

Attenuated Cardiovascular, Neuroendocrine, and Behavioral Responses After a Single Footshock in Central Amygdaloid Lesioned Male Rats

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ROOZENDAAL, B., J. M. KOOLHAAS AND B. BOHUS. *Attenuated cardiovascular, neuroendocrine, and behavioral responses after a single footshock in central amygdaloid lesioned male rats.* *PHYSIOL BEHAV* 50(4) 771–775, 1991.—The effect of bilateral electrolytical CEA lesioning on behavioral, cardiovascular and neuroendocrine changes has been studied in male Wistar rats before, during and shortly after a brief aversive stimulus of an unavoidable footshock. Blood samples were withdrawn via a permanent heart catheter. Lesioning of the CEA abolished completely the immobility response normally seen after a footshock. Lesions failed to affect the early tachycardiac response compared to sham-lesioned controls, but the poststress recovery was attenuated, probably due to diminished vagal activation. Furthermore, the magnitude of the responses of all measured hormones (epinephrine, norepinephrine, corticosterone and prolactin) appeared to be attenuated in the lesioned rats. These results suggest that the CEA plays an important and general role in the behavioral, autonomic and hormonal output during a brief unavoidable, unconditioned footshock. This is in contrast with the selective role of the CEA in vagal (parasympathetic) and on inhibitory (immobility) behavioral responses following conditioning.

Central amygdaloid nucleus	Electrolytic lesion	Acute footshock	Heart rate	Epinephrine	Norepinephrine
Corticosterone	Prolactin	Immobility behavior			

THE central nucleus of the amygdala (CEA) seems to be selectively involved in the passive component of the behavioral and the accompanying parasympathetic response during conditioned environmental challenges. For example, bilateral CEA lesioning disrupts the typical parasympathetic response, i.e., bradycardia, together with immobility behavior normally elicited as a consequence of emotional stress of fear of an aversive electric shock in rabbits (12) and rats (22,23). In addition, a CEA lesion attenuates the conditioned, stress-induced and vagus-mediated gastric ulceration in rats (9). However, conditioned-sympathetic and adreno-medullary activation, indicated by plasma norepinephrine and epinephrine levels, and the adreno-cortical (corticosterone) response appeared to be unaffected by CEA manipulation (Roozendaal et al., in preparation). Recently, we showed that the CEA is involved not only in conditioned stress-induced parasympathetic responses, but also in other, nonstress-related conditioned parasympathetic responses. Thus the conditioned cephalic insulin response is abolished by CEA lesioning (24). These findings altogether suggest a general role for the CEA in controlling conditioned-parasympathetic output.

However, the influence of lesioning of the CEA on acute, unconditioned-stress responses has received little attention. Therefore, the present study was designed to investigate a possible effect of bilateral electrolytical lesioning of the CEA on behavioral, hormonal and autonomic responses before, during and after a brief unavoidable footshock in male Wistar rats. The footshock

with the same parameters served as the unconditioned stimulus in a conditioning experiment with a one-trial learning procedure in rats.

METHOD

Animals

Thirty-two young adult (8–10 weeks old) male Wistar rats, weighing 280–320 g, were used. During the experiments the rats were housed individually in clear perspex cages (25 × 25 × 30 cm) with a sawdust-covered floor. Food and water was available ad lib in a temperature-controlled environment of $21 \pm 1^\circ\text{C}$, with lights on from 0830 to 2030 h. The experiments were carried out during the light period of the cycle (between 1000 and 1400 h).

Electrolytical Lesion of the CEA

The animals were anesthetized with ether and placed in a David-Kopf stereotaxic apparatus. The lesions were made with a monopolar stainless steel electrode (outer diameter of 0.2 mm and an uninsulated tip of 0.1 mm). The electrode was aimed at the central amygdala (coordinates: 6.7 mm rostral to interaural, lateral 4.0 mm to the midline, and ventral 7.0 mm below dura) (20).

The lesions were made with an anodal current of 1.25 mA

during 5 s. In the sham-operated controls, the electrode was lowered 6.0 mm below dura and no current was passed. The animals were allowed one week for postsurgical recovery.

Implantation of the Cannulae

Sixteen (8 lesioned and 8 sham-lesioned) rats were provided 1–3 days after lesioning with a silicon heart catheter through the jugular vein (28). This method allows frequent blood sampling in unanesthetized and undisturbed, freely moving rats.

