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Brain–Corticosteroid Hormone Dialogue: Slow and Persistent

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SUMMARY

1. The stress response system is shaped by genetic factors and life experiences, of which the effect of a neonatal life event is among the most persistent. Here we report studies focused on the "nature-nurture" question using rat lines genetically selected for extreme differences in dopamine phenotype as well as rats exposed as infants to the traumatic experience of maternal deprivation.

2. As key to the endocrine and behavioural adaptations occurring in these two animal models the hormone corticosterone (CORT) is considered. The stress hormone exerts slow and persistent genomic control over neuronal activity underlying the stress response system via high affinity mineralocorticoid (MR) and glucocorticoid receptors (GR). This action is exerted in a coordinate manner and involves after stress due to the rising CORT levels progressive activation of both receptor types.

3. Short periods of maternal separation (neonatal handling) trigger subsequently enhanced maternal care and sensory stimulation. However, a prolonged period (24h) of depriving the infant of maternal care disrupts the stress hyporesponsive period (SHRP) and causes an inappropriate rise in CORT. During development exposure to CORT and to sensory stimulation has long-lasting consequences for organization of the stress response system.

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4. We find that these factors embodied by mother-pup interaction are critical for dopamine phenotype, CORT receptor dynamics and neuroendocrine regulation in adult life. The findings provide a conceptual framework to study dopamine-related psychopathology against a background of genetic predisposition, early life events, stress hormones and brain development.

INTRODUCTION

In response to stress the brain activates the sympathetic-adrenal-medullary system and the hypothalamic-pituitary-adrenal (HPA) axis. These systems coordinatively facilitate recovery of homeostasis and adaptation by interactions on multiple levels. Circulating amines and ascending aminergic projections in brain mostly stimulate HPA activity. Alternatively, components of the HPA axis e.g. high levels of circulating corticosteroids (CORT), facilitate and sensitize central and peripheral amine functions.

There are considerable individual variations in the response to stress, which are usually recorded as differences in behavioural adaptations and in dynamics of the various components of the HPA axis and sympatho-adrenal system (Bohus, 1993). These variations concern the composition of the CRH and co-secretagogue (e.g. vasopressin, angiotensin) cocktail in hypothalamus. They also include patterns of endproducts (ACTH, β-endorphin) of pro-opiomelanocortin (POMC) processing in pituitary and brain (De Wied and De Kloet, 1987). Moreover, profound differences in adrenocortical responsiveness to ACTH may occur, while the fraction of biologically active free CORT and CORT-binding globulin (CBG) also appear to be dynamic regulatory components of HPA activity (Dallman *et al.*, 1992). Finally, the properties of CORT receptors in the brain pituitary also show profound individual variations (De Kloet, 1991).

It is thought that the individual differences in behavioural performance and (neuro)endocrine responses have a genetic background and are shaped by the experience of environmental events. Traumatic life events evoke long-lasting changes, in particular when experienced in early life. As will be pointed out in this contribution, mother-pup interaction is a critical determinant in neonatal life events (Levine and Lewis, 1959; Levine, 1994). Separation of mother and pup has shown profound effects on brain organization and its impact may be greatly amplified by genetic predisposition. However, experimental data to substantiate the mechanism and the impact of such "nature-nurture" interactions are relatively sparse.

Here we will report some of our experiments designed to examine genetic influence and mother-pup interaction on the individual variation in the stress response system. First, our recent observations with rat lines genetically selected for susceptibility to apomorphin are reported. These rat lines were selected in the eighties by Alexander Cools (Cools *et al.*, 1990) and appear to be an excellent animal model to study the role of dopamine and CORT in individual responses to stress. Second, the effect of mother-pup interaction on brain development is considered. It is only a few years ago that Seymour Levine (Stanton *et al.*, 1987;

Levine et al., 1991; Levine, 1994) reported the crucial experiments showing the profound influence of maternal deprivation on the organization of the stress response system. Third, the role of brain CORT receptor function is highlighted in the stress system organization. Recently, novel principles were uncovered of gene-mediated control of neuronal activity exerted by CORT (Joëls and De Kloet, 1989; 1990; 1992; 1994; 1995). These findings are fundamental for understanding the role of CORT action in brain development, stabilization of neuronal activity and maintenance of homeostasis.

