged animals. Post-stress recovery rate of plasma NE was delayed in aged rats.

Resting MAP is slightly elevated while HR shows a tendency to be lower in aged rats, but the differences failed to reach significance. Conflicting data are available for MAP and HR in aged rats. Increased basal MAP was reported by Chiueh et al. (9), others (16,26) failed to observe changes in this parameter. One of the possible variables in causing these different findings may be the increasing heterogeneity in a group of aged animals. This is reflected in the larger SEM. Another variable may be the previous "stress history" of the animals. In a study concerning conditioned stress responses subsequent to this experiment (22), the same aged rats showed a significantly higher basal MAP than the young ones. The difference could be ascribed to a decreased basal MAP in the young animals. This may mean that old male rats less easily adapt to handling and other experimental procedures as far as the blood pressure regulation is concerned. The finding that resting HR falls with age is consistent with one study (9) but conflicting with others that report no change (16) or an increased HR in aged rats (26).

Pre-stress basal plasma levels of NE but not of E were elevated in aged rats. This finding confirms earlier reports (9,32). Higher levels of plasma NE may reflect an age-related decrease in the metabolic clearance rate, due to changes in regional circulation (17). Most data, however, suggest an age-related increase of NE spillover (32). Such an increase might be of physiological significance because of decreased responsiveness of end organs with age (20).

Pre-stress basal plasma levels of CORT were also elevated in the aged rats which is in agreement with a number of former reports (15,24,31). Two types of receptors for corticosterone in the brain appear to be involved in the regulation of ACTH and corticosterone secretion (29). Since hippocampal type I receptors are occupied even with low circulating titers of CORT (30), it is tempting to speculate that the type I receptor mediates a tonic inhibitory influence on the HPA axis (10,14). Some studies report a decrease in hippocampal type I receptors in aged rats (21,24). The diminished inhibition of CORT release might be a cause for the observed increase in basal CORT levels in the aged rats.

The response of the cardiovascular system to transport and placement in an open field is not markedly different in aged rats.

The age-related difference in NE and CORT stress response failed to reach significance. This probably is caused by the large variability in responsiveness in aged rats. In the study of Korte et al. (22) subsequent to this experiment this variability was reduced and NE responsiveness to conditioned stressors appeared to be diminished in aged rats. In the present experiment the slightly blunted NE and CORT responses to transportation assume an age-dependent change in stress-induced arousal. Due to the large variability in aged rats this assumption, however, needs further extended investigation involving larger groups of animals.

Post-stress recovery of plasma NE response was delayed in the aged rats which is in agreement with other studies in rats (26). A delayed recovery can be caused by a decreased clearance rate of NE from the blood. Borton and Docherty (5) reported a decreased systemic neuronal uptake of noradrenaline in aged rats. Since an increase in NE uptake was seen in the hindlimb of aged rats (20), there may be tissue-specific alterations in reuptake mechanisms during aging. Studies in humans report age-related decreases in clearance rate, due to changes in regional blood flow (17,18). A prolonged release of NE can also be a cause of a delayed recovery. This may be caused by a decreased negative feedback through prejunctional alpha-2 adrenergic receptors (6). A final possibility is that the sluggish central catecholaminergic response to stress in aged rats is reflected not only in a diminished CORT and NE response but also in a slower inhibition of the plasma hormone responses. Whether the differences seen between young and aged rats' endocrine parameters are developmental in nature can only be solved by studying rats of intermediate age.

The present experiments were designed to study the dynamics of the physiological and endocrine stress response to the sudden, but mild stress of background auditory stimulus change. Former behavioral and cardiac rate studies have justified the use of this paradigm both in young and aged rats (8,19,34). Surprisingly, not only a slowly reacting system like the pituitary-adrenal axis failed to change rapidly, but also blood pressure, heart rate and plasma catecholamine responses were absent. Responses to transfer and/or to the novel environment were very marked, however, at all parameters studied here. The absence of a dynamic response to the sudden stimulus change probably can be explained by the different experimental conditions in this study. It occurred to us that the orienting response to the mild stress of a sudden change in the environment is very sensitive to distracting stimuli like

sound and movement. Heart rate responses formerly studied to the occurrence of sudden silence were always biotelemetrically monitored with minimal disturbance of the animal. In the present study the rats were connected to tubes for blood sampling and cardiovascular monitoring and two experimentators had to stand next to the open field. This situation most likely provided too much distracting factors, inhibiting behavioral and physiological responses to switching off the background noise

Together with the previous studies concerning the age-related reduction of vagal stress responses, the present findings indicate that the decrease in parasympathetic stress responsiveness in aged rats not necessarily leads to a disturbed balance in autonomic stress responses indicating that in aged rats probably compensatory mechanisms evolve. Whether these processes occur in the central nervous system or by adaptations in peripheral organs needs further investigation.

