Major Problems in Neuroendocrinology

An International Symposium

Edited by

E. BAJUSZ and G. JASMIN, Montreal

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Major Problems in Neuroendocrinology

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Editorial Foreword

Neuroendocrinology, the science or experimental analysis of neuroendocrine relationships, is primarily concerned with the physiology and pathophysiology of the endocrine glands and of the central nervous system as related to each other and to the function of the organism as a whole. In its broadest aspect, neuroendocrinology is a truly interdisciplinary science, that is, it embraces many different scientific disciplines from clinical medicine and biological studies on the one hand, to electrophysiology and protein chemistry on the other. Thus, neuroendocrinology is not a newly created entity or a separate, well defined chapter of modern biology and medicine, but rather an invasive trend of research, a correlative way of thinking. It spontaneously emerged from a number of scattered clinical and experimental observations indicating that the central nervous system exerts a regulatory function on the pituitary and thereby and/or directly also on other endocrine glands. Further developments have made it increasingly clear that the nervous system itself is, in a sense, a complex endocrine system producing humoral substances for the control of endocrine functions and, most probably also, a number of other bodily activities.

Although, it is commonly still customary to limit the field of neuroendocrinology to the description and analysis af some main pathways through which, it is thought, the central nervous system regulates the activities of endocrine end-organs, it should be recognized that this is an artificial way of handling neuroendocrine relationships. In reality, not only does the nervous system play an important role in governing the secretory activities of endocrine glands, but the reverse can also be true: the hormonal substances of endocrine organs may basically alter the function of purely neural mechanisms. Moreover, neither neuroendocrine adaptive reactions nor higher nervous activities (such as, for example, the complex behavioral processes) can appropriately be studied and understood solely on the basis of their relationship to neural structures; the hormonal influences responsible for the tonization of the underlying nervous mechanisms must always be taken into consideration, also. The fact that the various pituitary, thyroid, adrenocortical and gonadal hormones exert a definite influence upon the activities of the central nervous system is well documented; yet it is often neglected in the evaluation and planning of neuroendocrine studies. Nevertheless, investigations of neuroendocrine relationships have already given a number of new general concepts in the light of which many of our earlier beliefs on the mechanism of physiological and pathological processes have now to be re-examined and reformulated. What appears to emerge is that there is an intimate interplay of psychic and somatic factors; and that systemic insults as well as neuro- and psychogenic stimulations arising with ever-increasing frequency under the pressure and artificial environmental conditions of this modern world may all invoke patterns of adaptation but may also terminate in maladaptive consequences.

The past decade has witnessed the beginning of a series of breakthroughs in several areas of neuroendocrinology as significant as those currently taking place in the physical sciences. Progress is being made despite the intrinsic difficulties of working in this area where so much must be learned by inference about systems which are not directly observable in the functioning organism. In addition to this major handicap to rapid advances, there are limitations inherent in the circumstances of the particular laboratories concerned with regard to means available, methods, and species of subjects. Thus, one laboratory studies rats exclusively, another dogs, the third monkeys or even man. One laboratory relies on blood plasma evaluations primarily, a second uses histological and histochemical techniques, a third clinical evaluation of subjective states, or investigative approaches of endocrinology, cardiology, electrophysiology, neurology or psychiatry. One laboratory straps its subjects into special 'conditioning chambers', a second flies its investigators to field locations, a third uses naturally diseased patients, artificially elicits 'disease models', or studies in vitro preparation, and so on. Generalizations from each of these situations must be carefully checked against repeated comparable studies elsewhere, contradictory findings evaluated as to the species used, method of treatment and other experimental interventions, technique as regards analysis of effects, etc. This wide range of differences in methodologies and of opinions concerning the significance of findings need not be discouraging but, rather, should stimulate frequent cross-checking, reviews, comprehensive evaluations, and regular unrestricted exchange of information, only through which can clarification possibly be achieved.

The organization of the present international written symposium was motivated not only by a realization of the primary importance of neuroendocrine studies, but also by the traditions of our University where the widely known 'Revue Canadienne de Biologie' symposium series originated. Nevertheless, the extent of the present symposium necessitated its publication in the form of a book and not—as has been customary—as a regular number of our scientific periodical.

