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# HIPPOCAMPAL GLUCOCORTICOID RECEPTOR AND BEHAVIOR: A CORRELATIVE STUDY IN RATS AND MICE

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**Summary**—A correlation has been demonstrated between binding capacity for [$^3$H]corticosterone in the hippocampus and the performance in passive and active avoidance in the rat, and impaired behavior in adrenalectomized rats by exogenous corticosterone is restored. On this basis we have studied the possible correlation between strain-dependent behavioral differences and the glucocorticoid binding capacity in the hippocampus in mice and rats. In Naples high- (NHE) and low-excitability (NLE) rat strains, genetically selected on the basis of divergent locomotor activity upon forced exposure to a spatial novelty situation, no differences were found in glucocorticoid maximal binding capacity while both strains had a lower capacity than Naples random-breed (NRB) control rats. However, the intra-strain correlative analysis of hippocampal glucocorticoid receptors number and behavioral scores demonstrated that motor and emotional indexes of arousal to novelty were positively correlated in NLE-and negatively in NHE-, while no correlation was present in NRB rats. Using two inbred strains of mice, C57BL/6 and Balb/c, extensively investigated for learning abilities, the lower active avoidance score of C57BL/6 was associated with a lower binding capacity for [$^3$H]corticosterone in the hippocampus. Altogether the above results support the involvement of the hippocampal glucocorticoid receptor in the modulation of some adaptive behavioral responses, while do not prove that genetic differences in behavior rest on parallel differences in binding capacity for glucocorticoid hormone.

The role of the limbic system and especially of the hippocampus in the expression of emotions and adaptive behaviors has been studied for nearly 40 yr, and a wealth of neurobehavioral data is now available.

Research on the influence of endocrine secretions on brain functions has demonstrated the activational property of steroid hormones in certain kinds of behavior, and established the part played by the hypothalamo–pituitary–adrenal axis in the adaptive reactions of the organism to the environment [1, 2].

The role of the hippocampus in the regulation of adaptive behavior and in the modulation of the hypothalamo–pituitary–adrenal axis involves the function of the neuronal receptor system for corticosterone, and *in vitro* biochemical knowledge of these receptors has been amply gained (reviewed in Ref. [3]). On the other hand, the knowledge of the receptor–hormone or receptors–hormone interaction at the *in vivo* level has not been yet fully attained.

In this paper the binding capacity of the glucocorticoid receptor system will be given as [$^3$H]corticosterone specifically bound in the cytosol, namely as the whole receptor population able to bind corticosterone. Therefore, no distinction will be made between the Type I, corticosterone-preferring and Type II, glucocorticoid-preferring [4]. In our opinion this approach is fully valid, considering that separate physiological functions of the two receptors have not yet been clearly defined.

The studies we present today, began almost 10 yr ago as a result of the following unusual observation: binding capacity of the glucocorticoid receptor system in the hippocampus, unlike most of the biological parameters in the rat, has an exceptionally wide range of distribution in a homogeneous population of rats [5].

As shown in Fig. 1, in between 150 and 500 fmol, more than 3 times the variation occurs. Due to the fact that these values were

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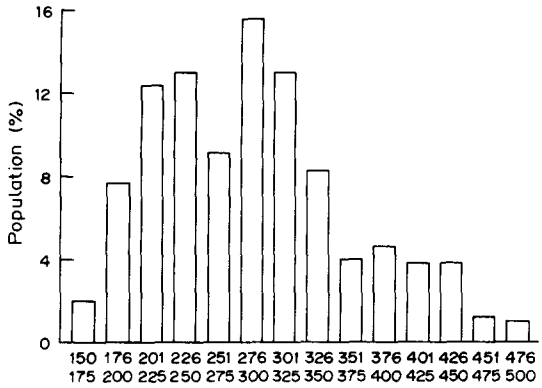


Fig. 1. Distribution of the glucocorticoid receptor binding capacity (fmol/mg protein), measured as [$^3$H]corticosterone (40 nM) maximally bound in the cytosol of a population of 120 male Wistar rats, 24 h after adrenalectomy.

obtained from a highly inbred strain of the Wistar rat, one could not consider this variation as determined by genetic factors, whereas environmental factors appeared a more plausible determinant.

We attempted to elucidate the meaning of this phenomenon investigating the basis of the physiological role of the glucocorticoid receptor in the hippocampus [6].