REFERENCES

1. Axelrod, J.; Reisine, T.D. Stress hormones: their interaction and regulation. Science 224: 452-459; 1984.
2. Bohus, B. Telemetred heart rate responses of the rat during free and learned behaviour. Biotelemetry 1:193-201; 1974.
3. Bohus, B. Acute cardiac responses to emotional stressors in the rat: the involvement of neuroendocrine mechanisms. In: Orlebeke, I.F.; Mulder, G.; van Doornen, L.I.P., eds. Psychophysiology of Cardiovascular Control. Methods, Models and Data. New York: Plenum Press, 1985:131-150.
4. Bohus, B.; Benus, R.F.; Fokkema, D.S.; Koolhaas, J.M.; Nyakas, C.; van Oortmerssen, G.A.; Prins, A.J.A.; de Ruiter, A.J.H.; Scheurink, A.J.W.; Steffens, A.B. Neuroendocrine states and behavioral and physiological stress responses. In: de Kloet, E.R.; Wiegant, V.M.; de wied, D., eds. Progress in Brain Research. vol.72. Elsevier Sci. Publ. 1987:57-70.
5. Borton, M.; Docherty, J.R. The effects of aging on neuronal uptake of noradrenaline in the rat. Naunyn-Schmiedebergs Arch. Pharmacol. 340:139-143; 1989.
6. Buchholz, J.; Duckles, S.P. Effect of age on prejunctional modulation of norepinephrine release. J. Pharmacol. Exp. Ther. 252:159-164; 1989.
7. Buckingham, R.E. Indwelling catheters for direct recording of arterial blood pressure and intravenous injection of drugs in the conscious rat. J. Pharm. Pharmac. 28:459-461; 1976.
8. Buwalda, B.Koolhaas, J.M.; Bohus, B. Age-related changes in cardiac response during orientation: the effect of amphetamine and vasopressin. Eur. J. Pharmacol. 183:2371; 1990.
9. Chiueh, C.C.; Nespor, S.M.; Rapoport, S.I. Cardiovascular, sympathetic and adrenal cortical responsiveness of aged Fischer-344 rats to stress. Neurobiol. Aging 1:157-163; 1980.
10. Dallman, M.F.; Akana, S.F.; Casciao, C.S.; Darlington, D.N.; Jacobson, L.; Levin. N. Regulation of ACTH secretion: variation on a theme of B. Recent Prog. Horm. Res. 43:113-173; 1987.