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., England) as described earlier (2). The transmitter was attached to a Velcro strap which was secured around the chest. The transmitter was connected to transcutaneous electrodes made of standard paper clips. The transmitted signals were received on a commercial FM receiver, amplified with half-amplitude cutoff frequencies at 10 and 100 Hz (Grass Pr CR preamplifier) and stored on tape.

For analysis, the recorded ECG samples were processed through a cardiometer pulse generator which generated a square wave pulse at each R-wave. The time between the onset of two consecutive pulses, the interbeat interval (IBI), was measured by a personal computer (Olivetti M24). IBIs falling within the range of 100 to 220 ms have been selected for computing the mean IBI of each sample period. Bradycardia was considered to be an increase in mean IBI. Decrease in IBI indicated tachycardia. During both recording and analysis the quality of the ECG signal was continuously monitored on an oscilloscope.

Experimental Design and Procedures

The animals were at random divided into two groups, each consisting of 8 CEA-lesioned and 8 sham-lesioned animals. One group was provided with transcutaneous electrodes for ECG recording. In the same group, also, immobility behavior was measured. The other group was provided with the jugular vein catheters for blood sampling. Both groups of animals were housed and handled identically.

During the first two days, each rat was transported to the experimental room and placed in the shock box and stayed in it for 30 min to habituate to the environment and experimental procedure. In one group the strap holding the transmitter was fixed around the chest of the rat. The other group of animals was connected with a polyethylene blood sampling tube. On day 3 the rats were placed in the shock box for 1 hour to minimize reactions to transfer. To measure base-line heart rate, ECG samples were recorded for 1-min sampling periods at $t = -10$ and -1 min. Immediately afterwards an aversive stimulus of a scrambled footshock (0.6 mA, AC for 3 s) was given. Additional ECG samples were taken at $t = 1, 3, 5$ and 10 min following this footshock. During the same sampling periods, immobility behavior was scored by direct observation. Immobility was described as motionless alertness.

The other group was transferred to the experimental room and connected with the blood polyethylene sampling tube for 1 hour. In this group preshock blood samples of 0.45 ml were withdrawn at $t = -15$ and -1 min for determination of plasma catecholamines, corticosterone and prolactin. After each sample the same quantity of heparinized-donor blood was given to avoid diminution of the blood volume with related changes in hemodynamics. Donor blood was obtained from unstressed rats with permanent

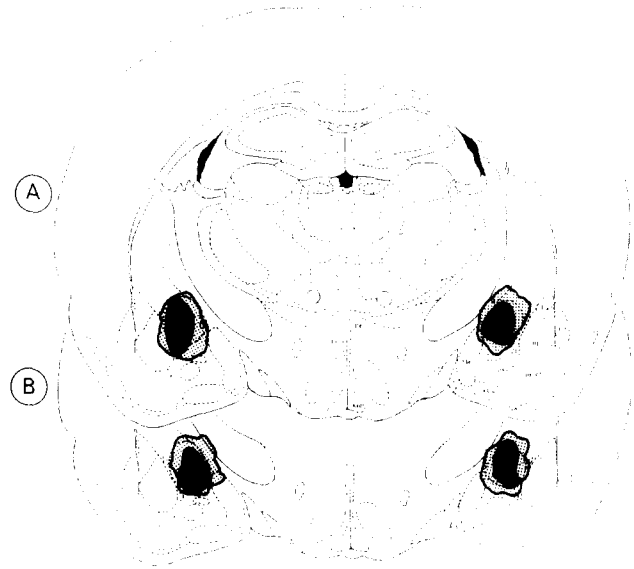


FIG. 1. Coronal section of the rat brain at 6.7 mm rostral to interaural showing a representative lesion (black) and the total area covered by the lesions (gray) in the cardiac (A: $n = 8$) and blood sampling (B: $n = 8$) animals.

heart catheters. Directly afterwards the rats were given the aversive stimulus of a footshock. Additional blood samples were taken at $t = 1, 3, 5$ and 20 min.