GENETICALLY SELECTED RAT LINES

The genetic influence on the stress response system involving dopamine has been questioned ever since 1985, when Cools initiated a breeding program based on pharmacogenetic selection of Wistar rats using as criterium stereotypic gnawing induced by the mixed D1/D2 agonist apomorphine. Apomorphine-unsusceptible (apo-unsus) rats, which virtually lacked a gnawing response accounted for about 25% of the total population. Another 25% were apomorphine-susceptible (apo-sus) showing vigorous gnawing in response to the drug. The remainder of the population consisted of rats with an intermediate gnawing score. In each generation the most outspoken apo-sus and apo-unsus rats enrolled a particular breeding procedure to maintain genotypic heterogeneity (Cools *et al.*, 1990).

Psychopharmacological studies suggested that the two rat lines represent the extremes in dopamine responsiveness co-existing in a normal rat population (Cools *et al.*, 1990). THmRNA abundance and D1/D2 receptor properties in the nigrostriatal and tubero-infundibular pathway differ significantly, thus providing a neurochemical basis to the line differences (Rots *et al.*, 1995a). Previous studies (Cools *et al.*, 1990) showed that rats of the two selected lines have considerable variation in behavioural adaptation and locomotor responses to stress. These findings suggest that discrimination by apomorphin susceptibility co-selects for stress responsiveness. Therefore, we hypothesized that susceptibility for apomorphine correlates with basal and stress-induced HPA activity.

Recent studies aimed to test this hypothesis showed that under basal a.m. conditions apo-sus rats had a higher amount of CRH gene transcripts in PVN and a higher ACTH level in plasma relative to their apo-sus counterparts (Rots *et al.*, 1995). However, similar concentrations of stress-induced circulating CORT were observed in both lines. Interestingly, the fraction of free CORT was decreased in these apo-sus rats, possibly due to an increased binding affinity of corticosteroid-binding globulin (CBG). Hippocampal CORT-selective mineralocorticoid receptors (MRs) were also increased (Sutanto *et al.*, 1989; Rots *et al.*, 1995b). Since MRs are involved in monitoring basal free CORT levels (Ratka *et al.*, 1989; Dallman *et al.*, 1992), their increase could be due to homologous upregulation in response to the reduced free CORT level. Collectively, the data suggest that the rat lines differ in apparent set point of basal HPA activity.

Subsequently the line difference in sensitivity of the HPA axis to stress was

tested by exposing rats to a novel environment. A relatively enhanced ACTH response was observed in apo-sus rats suggesting that the sensitivity of their stress response system to trigger an ACTH response was increased. Adrenal CORT secretion did not show a line difference, since under basal as well as stress conditions the extent of CORT secretion did not match that of ACTH stimulation. In fact, the apo-sus adrenal required much higher ACTH to maintain the same total CORT level under basal and stress conditions. Thus, hyporesponsiveness of the apo-sus adrenal to ACTH relative to their apo-unsus counterparts is the critical variable here (Rots *et al.*, 1995; 1995b). Yet, there were no line differences if adrenal function was directly tested with exogenous ACTH. Apparently, in the two lines stress mobilizes to a different extent factors synergizing with ACTH stimulation. For the apo-sus animal it is hypothesized that these synergizing factors of probable medullary origin (e.g. CRH, amines) (Vinson *et al.*, 1994) are relatively reduced rendering hyporesponsive adrenals as net results.

Stress-induced ACTH levels of apo-sus rats remained elevated much longer as compared to apo-unsus animals. Also the plasma total and free CORT levels were significantly elevated for longer time intervals. This reduced ability of apo-sus animals to terminate stress-induced ACTH and free CORT responses is indicative for CORT feedback resistance relative to their apo-unsus counterparts. The possible site of this CORT resistance was evaluated by conducting humoral challenge tests of pituitary and adrenocortical function. There was no line difference in the CORT response to exogenous CRH and ACTH suggesting that the feedback resistance of apo-sus rats resides in the brain (Rots *et al.*, 1995b).