11. Dawson Jr., J.M.; Kontur, P.; Monjan, A. High-performance liquid chromatographic (HPLC) separation and quantitation of endogenous glucocorticoids after solid-phase extraction from plasma. Hormone Res. 20:89-94; 1984.
12. De Boer, S.F.; Van der Gugten, J. Daily variations in plasma noradrenaline, adrenaline and corticosterone concentrations in rats. Physiol. Behav. 40:323-328; 1987.
13. De Boer, S.F.; Slangen, J.L.; Van der Gugten, J. Adaptation of plasma catecholamine and corticosterone responses to short-term repeated noise stress in rats. Physiol.Behav. 44:273-280; 1988.
14. De Kloet, E.R.; Reul, J.M.H.M. Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems. Neuroendocrinol. 12:83-105; 1987.
15. DeKosky, S.T.; Scheff, S.W.; Cotman, C.S. Elevated corticosterone levels:a possible cause of reduced reduced axon sprouting in aged animals. Neuroendocrinol. 38:33-38; 1984.
16. Docherty, J.R.; Fitzgerald, D.; O'Malley, K. Age-related reduction of baroreflex tachycardia without loss of ß-adrenoceptor-mediated tachycardia in Sprague-Dawley rats. J. Cardiovasc. Pharmacol. 8:376-380; 1986.
17. Esler, M.; Skews, H.; Leonard, P. Age-dependence of noradrenaline kinetics in normal subjects. Clin.Sci. 60:217; 1981.
18. Featherstone, J.A.; Veith, R.C.; Halter, J.B. Effect of age and alpha-2 adrenergic stimulation on plasma norepinephrine kinetics in man. Clin.Res. 32:69A; 1984.
19. Hagan, J.J.; Bohus, B. Vasopressin prolongs bradycardiac response during orientation. Behav. Neural Biol. 41:77-83; 1984.
20. Handa, R.K.; Duckles, S.P. Age-related changes in adrenergic vaoconstrictor responses of the rat hindlimb. Am. J. Physiol. 253:H1566-H1572; 1987.
21. Issa, A.M.; Rowe, W.; Gauthier, S.; Meaney, M.J. Hypothalamic-pituitary-adrenal activity in aged, cognitively impaired and cognitively unimpaired rats. J. Neurosci. 10:3247-3254; 1990.
22. Korte, S.M.; Buwalda, B.; Bouws, B.; Koolhaas, J.M.; Maes, F.W.; Bohus, B. Conditioned neuroendocrine and cardiovascular stress responsiveness accompanying behavioural passivity and activity in aged and in young rats. Physiol. Behav., in press.
23. Landfield, P.; Waymire, J.; Lynch, G.; Hippocampal aging and adrenocorticoids: a quantitative correlation. Science 202:1098-1102; 1978.
24. Lorens, S.A.; Hata, N.; Handa, R.J.; van de Kar, L.D.; Guschwan, M.; Goral, J.; Lee, J.M.; Hamilton, M.E.; Bethea, C.L.; Clancy jr., J. Neurochemical, endocrine and immunological responses to stress in young and old Fischer 344 male rats. Neurobiol. Aging 11:139-150; 1990.
25. Mason, J.W. A review of psychoneuroendocrine research on the pituitary-adrenal cortical system. Psychosom. Med. 30:576-607; 1968.
26. McCarty, R. Age-related alterations in sympathetic-adrenal medullary responses to stress. Gerontol. 32:172-183; 1986.
27. Muscholl. E. Peripheral muscarinic control of norepinephrine release in the cardiovascular system. Am. J. Physiol. 239:H713-H720; 1980.
28. Nyakas, C; Prins, A.J.A.; Bohus, B. Age-related alterations in cardiac response to emotional stress: Relations to behavioral reactivity in the rat. Physiol. Behav. 47:273-280; 1990.
29. Reul, J.M.H.; de Kloet, E.R. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117:2505-2511; 1985.

30. Reul, J.M.H.; van den Bosch, F.R.; de Kloet, E.R. Relative occupation of type-I and type-II corticosteroid receptors in rat brain following stress and dexamethasone treatment: functional implications. J. Endocrinol. 115:459-467; 1987.

31. Sapolski, R.M.; Krey, L.C.; McEwen, B.S. The neuroendocrinology of stress and aging: The glucocorticoid cascade hypothesis. Endocrine Rev. 7:284-301; 1986.

32. Sato, A.; Sato,Y.; Suzuki, H. Changes in sympatho-adrenal medullary functions during aging. In: Liss, A.R. ed. Organization of the Autonomic Nervous system: Central and Peripheral Mechanisms. 1987; 27-36.

33. Smedes, F.; Kraak, J.C.; Poppe, H. Simple and fast solvent extraction system for selective and quantitative isolation of adrenaline, noradrenaline and dopamine from plasma and urine. J. Chromatography 231:25-39; 1982.

34. Steenbergen, J.M.; Koolhaas, J.M.; Strubbe, J.H.; Bohus, B. Behavioral and cardiac responses to a sudden change in environmental stimuli: Effect of forced shift in food intake. Physiol. Behav. 45:729-733; 1989.

35. Steffens, A.B. A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. Physiol. Behav. 4:833-836; 1969.

36. Weeks, J.R.; Jones, J.A. Routine direct measurement of arterial pressure in unanesthetized rats. Proc. Soc. Exp. Biol. Med. 104: 646-648; 1960.

37. Wiersma, J.; Kastelijn, J. A chronic technique for high frequency blood sampling/transfusion in the freely behaving rat which does not affect prolactine and corticosterone secretion. J. Endocrinol. 107:285-292; 1985.