Chemical Determinations

Blood 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 150 μl of the supernatant was stored at -20°C for corticosterone and prolactin measurements and 100 μl at -80°C for the catecholamine measurement. Plasma corticosterone was measured by means of reversed-phase high performance liquid chromatography (HPLC) (6). Plasma concentrations of prolactin were determined by a double antibody radioimmunoassay for rat prolactin, using the method of Kwa et al. (15,16). NIDDK-rPRL-RP-2 was used as reference preparation and NIDDK-anti-rPRL-S9 as antiserum. Determination of plasma catecholamine concentrations was performed by HPLC in combination with electrochemical detection (ECD), with a minor modification of the method by Smedes et al. (27). The HPLC-ECD system included a LKB 2150 pump (LKB instruments, Bromma, Sweden), a Rheodyne injection valve with a 100- μl loop, a 25-cm analytical column (Nucleosil C18, Macherey-Nagel, Gimex Ned), held at 40°C by a column stove (LKV), a 5100-A electrochemical detector with a 5020 guard cell and a 5011 high sensitivity detector cell (ESA), and a BD 41 two-channel flat bed recorder (Kipp). The guard cell potential in front of the injection valve was $+450$ mV, the potentials of the working electrodes of the detector cell were -50 and $+350$ mV, respectively. The mobile phase contained 0.034 M citric acid, 0.043 M Na_2HPO_4 , 0.07% heptanesulfonic acid-sodium salt, 0.02% EDTA, and 3% methanol: 95% H_2O (pH 4.1). Absolute detection levels for epinephrine and norepinephrine in plasma were 0.010 and 0.005 ng/ml, respectively.

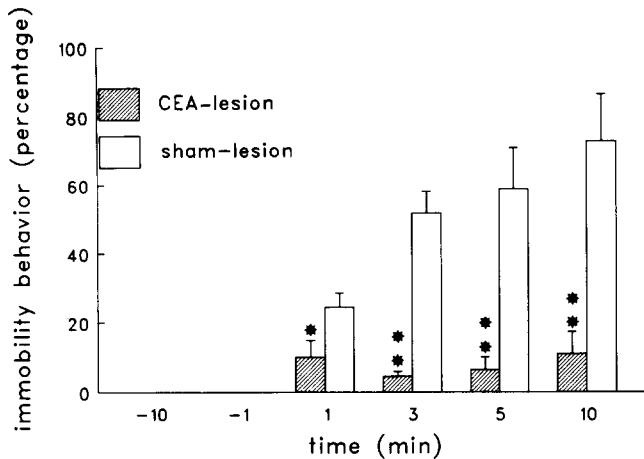


FIG. 2. The duration of immobility (\pm S.E.M.) during the 4 sampling periods following an aversive stimulus of a footshock in both CEA-lesioned ($n=8$) and sham-lesioned animals ($n=8$). * $p<0.05$; ** $p<0.01$.

Histology

At the completion of the experiments, the CEA-lesioned animals were deeply anesthetized with sodium pentobarbital (90 mg/kg IP) and perfused intracardially with saline followed by a 4% formaldehyde solution. The brain was removed from the skull and fixed in 4% formaldehyde for at least 24 h. Subsequently, frozen sections of 40 μ m were cut and the lesion place was examined on unstained sections. A minimum of 50% CEA damage as determined 6.7 mm rostral to interaural line was used as a selection criterion for correctly lesioned animals.

Statistics

Behavioral data were analyzed with the Mann-Whitney U-test (two-tailed, corrected for ties). Hormonal and cardiac data of the rats were evaluated for significance using analysis of variance with repeated measures (ANOVA). The repeated measures ANOVAs were followed by the Student's *t*-test. A probability level of $p<0.05$ was taken as statistical significance for all tests.

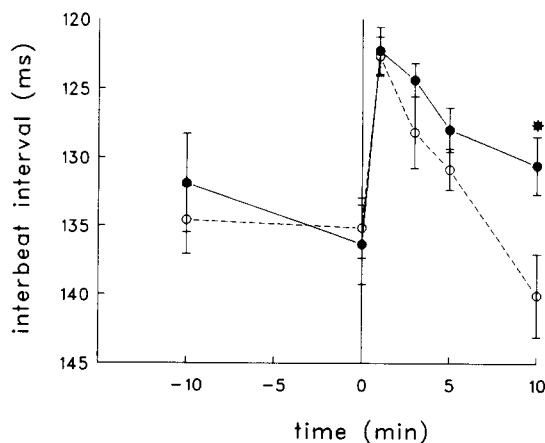


FIG. 3. Changes in interbeat interval (IBI) (\pm S.E.M.) as a consequence of an acute footshock in CEA-lesioned (\bullet ; $n=7$) and sham-lesioned animals (\circ ; $n=8$). * $p<0.05$.