The basal free corticosterone level was lower in apo-sus rats, while in contrast stress-induced free corticosterone was elevated. The binding to CBG seems therefore a dynamic regulatory factor in determining the line difference in bio-availability of CORT. Consequently, one would predict also profound line differences in brain CORT receptors. Indeed, Sutanto *et al.*, (1989) showed previously that binding to hippocampal MR and glucocorticoid receptors (GR) as well as to GR in frontal cortex was increased in apo-sus rats. There are data suggesting modulation of steroid receptor function by dopamine (Antakly *et al.*, 1987; Seger *et al.*, 1988; Maccari, 1990), but the contribution of this phenomenon to the present line differences remains to be established.

In conclusion, genetic selection of rats for extreme differences in dopamine responsiveness co-selects for adrenocortical responsiveness to ACTH and corticosteroid feedback efficacy.

DEVELOPMENT OF THE STRESS RESPONSE SYSTEM

The next question we asked was whether differences in dopamine responsiveness are a determinant of variations in the HPA axis, or alternatively whether neonatal HPA differences alter responsiveness of the dopamine pathways. An initial answer to both questions was obtained by comparing the development of HPA activity and dopamine in both lines. A line difference in ACTH is

detectable at around the age of weaning. At day 18 the basal a.m. levels of ACTH are significantly elevated in apo-sus rats. In apo-sus rats CORT receptor transcripts are transiently elevated at 10 days, but at older ages hippocampal MR and GRmRNA are not different in spite of the higher binding capacity of these receptors. Interestingly, the development of the line difference in HPA activity precedes the divergence in dopamine phenotype since TH mRNA was not different in rats of both lines at 18 days of age. This observation raises the interesting possibility that the genetic selection for dopamine may be secundarily to development changes in the HPA axis (Rots *et al.*, 1985c).

Between day 4 and 14 the HPA axis in the rat is hyporesponsive to stress. During this stress hyporesponsive period (SHRP) circulating CORT and ACTH levels are extremely low. Stressors which evoke a pronounced CORT response during adulthood, are only weakly active in the infant. CBG levels are not detectable during the SHRP. GR levels in brain are generally low at first and reach adult levels around the fourth week of life (Rosenfeld *et al.*, 1988; 1988a). GR microdistribution changes dramatically during development. For instance, the nucleus suprachiasmaticus and the hippocampal CA3 region express high levels of GR during the first week, and then practically disappear from these regions (Van Eekelen *et al.*, 1987; 1991). MR expression is low the first day, but then rapidly rises until adult levels after one week. In view of the very low circulating B levels during the SHRP most of the CORT actions involved in stabilization of the stress response system are mediated via MR (Rosenfeld *et al.*, 1990; 1993a).

The diminished responsiveness to stress during the SHRP appears to be due to a multifactorial regulatory system, which depends on internal (endocrine, neural) and external (maternal) inputs to maintain overall quiescence. The major rate-limiting factors in HPA activation appear at the level of the brain and the adrenal (Rosenfeld *et al.*, 1992). Only some stimuli, e.g. interleukin-1, histamine, are able to trigger a neonatal CORT response (Levine *et al.*, 1994). Under most conditions, however, acutely elevated ACTH or exogenously administered ACTH do not trigger an adrenocortical response. The proximal cause of the SHRP is, therefore, at the level of the adrenal due to reduced sensitivity to ACTH.

Studies using maternal separation have demonstrated that the mother regulates HPA responses in the infant. These HPA responses slowly develop as a function of time after maternal separation. A normally reared pup does not display CORT responses to saline, novelty and acute maternal separation, unless maternal deprivation is prolonged for at least 8 up to 24 h. The phenomenon appears to involve priming (sensitization) of the adrenal to ACTH and stress. Besides duration, also increasing age of the infant exposed to maternal separation causes HPA activity. The ACTH and CORT responses to ether, novelty and saline injection immediately following 24 hours of maternal deprivation are larger at 9, 12 and 16 days of age, than at day 3 (Levine *et al.*, 1991).