Mere perusal of the Index reveals that this symposium volume deals with various aspects of basic neuroendocrine studies, presents timely information on a number of highly controversial subjects of neuroendocrine correlations, and shows some major lines along which investigations are at the moment being conducted. Since neuroendocrinology has already acquired such a broad significance as to encompass almost the entire scope of biology and medicine as well, it was obviously not possible to cover the subject in every detail in one publication. Instead, the original and critical review articles of a carefully selected group of 49 eminent investigators from 13 countries have been included here in an effort to correlate the work done in any one field with that done in others. It is especially noteworthy that among our contributors are biochemists, theoretical and clinical endocrinologists, neurologists, physiologists, histologists and pathologists, in other words, representatives of a number of different disciplines but all engaged in neuroendocrine studies. We sincerely believe that a volume arising from such a mutual effort, to which each contributor adds something that is uniquely his own, is extremely valuable for the successful development of neuroendocrinology. It should serve also to stress that science has no limiting boundaries, neither with regard to disciplines nor to countries.

We would like to sincerely thank the Contributors, not only for their valuable co-operation, but also for their forbearance of some, albeit minor, editorial alterations that were necessary to ensure uniformity and brevity. It is a great pleasure, furthermore, to express our gratitude to the S. Karger AG, our publishers, whose understanding and co-operation enabled us to publish this volume speedily because of the rapidly changing character of the field. But even so, we felt it to be our editorial duty to bring the publication as up-to-date as possible; with this purpose in mind one of us (E.B.) has under-taken the preparation of a 'concluding article' by incorporating a review of relevant papers that appeared during the preparation of the present symposium volume and also by giving references to such topics of neuroendocrinology that were not covered by other contributors.

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Neural, Neuroendocrine and Hormonal Interactions

Major Problems in Neuroendocrinology, ed. by E. BAJUSZ and G. JASMIN, pp. 1–16
(S. Karger, Basel/New York 1964).

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Neuroendocrine Interrelationships and Behavioural Processes

K. LISSÁK and E. ENDRÖCZI

The adaptative changes elicited by environmental stimuli may be characterized by the alterations occurring in the vegetative or metabolic processes as well as in the behavioural reactions of higher vertebrates. The organization of motivation, approaching and aversive behaviour, constitutes an inseparable unit of neural and humoral integration which includes inductive, facilitatory and inhibitory interactions; the whole complex has simply been termed adaptation.

Neurophysiological and neuroendocrinological investigations have provided abundant evidence to demonstrate the role of neuroendocrine interrelationships in the elaboration of complex behavioural reactions. The humoral factors play an important role in the induction, facilitation or inhibition of approaching or aversive motivation. However, they produce their effects permissively. They can change the biochemical gradients of the neural net and thereby influence the neural patterns.

The present paper gives a short account of investigations performed in our laboratories in Pécs over the past years and deals with the role of endocrine factors in the development of complex learned behavioural reactions.

Some Recent Neuroanatomical Considerations Concerning Pituitary-Adrenocortical Function

The methods of modern neurophysiology enable us to study the intrinsic mechanisms of regulatory processes in waking animals. Thus stimulation experiments with chronically implanted electrodes have shown that the midbrain and forebrain structures can exert a facilitatory as well as an inhibitory influence on pituitary-adrenocortical function. Relatively short stimulation of these structures can induce rapid ACTH release while stimulation of other structures elicits a long inhibitory period in pituitary-adrenocortical function. Fig. 1 summarizes our observations and shows the neuroanatomical distribution of facilitatory and inhibitory loci involved in pituitary-adrenocortical regulation.