As shown in Fig. 2, in an homogeneous population of Wistar rats, two sub-groups could be selected according to the level of performance in a active avoidance task: good, meaning 15 or more avoidances out of 30 consecutive trials (given by only 25% of the population) or poor, meaning less than 10 avoidances (given by 50% of the population). Within this population, the distinction between "poor" and "good" learners was closely paralleled by a clear cut distinction in binding capacity. One hippocampus of each rat was used in determining individual maximal binding capacity and the other in studying binding characteristics by Scatchard analysis of the saturation curve.

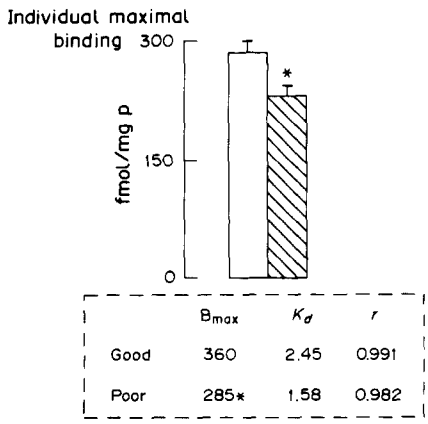


Fig. 2. [$^3$H]Corticosterone (40 nM) maximally bound in hippocampal cytosol (means ± SE) and Scatchard analysis (0.6–40 nM) of binding parameters ($B_{max}$, as fmol/mg p and $K_d$, as nM) in male rats, 15 days after learning conditioned active avoidance. Open columns: good > 15 out of 30 trials; and solid columns: < 10 avoidances out of 30 trials. *$P < 0.05$, Student's $t$-test.

As shown in the figure, the binding difference between good and poor learners is due to a difference in the number of binding sites, with no change in the affinity.

So, there was a reason to believe that in the individual adult rat the level of the binding capacity for corticosterone in the hippocampus and the correlated regulation of attentional processes, might be the resultant of postnatal experiential events, or of a peculiar condition of the mother–offspring pituitary–adrenocortical interrelationship, affecting perinatal development and maturation of the hippocampus. It is known, indeed, that improper contacts of hormones with organisms in their early life may affect the development and the expression of body activities in adulthood.

Such consequences can be of great importance in the case of adrenocortical hormones, with regard to both behavior and activity of the hypothalamo–pituitary–adrenocortical axis in adulthood.

On these grounds, it was deemed important to ascertain whether improper contact with exogenous corticosterone in early life could affect the glucocorticoid receptor system in the hippocampus and, concomitantly, pituitary–adrenal activity and behavior in the adult rat [7, 8]. Because in most of the studies concerning this aspect pharmacological doses of glucocorticoids have been used, making doubtful their physiological relevance, we used an approach of physiological relevance: infant rat exposure to glucocorticoid was realized by administering 200 $\mu$g/ml of corticosterone, starting from day 2 post-partum, in the drinking water of the lactating mother, resulting in a daily intake of about 25 mg/kg body wt. The hormone passes into the mother's milk, in equilibrium with the plasma level, and from the milk into the pup [7–9].

As shown in Fig. 3, the enhancement of the plasma level of the hormone in the mother as well as in the suckling is in within the physiological range. This approach avoids any physical contact of the experimenter (handling, injections, etc.) with the pups, which has been proven to produce modification in neurochemical or behavioral parameters [10].

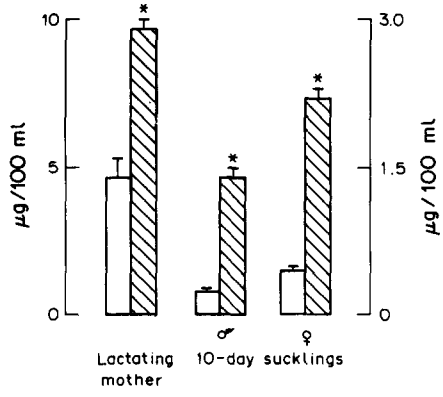


Fig. 3. Means ± SE of morning plasma corticosterone level in lactating rats drinking corticosterone-enriched water (200 μg/ml) and in their sucklings. $n = 8$–10. $^*P < 0.05$, ANOVA.