Maternal deprivation also revealed which factors actually are responsible for the activation of the HPA axis. It appeared that tactile stimulation suppresses neural pathways involved in suppression of ACTH release. Feeding has yet other effects, which are predominantly peripheral, as appeared from the enhanced adrenocortical responsiveness following mother deprivation (Rosenfeld *et al.*,

1993; Suchecki *et al.*, 1994; Levine, 1994). The impact of the different aspects of maternal care depends on the duration of the separation and the postnatal age this is endured. Brain organization appears stimulated by repeated daily short deprivations and the subsequent daily intensified maternal care. This procedure is called neonatal handling and is thought to facilitate maturation of specific neural (limbic) pathways (Meaney *et al.*, 1994; Levine, 1994). On the other hand 24 h of maternal deprivation evokes increased CORT at a time the hormone level otherwise would be low and unperturbed.

In conclusion, first, the preliminary study suggests that the line difference in apomorphin susceptibility develops due to diverging HPA activity during development of the two lines. Second, this could imply that the difference in dopamine phenotype develops as a consequence of a genetically determined mother-infant interaction evoking differential sensory stimulation and HPA activation during early life.

LIVE EVENTS AND BRAIN DEVELOPMENT

Do stimuli that disrupt the SHRP have the ability to alter brain development and subsequently behaviour and physiological responses in later life? Maternal behaviour ensures during development a quiescent stress response system in the newborn rat, which is characterized by low and constant CORT levels. Maternal deprivation of the infant for 24 hours disrupts the SHRP. As a result inappropriate high levels of CORT during maternal deprivation are thought to interfere with normal brain development.

During development CORT administration has permanent effects on growth and differentiation of the brain. The steroid inhibits protein synthesis, glucose uptake, neuro- and gliogenesis. Neuronal "birthdays" are altered and myelinogenesis, formation of dendritic spines, axonal growth and synaptogenesis is retarded. CORT is critical for neurotransmitter phenotype (Bohn, 1984). For instance, without GR (in homozygous mutants) the adrenal medulla was poorly developed (G. Schütz and T. Cole, unpubl. observation). Low levels of CORT facilitate differentiation of previously established phenotype, e.g. increase tyrosine hydroxylase (TH) in sympathetic neurons and phenyl-N-methyl transferase (PNMT) in chromaffin cells.

In contrast, repeated short-term separation of mother and infant by neonatal handling triggers sensory stimulation, which also alters brain organization (Hofer, 1984). Thus, depending on the duration of maternal separation the infant may experience changing effects of sensory stimulation and/or CORT. As adults the consequence of short- and long-term maternal deprivation is strikingly different, since emotional and adrenocortical reactivity seem oppositely affected. The underlying mechanism of this bimodal effect of mother-pup interaction is poorly understood. Little is known at what point in time the impact of mother-infant interaction changes the stress response system from an efficient to a sluggish operation.

We designed a series of experiments to test the hypothesis that long-term

maternal deprivation during early development has effects on central nervous system function at adulthood. Three days old unselected pups were deprived from their mother for 24 hours, subsequently they were allowed to grow up normally. As young adults (2 months) the mother-deprived rats had reduced basal CRH mRNA expression. However, basal plasma ACTH and CORT levels were significantly elevated. Adrenal weight was also increased (Rots *et al.*, 1995d).

Long-lasting effects of maternal deprivation have also been found on CORT receptor levels. 24 Hours of deprivation starting at postnatal day 3 resulted in reduced GR mRNA in PVN and anterior pituitary and reduced hippocampal GR binding in male adult rats. These HPA parameters in males suggest that hypercorticism accompanied by GR downregulation has developed in the adult animals previously deprived from the mother. Adult female rats showed the opposite effect and an increased Bmax of hippocampal GR was found.

Most interestingly, however, is the finding that the mother-deprived rats showed as adults increased susceptibility for apomorphine. Administration of the drug evoked excessive gnawing behaviour in these animals. Thus, 24 hours of maternal deprivation at postnatal day 3 results in an adult animal which is characterized by hypercorticism and increased nigrostriatal dopamine responsiveness (Rots *et al.*, 1995d).

In conclusion, these findings are of great interest in view of data obtained from rat lines genetically selected by high and low susceptibility of apomorphine-induced gnawing. The apo-sus rats resemble the mother-deprived rats in enhanced dopamine responsiveness, and preliminary data show feedback resistance in both cases. However, there are also profound differences. Adrenocortical responsiveness to ACTH and CORT receptor number are increased after maternal deprivation, but decreased in the apo-sus rat line.