All of the experiments mentioned above were carried out on cats bearing chronically implanted electrodes. The details of the method

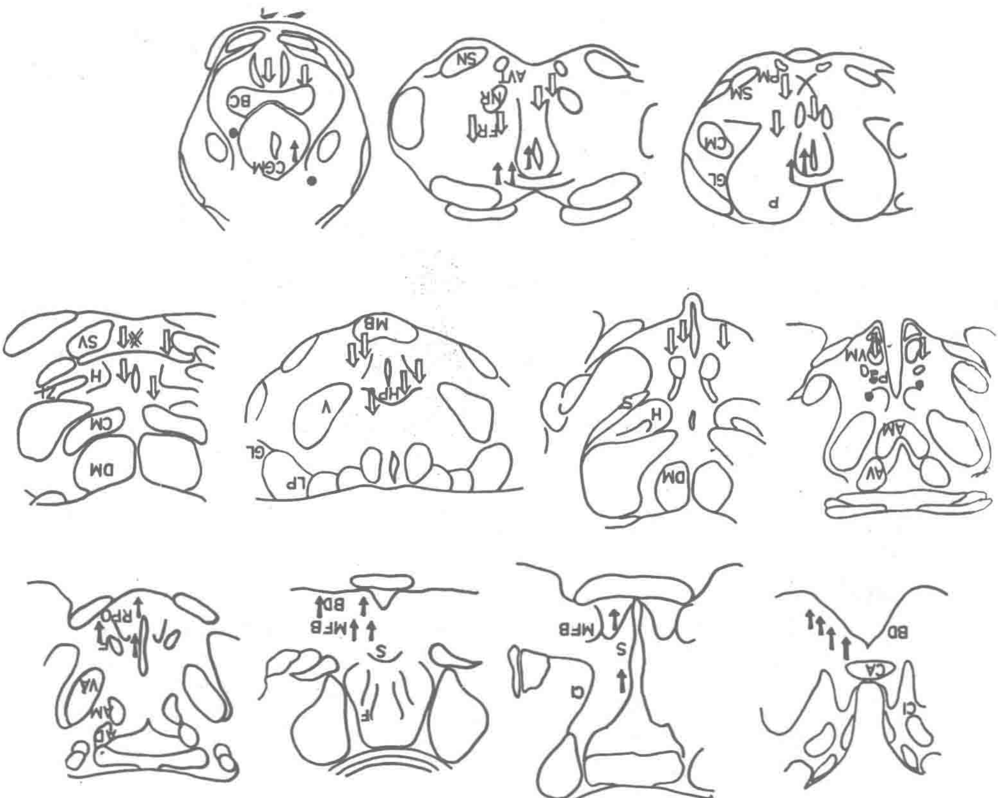

Fig. 1 The effects of electrical stimulation of the diencephalic and mesencephalic structures on the pituitary-adrenocortical function in cats. The black arrows show the anatomical locations where the stimulation induced a decrease of the corticosteroid output. The white arrows correspond to the activating points. At the stimulation of black points the pituitary-adrenocortical activity failed to change significantly.

have been described elsewhere (ENDRÖCZI AND LISSÁK; LISSÁK AND ENDRÖCZI, 1959, 1960; ENDRÖCZI AND LISSÁK, 1962). Stimulation was done with a rectangular impulse generator and lasted 15 minutes (0.5–1.5 volts, 0.5 m/sec and 30 c/sec). 45 minutes after the cessation of stimulation the animal was anaesthetized with Nembutal intraperitoneally, and the adrenal venous blood collected through a polyethylene tube inserted into the lumboadrenal vein. Estimation and paper chromatographic separation of the corticosteroids have been described in our earlier papers (ENDRÖCZI AND LISSÁK, 1959; ENDRÖCZI AND YANG, 1960). A marked ACTH release was observed after stimulation of the caudal and posterior hypothalamic nuclei, the ventral tegmentum and the mesencephalic reticular formation. Similarly ACTH secretion increased significantly after stimulation of the central and basomedial amygdaloid nuclei and one of the subcallosal descending fornices. In contrast to these findings a marked drop in ACTH release was observed due to stimulation of the following structures: basal septal area, antero-lateral hypothalamus including the preoptica region, the medial forebrain bundle and the dorsal hippocampus. It is worth mentioning that hippocampal stimulation showed a frequency reversal characteristic in its action; higher stimulatory frequencies activated pituitary-adrenocortical function (LISSÁK AND ENDRÖCZI, 1961, 1962). An inhibitory zone in the dorsal tegmental area was also detected in these investigations, however, it partly overlapped the facilitatory action of the reticular formation. Analysis of the inhibitory form of the regulation showed that hippocampal stimulation not only decreased ACTH secretion but also blocked the activating effects of neural and humoral stressors (ENDRÖCZI, LISSÁK, KOVÁCS AND BOHUS, 1959; LISSÁK AND ENDRÖCZI, 1960, 1961; ENDRÖCZI AND LISSÁK, 1962).