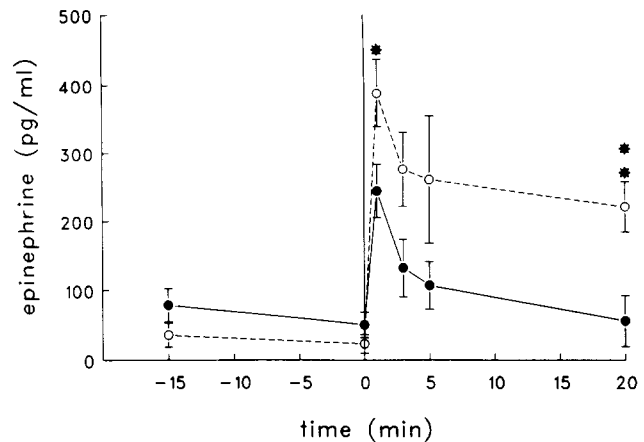


FIG. 4. Changes in plasma levels of epinephrine as a consequence of the aversive stimulus of an acute footshock in CEA-lesioned (\bullet ; $n=6$) and sham-lesioned animals (\circ ; $n=8$). * $p<0.05$; ** $p<0.01$.

RESULTS

Histological examination of the lesion sites in both experiments revealed that none of the rats had to be excluded from further analysis (Fig. 1).

Immobility Response

Before the shock, none of the animals showed much immobility. Most of them were sleeping or exploring the shock box (data not shown). In the initial phase after the inescapable footshock, all animals were actively trying to escape from this environment. However, in the later phase the sham-lesioned animals spent increasingly more time on immobility behavior. The CEA-lesioned animals persisted in exploratory and escape behavior. Already at $t=1$ min this difference is significant ($p<0.05$), and remains significant at all subsequent sampling periods $t=3$, 5 and 10 min ($p<0.01$) (Fig. 2).

Cardiac response

A significant lesioning \times time interaction was found in the heart rate response, $F(4,52)=3.46$, $p<0.05$. No differences in heart rate were seen during the prestress period. In the initial phase after the footshock, a similar tachycardiac response, i.e., decrease in IBI, was found in both groups. In the sham-lesioned animals this tachycardia successively diminished and even shifted to a bradycardia relative to prestress base-line. The CEA-lesioned animals, however, failed to show this bradycardia. A significant difference in IBI was found at $t=10$ min ($p<0.05$) (Fig. 3).

Catecholamine Responses

No significant differences in prestress plasma epinephrine and norepinephrine were found. Immediately after the footshock, both groups reacted with a rapid increase in catecholamines. However, in the sham-lesioned animals, the responses were significantly larger, both for epinephrine, $F(1,39)=4.84$, $p<0.05$ (Fig. 4), and norepinephrine, $F(1,42)=19.90$, $p<0.01$ (Fig. 5). Statistical analysis revealed significant differences for epinephrine at $t=1$ ($p<0.05$) and 20 min ($p<0.01$) and for norepinephrine at $t=1$ ($p<0.05$) and 3 and 5 min ($p<0.01$). At $t=20$ min, norepinephrine concentrations were returned to base-line levels in both the CEA-lesioned and sham-lesioned rats.

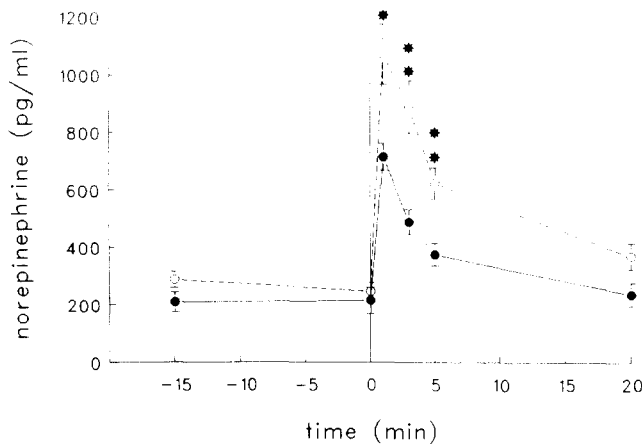


FIG. 5. Changes in plasma levels of norepinephrine following an acute footshock in CEA-lesioned (●: $n=8$) and sham-lesioned animals (○: $n=8$). * $p<0.05$; ** $p<0.01$.