BRAIN CORTICOSTEROID RECEPTORS AND HOMEOSTASIS

If CORT and CORT receptor dynamics are changed in genetically selected lines and after maternal deprivation of pups, what is the implication for its modes of operation in homeostasis and behavioural adaptation? Definite answers to this question cannot be given, but a substantial advance in knowledge required for such answers has recently occurred in the molecular and cellular action of corticosteroid hormones in brain. The action of CORT in limbic circuitry through gene regulation is mediated by high affinity (Kd = 0.3 nM) MR and lower affinity (Kd = 3 nM) GR. Basal a.m. CORT levels predominantly occupy MRs, while high CORT levels during stress and the circadian peak progressively occupy GRs in addition to MRs. Accordingly, the brain CORT receptors are equipped by their differential affinity to monitor a wide range of circulating CORT concentrations (Reul and De Kloet, 1985; De Kloet and Reul, 1987; De Kloet, 1991).

Confocal microscopy revealed in individual hippocampal CA1 neurons a clustered pattern of intranuclear CORT receptor distribution. Using two different fluorochromes and novel image analysis techniques clusters containing single species of either MR and GR were found as well as clusters showing

significant overlap in immunostaining of both types. This observation suggests a close interaction between MR and GR in control of neuronal function (Van Steensel, 1995). Transient transfection of a neuroblastoma cell line with MR and GR and an MMTV promoter construct also suggests close interaction of MR and GR in transcriptional regulation. Co-expressed MR and GR display at ED50 concentrations of cortisol enhanced transactivation properties as opposed to cells with individually expressed receptor types. Moreover, co-expressed MR and GR did bind with higher affinity to glucocorticoid response elements (GRE's). The findings were interpreted as cooperativity between heterodimers of MR and GR, which would considerably increase complexity in CORT control of gene regulation (Trapp *et al.*, 1994).

It is of interest that a linear dose response of CORT is observed with the MMTV promoter in the transfected cell lines. In contrast, in hippocampal neurons containing both receptor types usually U-shaped dose response relationships are noted with increasing concentrations of CORT (Joëls and De Kloet, 1994). Such effects of CORT on the activity of hippocampal CA1 neurons develop slowly within 30–60 minutes and last several hours. The hormone does not change resting membrane potentials, but changes neuronal excitability. The CORT effects are therefore only detectable after a shift from resting level induced by synaptic stimulation or exposure to transmitters. Inclusion of specific agonists and antagonists revealed that this U shape is explained by a coordinated MR and GR-mediated response.

In young adult rats predominant MR occupancy allows only small calcium currents (Karst and Joëls, 1994) as well as transmitter responses mediated by e.g. 5HT1A receptors (Joëls and De Kloet, 1990; 1991) and muscarinic cholinergic receptors (Hesen *et al.*, 1993). Additional GR occupancy with higher CORT concentration increases calcium currents and transmitter responses. A similar increase occurs after depletion of both receptor types after adrenalectomy (ADX), although this ADX effect particularly concerns the low-threshold calcium current. In parallel with these calcium conductances also the calcium-dependent potassium conductance is changed dose-dependently (Joëls and De Kloet, 1989; 1990). Moreover, synaptic responses to amino acids achieved via ligand-gated ion channels are most stable with predominant MR occupancy. These responses rapidly deteriorate, particularly after repeated stimulation, in the absence of steroid or after simultaneous MR and GR activation by high amounts of CORT (Joëls and De Kloet, 1993).

The physiological consequence of the stabilizing MR-mediated action is that these receptors appear to be important for sustaining the main stream of information carried by amino acids. In spite of the relatively high neuronal firing the integrity of the neuronal network is maintained. Increased CORT after stress or during the circadian rise may serve to suppress transiently raised excitability. First, this is achieved by reducing amino acid input, particularly upon repeated stimulation. Second, via increased calcium-dependent potassium conductance. Third, via increased serotonergic and reduced adrenergic input (Joëls and De Kloet, 1992; 1994; 1995).