Threshold stimulation of various brain structures resulted in well-defined behavioural reactions and characteristic emotional manifestations (fear, rage, vegetative signs). There was no close parallelism between the emotional reactivity and the direction of the endocrine responses elicited by stimulation. Stimulation of the anterolateral hypothalamic region in the presence of inhibition of pituitary ACTH function induced aggressivity, however, the rage reaction elicited by stimulation of the medial amygdaloid complex of nuclei was accompanied by a marked increase in ACTH secretion. A similar inverse correlation between the behavioural and endocrine responses has been found on stimulation of other parts of the hypothalamic and midbrain structures.

These findings suggested a more discrete organization of the neuroendocrine processes in the brain than had been previously proposed and argued against a 'centre-like' localization of endocrine regulation at the level of subcortical integration.

Approximately the same neuroanatomical relationships of pituitary-adrenocortical regulation were found in the experiments with chemical stimulation where cholinergic and adrenergic substances were injected through a microcannula into the various subcortical areas. In a total volume of 0.01 ml, 5 μg of carbamylcholine or acetylcholine and 10 μg of eserine sulphate were injected into the antero-lateral hypothalamus, the preoptic region or the basal septum. This resulted in a marked decrease in ACTH secretion but failed to exert a significant effect in the area of the supraoptic or paraventricular nuclei. The injection of cholinergic drugs resulted in a moderate increase, but that of 2 μg of adrenalin or noradrenalin produced a high ACTH release at the level of the rostral reticular formation and the posterior hypothalamus. The injection of ephedrine under the same conditions led to similar results. At the subcollicular level or caudalward from it the lower parts of the brainstem proved to be insensitive to adrenergic drugs in so far as no pituitary activation followed the injection of adrenalin or noradrenalin. These findings confirm the former suggestions, i.e. that at the level of the posterior hypothalamus and the rostral mesencephalic reticular formation the midbrain only has a relatively small area which is sensitive to adrenergic drugs. The injection of adrenergic substances did not induce visible behavioural reactions, except that the administration of noradrenalin into the posterior hypothalamus elicited aggressive reactions (attacks) lasting one hour after the injection. These experiments with chemical stimulation convinced us that the regulative patterns of endocrine and behavioural integrative processes might easily be separated on the basis of the chemosensitivity of neural structures to the chemical mediators, and they also revealed the discrete intrinsic organization of these mechanisms.

The facilitatory and inhibitory influence of forebrain structures on pituitary ACTH secretion has also been observed by other authors in recent years, and their findings fit well into our observations relating to the neuroanatomical basis of these processes. Thus MASON *et al.* (1958), SUZUKI, ROMANOFF, KOELLA AND LEVY (1960) and SLUSHER AND HYDE (1961) reported the inhibitory effect of anterior hypothalamus stimulation on the adrenocortical steroid output in