Corticosterone Response

ANOVA revealed a significant lesioning \times time interaction, $F(3,42)=8.17$, $p<0.0005$. Before the delivery of the footshock, plasma corticosterone concentration was somewhat, but significantly higher in the CEA-lesioned rats at $t=-1$ min ($p<0.05$). After the footshock, the animals developed a slow, but long lasting increase in corticosterone. In the sham-lesioned animals, this response was larger compared to the rise in the CEA-lesioned animals. A significant difference in plasma corticosterone concentration was found at $t=20$ min ($p<0.05$) (Fig. 6).

Prolactin Response

No differences were found before the rats received the inescapable footshock. An increase in prolactin level was observed as a consequence of the shock in both groups. However, this increase was significantly larger and remained higher for a longer period of time in the sham-lesioned animals compared to the CEA-lesioned rats, $F(1,33)=8.64$, $p<0.05$. Significant differences in plasma prolactin level were reached at $t=1$ ($p<0.05$)

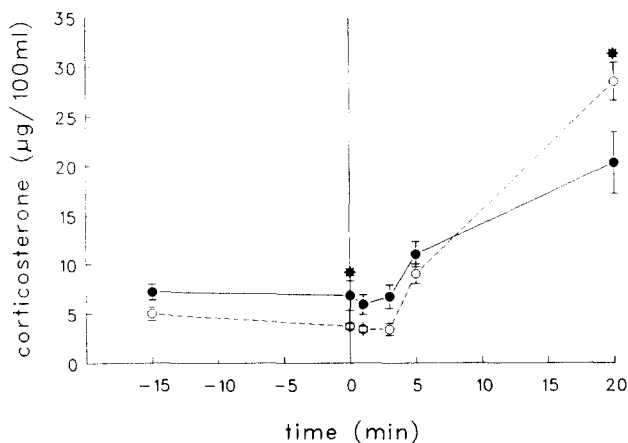


FIG. 6. Changes in plasma corticosterone levels as a consequence of an acute footshock in CEA-lesioned (●: $n=8$) and sham-lesioned animals (○: $n=8$). * $p<0.05$.

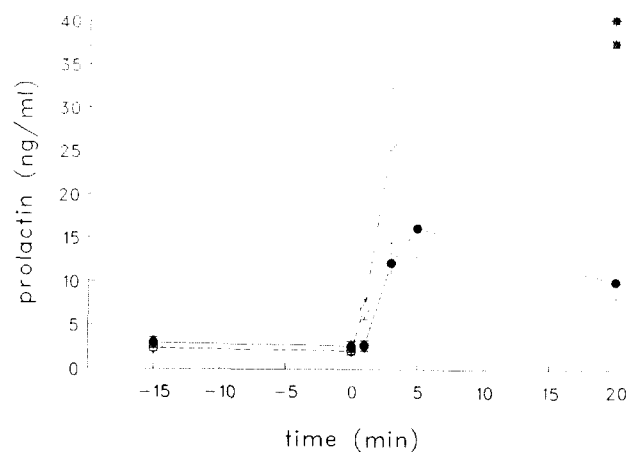


FIG. 7. Changes in plasma prolactin levels as a consequence of an acute footshock in CEA-lesioned (●: $n=8$) and sham-lesioned animals (○: $n=7$). * $p<0.05$; ** $p<0.01$.

and 20 min ($p<0.01$) (Fig. 7). At other intervals, a remarkably large S.E.M. was found in the sham animals.

DISCUSSION

The present study demonstrates for the first time that the CEA is not exclusively involved in conditioned responses to stressful stimuli. There seems to be, also, a rather general, non-selective amygdaloid modulatory influence on acute, unconditioned-stress responses. This suggests that the CEA has a complex and rather differentiated control on both unconditioned and conditioned responses. The behavioral, cardiovascular and neuroendocrine stress responses to an inescapable shock were one way or the other affected by CEA lesioning, while our previous studies demonstrated that complex stimuli that signal a previous aversive stimulus of inescapable footshock are selectively affected by the lesion: only the passive component of the behavioral and the accompanying parasympathetic response, i.e., immobility and bradycardia, were abolished (22).