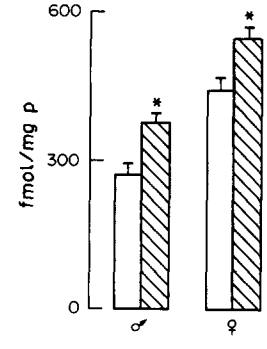


Fig. 5. [$^3$H]Corticosterone binding capacity ($B_{max}$) in the hippocampal cytosol (6 animals per sample) from 90-day-old male (open columns) and female corticosterone-nursed rats (solid columns). $^*P < 0.05$, ANOVA.

Upon reaching adulthood, this type of offspring, "corticosterone-nursed", showed some sex-related anomalies. As shown in Fig. 4, at 90 days of age corticosterone-nursed males had a lower basal adrenocortical activity and a reduced body weight in comparison to controls. In females, no differences in body weight was found, while basal adrenocortical activity was higher in comparison to controls. Furthermore, as shown in Fig. 5, corticosterone-nursed rats, both male and female, had in adulthood a significantly higher binding capacity for the glucocorticoid hormone in the hippocampus, due to an increase in the number of binding sites. Concomitantly, as shown in Table 1, corticosterone-nursed adult offspring, both males and females, had a significantly higher performance in passive avoidance test.

Thus, it appeared that improper contact of the hormone with the hippocampus, in a critical period for the development and maturation of this brain area, has an imprinting effect on the glucocorticoid receptor. Since the function of the hippocampus is fundamental for adaptation,

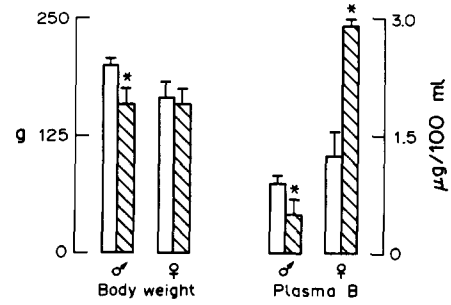


Fig. 4. Mean values ± SE of body weight and plasma corticosterone levels in ninety day old rats, control-nursed (open columns) and corticosterone-nursed (solid columns). $n = 20$–24. $^*P < 0.05$, ANOVA.

in its behavioral and hormonal components, and relies on the integratory role of the glucocorticoid receptor system any permanent alteration of this receptor will lead to altered adaptive responses.

Individuality of the adaptive responses in adult animal depends not only upon the interaction with the environment in early life, but also upon genetic mechanisms. In fact, strain dependent differences in avoidance learning have been reported by a number of studies [11].

The purpose of our study was to investigate whether the genotype-dependent behavioral differences of C57/BL and Balb/c mice in active avoidance learning are related to differences in the capacity of hippocampal corticosterone receptor system [12].

As shown in Fig. 6, C57/BL attained lower avoidance scores than Balb/c, thus confirming previous results [13]. Moreover, C57/BL mice had a lower maximal individual [$^3$H]corticosterone binding in the hippocampus.

This result suggest that, as in the rat, a functional relationship exists between binding capacity for [$^3$H]corticosterone and the degree of active avoidance learning in the mouse, with a statistically significant correlation coefficient either within each single group, or in the whole population, and that such a relationship might

Table 1. Passive avoidance learning in 90-day-old corticosterone-nursed rats

| | Males (58) | Females (70) |
|---|---|---|
| Control nursed | 10–83 | 31–227 |
| | (15) | (14) |
| | 280* | 250* |
| Corticosterone nursed | 23–300 | 196–300 |
| | (15) | (14) |

Median and interquartile values of step-through latency (s) in the 24 h retention test.
$^*P < 0.05$, Mann–Whitney test. In parentheses: number of animals.

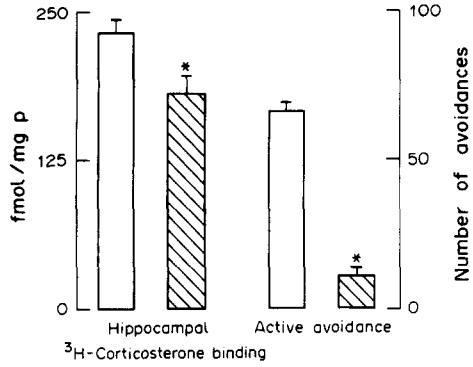


Fig. 6. Maximal individual [$^3$H]corticosterone binding capacity and active avoidance scores in mice of the Balb/c (open columns) and C57/BL (solid columns) strains. Mean values ± SE. *Significantly different ($P < 0.001$) from the other strain, Student's $t$-test.