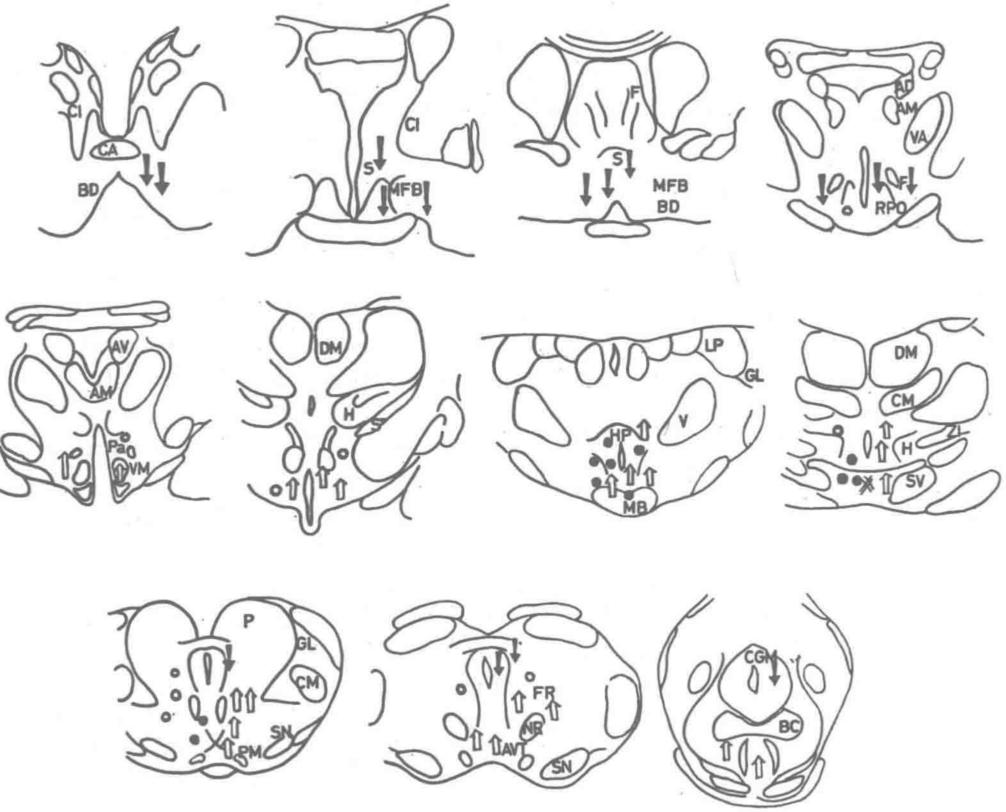

Fig. 2 The figure shows the effects of cholinergic and adrenergic chemical stimulation and the anatomical locations of cannulas in the diencephalon and mesencephalon of cats. Black arrows: the depressing action of cholinergic stimulation. White arrows: the activating action of cholinergic stimulation. Black points: the activating influence of adrenergic stimulation. Circles: O no significant change following adrenergic stimulation.

cats, dogs and monkeys. The facilitation of caudal hypothalamic and midbrain stimulation was also observed in different species by several authors (Colfer, Degroot and Harris, 1950; Harris, 1955; Lissák and Endröczi, 1960).

The Functional Interrelationship between Behavioural Reactions and Pituitary-Adrenocortical Activity

The introduction of ACTH and corticosteroid therapy into medical practice has provided a large number of observations concerning psychological and neuropathological disturbances during treatment (Cleghorn and Graham, 1949; Cleghorn, 1953). Similar psychotic alterations and EEG abnormalities were detected in patients suffering from Addison's disease or hyperadrenocorticism (Engel and Margolin, 1941; Hoefer and Glaser, 1949; Wayne, 1954).

Woodbury *et al.* (1952, 1954) demonstrated in rats that the corticosteroids can exert both facilitatory and inhibitory influences on the convulsive threshold of the central nervous system. Corticosterone itself, a constant component in the adrenal venous blood of different species, has no action upon the convulsive threshold; however, it counteracts the decreasing effect of hydrocortisone and cortisone. In this respect it is an important fact that the ratio of corticosterone to hydrocortisone varies widely with each species, and, within certain limits, even for the same species (Lissák and Endröczi, 1960; Endröczi, Medgyesi and Lissák, 1958). As has recently been reported by Heuser and Eidelberg (1961) behavioural and electric seizures can be elicited by the administration of a single dose of 17-hydroxy-11-desoxycorticosterone succinate (Reichstein's substance) in cats and monkeys, but the injection of hydrocortisone or cortisone fails to exert such an effect under acute experimental conditions. However, the facilitatory influence of cortisone on the electric seizure activity of forebrain structures induced by dorsal hippocampal stimulation has been demonstrated by Endroczi and Lissák (1961, 1962) in waking cats bearing chronically implanted electrodes.

Wide individual variations in the psychotic changes during ACTH and corticosteroid therapy can be observed in the severity as well as in the quality of the symptoms. According to Cleghorn and Pattee (1954), therapeutic doses of cortisone induced a marked depression in 5% of patients and a euphoric state was observed in more than $^2/_3$ of the vases during prolonged administration. Opposite effects of ACTH administration on mood have also been reported by Wacholder (1957).

