Genetic and environmental influences on behavioral and neurochemical aspects of emotionality in rats

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Summary. Three pairings of rats (two derived from divergent, selective breeding and one from divergent environmental conditions) were compared with regard to behavioral and hormonal parameters. Striking differences were observed: results obtained in our own laboratory as well as those found in a review of the literature pointed to higher emotionality (e.g., increased defecation and corticosterone secretion, etc.) in Roman low-avoidance, Wistar-Kyoto and group-housed rats, as compared to their respective counterparts, Roman high-avoidance, spontaneously hypertensive, and individually housed Wistar rats. Concomitant receptor binding studies reviewed here (^{3}H-diazepam- and ^{3}H-imipramine-binding sites) have revealed, however, less consistent intrapair differences.

Key words. Locomotor activity; defecation; corticosterone; emotionality; RHA vs RLA rats; SHR vs WKY rats; individual housing; selective breeding; receptor binding.

1. Introduction

Although psychiatrists tend to group mental aberrations into diagnostic classes, and to conduct neurochemical studies on such 'defined' sub-groups, mental disturbances are more likely to represent a summation of many affective peculiarities, with one and the same symptom being detected in diverse diagnostic sub-classes. For example, signs of social withdrawal can be observed in schizophrenic patients, in depressed patients and in alcoholics. Similarly, states of anxiety are present in patients who belong to different diagnostic sub-groups. It can be seriously argued, therefore, that the consideration of 'social withdrawal' or 'anxiety', to mention only the two examples cited above, would be superior, as entities, to the use of terms like 'schizophrenia' or 'depression' when trying to uncover relationships between mental abnormalities and neurochemical parameters.

These same entitites are also observable in experimental animals. 'Social withdrawal' and 'anxiety' both represent evolutionary patterns which, when considered in a species-specific context, are comparable in humans and animals. Thus, studies on aberrant patterns in laboratory animals may represent a beneficial contribution to a better understanding of human psychopathological states.

A strong genetic influence has also been noted in many psychiatric disturbances. Comparisons between twins (monozygote versus dizygote), and familial analyses, have clearly indicated that an increase in consanguinity often parallels an increase in morbidity risk[2, 111]. Concordance for monozygotic twins, however, has always been clearly below 100%. Therefore, besides genetic disposition, other circumstances may induce (or inhibit) the initiation of a given disturbance. Among these, environmental conditions and/or hormonal alterations and/or stress have been proposed as triggering factors[33]. Thus, assuming that psychiatric symptoms are ultimately caused by neurochemical alterations, then it must be inferred that both environmental circumstances and genetic factors may influence neurochemical processes.

Compared to human studies, an advantage of studies using rodents is that the genetic background, as well as environmental circumstances of the latter, can more easily be controlled. On the one hand, by selective breeding the genetic background can be experimentally manipulated and, on the other hand, environmental conditions can more reliably be kept constant and/or defined. We have compared groups of rats which have emerged from selective breeding programs (genetic manipulation) and other rats which have differed only with respect to housing condition (environmental manipulation), in regard to both behavioral, and neurochemical, parameters. The present review attempts to demonstrate the parallels (and dissimilarities) between these groups of rats, with regard to some criteria which are thought to be relevant in biologically-oriented psychiatry.

2. Description of the rats used

2.1 Roman high-avoidance and Roman low-avoidance rats

Using two-way active avoidance (shuttlebox) performance as the selection criterion, the Swiss line of Roman high avoidance rats (RHA/Verh) has been selected and bred since 1972 on the basis of rapid acquisition of the response, whereas the Swiss line of Roman low-avoidance rats (RLA/Verh) is selected and bred on the basis of the failure to acquire that response[26].

2.2 Spontaneously hypertensive (SHR) and normotensive (WKY) rats

In 1959 Okamoto and Aoki[79] started with the selective inbreeding of Wistar-Kyoto rats (WKY) from which a strain of spontaneously hpertensive rats (SHR) was finally produced[71, 72]. The animals' blood pressure was used as a selection criterion, whereby SHR develop arterial hypertension at the rate of 100%. Most researchers agree with Trippodo and Frohlich[102] that "until a better experimental model is made generally available, we feel justified in taking the affirmative position that the SHR is indeed an excellent laboratory counterpart of essential hypertension." The SHR and WKY rats used at our laboratory were obtained from Roche-Füllinsdorf, Basel.

2.3 Individually and group-housed rats

Individually and group-housed rats were derived from a local Wistar strain (Füllinsdorf Albino). Housing condition was the sole variable[42].

3. Cross comparisons regarding the selection criteria among these rats

3.1 RHA/Verh and RLA/Verh rats

Blood pressure. In collaboration with Dr F. Hefti (Hoffmann LaRoche, Basel) systolic arterial blood pressure was determined in RHA/Verh and RLA/Verh rats. In this preliminary study (6 rats per line), a higher blood pressure was found for RLA/Verh (159 mm Hg), as compared to RHA/Verh rats (134 mm Hg) (unpublished results). Differences in stress reaction to the experimental conditions (pre-warming, immobilization) may have influenced these findings, however, as the only other report comparing blood pressure between RHA and RLA rats has revealed a marginally higher systolic blood pressure ($0.1 > p > 0.05$) for RHA rats[55].

3.2 SHR and WKY rats

Avoidance behavior. Our (unpublished) observations have indicated that SHR acquired active avoidance quicker than did normotensive WKY rats at 3 different shock intensities, a finding which is in accordance with Knardahl and Sagvol-

den[65]. Those authors concluded, in addition, that it is not blood pressure per se which affects the learning of an active avoidance task. Oehme et al.[78], on the other hand, described a disordered avoidance learning in SHR rats.