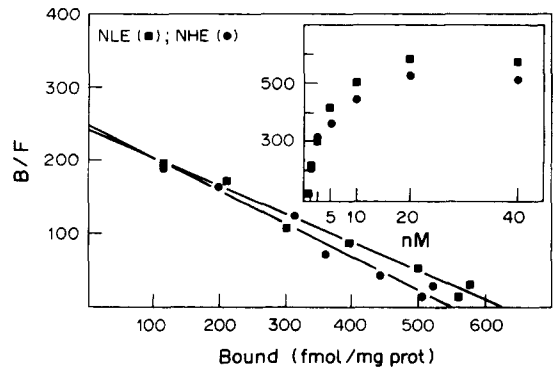


Fig. 7. [$^3$H]Corticosterone binding in hippocampal cytosol obtained from NHE ($n = 6$) and NLE ($n = 6$) rats. Scatchard plots and *in vitro* saturation analysis in the inset.

depend on genetic factors expressing behavioral regulation. On the other hand, we have found that morning plasma corticosterone levels were significantly lower in C57/BL, compared to Balb/c (Table 2).

This might indicate that the genetic determinant is acting mediately, through differences in the activity of the hypotalamo–pituitary–adrenal axis, possibly during neonatal life, which can permanently affect the binding capacity in adulthood.

Data presented up to this point, have shown that binding capacity of glucocorticoid hormone in the hippocampus, could be influenced by environmental factors during perinatal life or by genetic factors.

In order to investigate more in depth the relationship between the genetic factor and the hippocampal glucocorticoid receptor in the expression of adaptive behaviors action, we have studied whether the genotype-dependent behavior of the NHE and NLE rat strains is accompanied with differences in the capacity of hippocampal glucocorticoid receptors [14].

As shown in Fig. 7, no differences were found in binding capacity, while both genetically selected strains showed lower binding capacity than control rats (NRB). Surprisingly enough, these results would exclude that the genetic differences in the behavior of these two strains of rats are parallel to distinct patterns in the glucocorticoid binding capacity in the hippocampus. However, a correlative analysis was made among individual maximal binding capacity for [$^3$H]corticosterone and the behavioral scores derived from exploration in the Lat-maze (Fig. 8). The arousal score was positively correlated in NLE and negatively in NHE, but not in NRB control rats. The positive–non-negative correlations are good evidence for an inverted U relationship between glucocorticoid receptors in the hippocampus and behavioral activation. Since the exploratory behavior in a Lat-maze has many individual components, it is difficult to evaluate the relative weight of each of them. We can only tentatively speculate that the differential genotype-dependent neurobehavioral correlative profile reveals a different meaning of exploration in the 3 strains.

While in the first part of this presentation, linear correlations between the capacity of binding and learned avoidance behavior were evident, in the second part we have observed how these correlations can also be non-linear in

Table 2. Morning plasma corticosterone level in mice of the Balb/c and C57BL76 strains

| Strain | Corticosterone ($\mu$g/100 ml) |
|---|---|
| Balb/c ($n = 10$) | 6.25 ± 0.3 |
| C57BL78 ($n = 10$) | 1.09 ± 0.7* |

Mean values ± SE. *Significantly different ($P < 0.001$) from the other strain, Student's $t$-test.

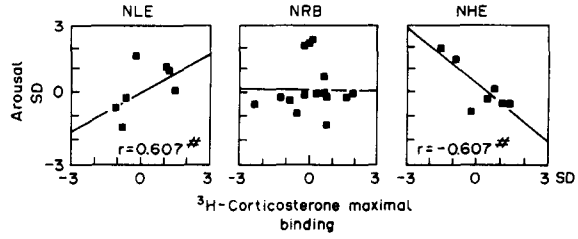


Fig. 8. Scatterplots of the correlation between arousal and [$^3$H]corticosterone binding capacity in the hippocampus. Abscissae and ordinates are given in units of standard deviation from the mean. *$0.05 > P > 0.01$ (two-tailed).

relation to a different type of behavior, namely the innate exploratory one.

In conclusion, our studies strongly indicate the complexity of the mechanism regulating the glucocorticoid binding capacity in the hippocampus and emphasize the fact that we are still far from the understanding of the physiological role of this system.

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