Accordingly, chronic over and underexposure to corticosteroids or receptor

defects have important consequences for the functioning of hippocampal neuronal circuits. Ion regulations become profoundly disturbed and morphological observations have clearly demonstrated apoptosis of dentate gyrus neurons after ADX. High CORT and stress reduce dendritic branching of CA3 neurons. High CORT and continuous depolarizations (e.g. epilepsia) or energy deprivation (e.g. ischema) may pose also a serious threat to viability of hippocampal neurons, as has been observed for the CA1 cells (Sapolsky, 1992; McEwen, 1994; McEwen, this volume).

The changes in hippocampal excitability and morphology are also critical for higher brain functions under control of CORT. If tested in the Morris water maze, MR- and GR-mediated effects were found on response selection and storage of spatial information, respectively (Oitzl *et al.*, 1992). CORT affects the inhibitory control of the hippocampus over basal and stress-induced HPA activity. MRs appear mostly involved in control of basal activity and the sensitivity or threshold of the stress response system, while additional GR activation on the level of the hippocampus exerts opposite effects (Ratka *et al.*, 1989; De Kloet, 1991; De Kloet *et al.*, 1993).

With respect to neuroendocrine regulation it is of relevance that GRs are widely localized in brain and pituitary with extremely high concentrations in PVN and corticotrophs. A large body of evidence clearly demonstrates that GRs in CRH neurons and pituitary corticotrophs suppress stress-induced HPA activity. In contrast, GRs in hippocampus rather seem to mediate disinhibitory influences. Furthermore, GRs in ascending projections mediate activating CORT effects on aminergic nerve impulse flow. On the level of the CRH neurons GR-induced dopaminergic, serotonergic and adrenergic neurons as well as limbic pathways, therefore attenuate GR-mediated feedback, rendering a feedback-resistance subject.

In conclusion, MR and GR mediate in close interaction coordinatively CORT control of neuronal activity underlying the stress response circuitry. Dysbalance in receptor activity or aberrant CORT levels that seem to be induced by traumatic events in early life increase vulnerability of the stress circuitry to adverse effects of negative events. A role of dopamine in this cascade involving life events, CORT receptors and brain development is indicated (see Fig. 1). Its implication for pathogenesis of neuropsychiatric disorders needs to be established (Mednick *et al.*, 1992; Ellenbroek *et al.*, 1995).

CONCLUDING REMARKS

Based on these recently discovered basic facts in the interaction between genetic background and the effect of early life events on brain development a number of critical questions for future research can be formulated. These include: How does genetic influence modulate mother-infant interaction? What is the underlying mechanism of the effect of mother-infant interaction on organization of the stress response system? To what extent are early experiences associated with individual differences in coping with stress? What are the implications of

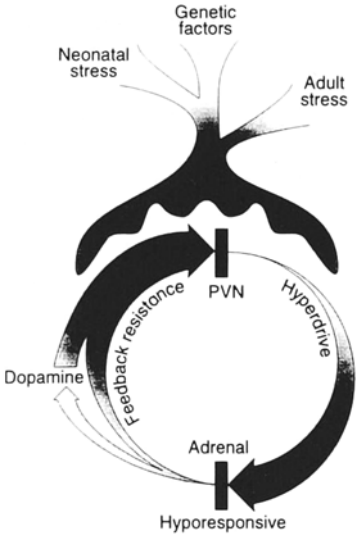

Fig. 1. The glucocorticoid-dopamine connection. The figure is based on findings with rat lines genetically selected for extreme differences in dopamine phenotype. Vertical bars indicate adaptations in adrenal cortex and paraventricular nucleus (PVN) to prevent corticosteroid hormone overexposure by hyporesponsiveness to ACTH and by resistance to glucocorticoid feedback, respectively, in the rat line with highest dopamine reactivity (i.e. apo-sus rats). Glucocorticoid sensitization of ascending dopaminene neurons is proposed to feed activation of the CRH neurons in these apo-sus animals. We postulate that a pathological condition may develop if these adaptations become impaired due to poor coping with (neonatal) stress (from N. Y. Rots: Stress and Dopamine: studies in genetically selected rat lines, Ph.D. thesis).

these genetic and ontogenetic modulations of the stress response system in terms of vulnerability for stress-related disorders in adult life and senescence? More specifically, how are these developmental changes in brain organization related to precipitation of specific neuropsychiatric disorders?

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