The CEA receives polysensory information (conditioning stimulus) from the anterior basolateral amygdaloid complex (29). The CEA may add an emotional charge to this "pure" polysensory pattern, which can be seen as conditioning. CEA destruction may be responsible for an impairment or abolishment of this conditioning process, leaving the initial real shock responses intact, but showing an early return to preshock levels of all parameters, except for the passive behavioral and accompanying parasympathetic responses which were totally abolished.

These different functions of the CEA may have their morphological substrate in different subdivisions of the CEA. Several recent studies have recognized four major subdivisions arranged from medial to lateral, on basis of cell density, neuronal morphology, neurotransmitter properties, and afferent and efferent projection sites (4, 18, 30). Another possibility is that both the unconditioned and conditioned response may be generated in the same subdivision(s), but that different neuronal subpopulations, using different neurotransmitter systems, are involved.

Our findings are in agreement with electrical and chemical stimulation studies. Electrical stimulation of the CEA can produce a complex pattern of behavioral and autonomic changes that, taken together, constitute a state that highly resembles fear. For example, it produces a cessation of ongoing behavior (1), it can also produce decreases in heart rate (1, 7, 13, 19), and blood

pressure (7,11). In addition, observations with chemical stimulation with L-glutamate and thyrotropin-releasing hormone revealed similar changes in heart rate, blood pressure (7) and in plasma catecholamines (3).

Studies of the past several years have provided detailed descriptions of the efferent targets of the CEA neurons in a number of mammalian species. A part of the CEA output pathways are likely to be involved in a monosynaptic, peptidergic innervation of the dorsomedial medulla, particularly to the nucleus of the solitary tract, dorsal motor nucleus of the vagus and the ambiguous nucleus (5, 10, 14, 21, 25, 26). This projection may be responsible for the control of parasympathetic output. In addition, recent evidence suggests that the CEA innervation of the periaqueductal gray is involved in the passive behavioral component (immobility) (17). Moreover, the innervation of the lateral parabrachial nucleus in the midbrain, and the lateral hypothalamic nucleus may be involved in the sympathetic responses (17). Finally, a new monosynaptic innervation of the

paraventricular nucleus of the hypothalamus has recently been described, which may be involved in the influence of the CEA in the pituitary-adreno-cortical system (8). The chemical nature of the latter connection is largely unknown.

The exact identification of input and output systems of the CEA conveying behavioral, physiological and neuroendocrine stress responses remains an inviting prospect.