The multidirectional actions of pituitary-adrenocortical activity on the central nervous system may be explained partly by the different effects of the corticosteroids and partly by their points of attack being situated at different levels in the brain mechanism. The first assumption is supported by a great number of observations, e.g. the administration of hydrocortisone or cortisone can induce epileptic seizure, but 11-desoxycorticosterone prevents idiopathic epileptic convulsions (Glaser, 1947; Cleghorn, 1954).

LIDDELL *et al.* (1935) were the first to demonstrate the effect of an adrenocortical extract on higher nervous activity in conditioned reflex experiments. In the past decades many investigators have pointed out the role played by corticosteroids in different forms of learned and motivated behaviour, however, our knowledge of the mechanisms implicated in these processes is rather restricted (ANDERSON AND PARMENTER, 1941; MIRSKY, MILLER AND STEIN, 1953; ENDRÖCZI, LISSÁK AND MEDGYESI, 1958; LISSÁK AND ENDRÖCZI, 1960). The present paper deals with our latest observations in this field and shows the intimate connections between motivated behaviour and pituitary-adrenocortical function.

Our earlier experiments showed that the administration of ACTH and corticosteroids increased the internal inhibitory processes of alimentation and avoided conditioned reflex activity (LISSÁK AND ENDRÖCZI, 1960), which was indicated by faster extinction in the non-reinforced stereotype during treatment in dogs and cats. Similar findings were reported by MIRSKY, MILLER AND STEIN (1953) in monkeys, however, their interpretation of the mechanisms involved in these events differs from ours. It is an interesting fact that corticosterone has no influence upon internal inhibitory processes, which may explain the ineffectiveness of ACTH in experiments on rats, in which this steroid is the main component in the adrenal venous blood. This kind of interpretation directs attention to the role that qualitative

Table I

The Proportion of Hydrocortisone to Corticosterone

	Cpd F/B
Man	2.3 – 10:1
Monkey	20 :1
Dog	2 – 5:1
Cat	1 – 2:1
Rabbit	0.05:1
Rat	only corticosterone
Mouse	only corticosterone
	+ LISSÁK, K. and ENDRÖCZI, E. (1960)

differences play in adrenocortical secretion and to its effect on higher nervous activity. Tab. 1 lists the ratio of hydrocortisone and corticosterone in various higher vertebrates.

The action of pituitary-adrenocortical function on internal inhibitory processes, e.g. extinction, may not be regarded as being dependent on the situation. Such action of ACTH could be observed in approaching as well as in aversive situations and led to the conclusion that the corticosteroids exerted their influence on the neuroanatomical connections which were involved in the development of internal inhibitory processes (Lissák and Endröczi, 1960).

In alimentary conditioned reflex experiments the cats were trained to jump up on to a bench, placed at 1½ feet from the floor, to reach the food. As a conditioned signal the light of a 15 watt bulb in the top of the feeding device was used. There similar feeding devices were arranged side by side at intervals of 50 cm for studying the spatial discrimination of the animals. The ground surface of the experimental chamber was about three and half square metres.

It is common knowledge that in the course of locomotor conditioned reflex training an animal will show intersignal motor activity which is more intensive in the early phase and decreases with advancing stabilization of the conditioned reflex response. This intersignal motor activity, or as it is called in our recent papers 'the spontaneous goal-directed motor activity' (Endröczi and Lissák, 1962; Lissák and Endröczi, 1962), shows wide individual variations. Taking into account the Pavlovian explanation of this motor activity, the decrease of intersignal activity in the course of conditioned reflex training should be regarded as the consequence of discriminative inhibitory processes. On the other hand; it is known that if one puts the animal in a so-called 'free-operant' situation, as Skinner or Miller does, the spontaneous goal-directed motor activity will become an essential component of learned behaviour. There is no doubt that intersignal locomotor activity reflects the intensity of irradiation and the concentration of facilitatory and inhibitory processes, stated in the classic Pavlovian terminology. However, analysis of such motor behaviour reveals some new aspects of learned and motivated behaviour and of conditioned reflex behaviour. Spontaneous goal-directed motor activity may be regarded as a driving force in the elaboration of conditioned reflex activity of the motor component of the motivation accompanying the development of specific temporary connections. It constitutes a tendency with an affective