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REFERENCES

- Applegate, C. D.; Kapp, B. S.; Underwood, M. D.; McNall, C. L. Autonomic and somatomotor effects of amygdala central N. stimulation in awake rabbits. *Physiol. Behav.* 31:353-360; 1983.
- Bohus, B. Telemetered heart rate responses of the rat during free and learned behavior. *Biotelemetry* 1:193-201; 1974.
- Brown, M. R.; Gray, T. S. Peptide injections into the amygdala of conscious rats: Effects on blood pressure, heart rate and plasma catecholamines. *Regul. Pept.* 21:95-106; 1988.
- Cassell, M. D.; Gray, T. S.; Kiss, J. Z. Neuronal architecture in the rat central nucleus of the amygdala: A cytological, hodological and immunocytochemical study. *J. Comp. Neurol.* 246:478-499; 1986.
- Danielsen, E. H.; Magnuson, D. J.; Gray, T. S. The central amygdaloid nucleus innervation of the dorsal vagal complex in rat: A phaseolus vulgaris leucoagglutinin lectin anterograde tracing study. *Brain Res. Bull.* 22:705-715; 1989.
- Dawson, R., Jr.; Kontur, P.; Monjan, A. High-performance liquid chromatographic (HPLC) separation and quantitation of endogenous glucocorticoids after solid phase extraction from plasma. *Horm. Res.* 20:89-94; 1984.
- Gelsema, A. J.; McKittrick, D. J.; Calaresu, F. R. Cardiovascular responses to chemical and electrical stimulation of amygdala in rats. *Am. J. Physiol.* 253:R712-R718; 1987.
- Gray, T. S.; Carney, M. E.; Magnuson, D. J. Direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular nucleus: Possible role in stress-induced adrenocorticotropin release. *Neuroendocrinology* 50:433-446; 1989.
- Henke, P. G. Facilitation and inhibition of gastric pathology after lesions in the amygdala of rats. *Physiol. Behav.* 25:575-579; 1980.
- Hopkins, D. A.; Holstege, G. Amygdaloid projections to the mesencephalon, pons and medulla oblongata in the cat. *Exp. Brain Res.* 32:529-547; 1978.
- Iwata, J.; Chida, K.; LeDoux, J. E. Cardiovascular responses elicited by stimulation of neurons in the central amygdaloid nucleus in awake but not anesthetized rats resemble conditioned emotional responses. *Brain Res.* 418:183-187; 1987.
- Kapp, B. S.; Frysinger, R. C.; Gallagher, M.; Haselton, J. R. Amygdala central nucleus lesions: Effect on heart rate conditioning in the rabbit. *Physiol. Behav.* 23:1109-1117; 1979.
- Kapp, B. S.; Gallagher, M.; Underwood, M. D.; McNall, C. L.; Whitehorn, D. Cardiovascular responses elicited by electrical stimulation of the amygdala central nucleus in the rabbit. *Brain Res.* 234:251-262; 1982.
- Krettek, J. E.; Price, J. L. Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. *J. Comp. Neurol.* 178:225-254; 1978.
- Kwa, H. G.; van der Gugten, A. A.; Verhofstad, F. Radioimmunoassay of rat prolactin: comparison of rat prolactin preparations isolated from (cranular fraction of) pituitary tumor transplants and from normal pituitary plates. *Eur. J. Cancer* 5:559-569; 1969.
- Kwa, H. G.; van der Gugten, A. A.; Sala, M.; Verhofstad, F. Effect of pituitary tumors and grafts on plasma prolactin levels. *Eur. J. Cancer* 8:39-54; 1972.
- LeDoux, J. E.; Iwata, J.; Cicchetti, P.; Reis, D. J. Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *J. Neurosci.* 8:2517-2529; 1988.
- McDonald, A. J. Cytoarchitecture of the central amygdaloid nucleus of the rat. *J. Comp. Neurol.* 208:401-418; 1982.
- Mogenson, G. J.; Calaresu, F. R. Cardiovascular responses to electrical stimulation of the amygdala in the rat. *Exp. Neurol.* 39:166-180; 1973.
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.
- Rogers, R. C.; Fryman, D. L. Direct connections between the central nucleus of the amygdala and the nucleus of the solitary tract: An electrophysiological study in the rat. *J. Auton. Nerv. System* 22:83-87; 1988.
- Roozendaal, B.; Koolhaas, J. M.; Bohus, B. Differential effect of lesioning of the central amygdala on the bradycardiac and behavioral response of the rat in relation to conditioned social and solitary stress. *Behav. Brain Res.* 41:39-48; 1990.
- Roozendaal, B.; Koolhaas, J. M.; Bohus, B. Central amygdala lesions affect behavioral and autonomic balance during stress in rats. *Physiol. Behav.* 50:777-781; 1991.
- Roozendaal, B.; Oldenburger, W. P.; Strubbe, J. H.; Koolhaas, J. M.; Bohus, B. The central amygdala is involved in the conditioned but not in the meal-induced cephalic insulin response in the rat. *Neurosci. Lett.* 116:210-215; 1990.
- Saper, C. B. Convergence of autonomic and limbic connections in the insular cortex of the rat. *J. Comp. Neurol.* 210:163-173; 1982.
- Schwaber, J. S.; Kapp, B. S.; Higgins, G. A. The origin and extent of direct amygdala projections to the region of the dorsal motor nucleus of the vagus and the nucleus of the solitary tract. *Neurosci. Lett.* 20:15-20; 1980.
- 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. Chromatogr.* 231:25-39; 1982.
- 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.
- Turner, B. H. The cortical sequence and terminal distribution of sensory related afferents to the amygdaloid complex of the rat and monkey. In: Ben Ari, Y., ed. *The amygdaloid complex*. Amsterdam: Elsevier; 1981:51-62.
- Turner, B. H.; Zimmer, J. The architecture and some of the interconnections of the rat's amygdala and lateral periallocortex. *J. Comp. Neurol.* 227:540-557; 1984.