3.3 Individually and group-housed Wistar rats

Blood pressure. An increased blood pressure has been observed after various periods of individual housing[9].

Avoidance. Looking at the effect of individual housing on shuttle-box avoidance, Lovely and Pagano[70] found a facilitated acquisition, as compared to group-housed controls. Acquisition of pole jump or conditioned avoidance responding, however, was found to be delayed in individually housed, female rats[23]. Our own (unpublished) comparison among RHA/Verh, RLA/Verh and Wistar rats, including individually and group-housed animals for each group, have revealed clear-cut intergroup differences in shuttlebox performance (RHA/Verh > Wistar > RLA/Verh). None of these groups, however, showed any effect of housing conditions.

4. Comparisons in regard to 'fear'

It has often been proposed that momentary fear may be estimated by observing a rat's behavioral and hormonal responses when exposed to a novel environment. Locomotor activity, defecation and plasma concentrations of corticosterone are the most widely assessed parameters. Although occasionally questioned[3], the validity of defecation as a parameter of fear has long been accepted[53,58] and has been emphasized in a recent report by Pellow et al., utilizing the elevated plus maze[82]. In this apparatus, the inhibition of entries into open arms, as opposed to closed arms, was considered to reflect the animals' fear (e.g., anxiolytic drugs dramatically attenuate the animals' innate aversion to the open arms). When these authors confined rats to either the two closed, or the two open, arms, a significant decrease in the number of entries and a significant increase in the time spent motionless and in the amount of defecation was found when rats were exposed to the latter. In addition, plasma corticosterone levels were significantly more elevated in rats confined to the open arms.

We have used these three parameters (locomotor activity, defecation and corticosterone levels) in order to determine whether RHA/Verh vs RLA/Verh, SHR vs WKY and individually- vs group-housed Wistar rats differ in fear experienced upon being exposed to novel environments. All groups of rats, at least in our laboratory, were on a L12:D12 light cycle (with food and water ad libitum), and all behavioral and hormonal observations were carried out in adult male rats during a narrow time interval at the middle of the dark phase.

4.1 RHA/Verh and RLA/Verh rats

RHA rats and their descendents, in several laboratories, have often been observed to be more active than their RLA counterparts, using the number of intertrial responses in the shuttlebox as a criterion for locomotor activity in a stress situation[18,21,89]. In our studies, RHA/Verh rats made more crossings than did RLA/Verh rats during a 10 min openfield test[40], and similar interline differences could be observed when using either an oversized macrolon cage or the shuttlebox as novel environment[48,49] as well as during long-term registration of 'spontaneous' activity[49]. These differences confirmed previous reports which had used either an openfield or an enclosed maze[7,29,74], and have been seen both during the dark phase and the light phase[7,49]. With regard to defecation scores, the two Roman rat lines have been reported to differ as follows: during exposure to three different novel environments (mild stress), mean defecation scores for RHA/Verh rats were below 1 bolus per 10 min whereas, for RLA/Verh rats, means of up to 7 boli were observed. Defecation in the RHA/Verh rats increased, however, in stronger stress situations (ether vapor, immobilization and inescapable footshock), so as to attenuate genetic differences[41]. In the same study, RHA/Verh rats showed less of an increase in corticosterone, ACTH and prolactin than did RLA/Verh rats when exposed to the novel environments. With the stronger stresses, however, equally high hormone levels (probably a 'ceiling' effect) were found[41]. The differences described above clearly reflect a dissimilar response to novel environments[40,41].

4.2 SHR and WKY rats

In accordance with most previous reports[24,66,72], we found SHR to be significantly more active in the openfield than WKY rats[40,50]. Others have found a reduced activity for SHR (vs WKY rats) upon initial testing in an openfield, which gradually turned into hyperactivity in subsequent trials[64,88]. No significant difference between SHR and WKY rats has been found for 'spontaneous' activity (refs 66, 71, and our unpublished data). As in previous studies[24,66,93], we have also observed a highly significant interstrain difference in defecation during openfield tests (SHR < WKY)[40]. Variable patterns in basal and stress-induced corticosterone secretion have been observed in SHR, as compared to normotensive rats[25,73,77,94,110]. In our laboratory, SHR and WKY rats did not differ in basal corticosterone plasma concentrations. A 10-min openfield test, however, induced a significantly higher rise in the WKY rats[40]. Most recently, we have observed that SHR more frequently enter the open arms of an elevated plus maze than do WKY rats and this reflects, once again, the dissimilar level of fear (emotionality) in these two groups of rats[50].

4.3 Individually and group-housed Wistar rats

The most prominent behavioral change induced by individual housing is the locomotor hyperactivity observed upon exposure to a novel environment[36,107]. An initial hypoactivity changing, upon repeated exposure to an openfield, to hyperactivity, has also been noted[22]. Our individually housed rats showed a markedly increased locomotor activity in an openfield[42], even throughout the whole L12:D12 cycle[43]. On the other hand, 'spontaneous' activity has been noted to be either slightly reduced in individually housed rats[38] or not[43], depending upon the method of measurement used (with no circadian differences seen in either study). In agreement with Syme[99], who could not establish any housing effect in female rats, others have indicated that there was no significant difference in spontaneous activity between individually and group-housed rats, once the initial hyperactivity (reaction to the novel environment) had disappeared[22]. In contrast to the quite equivocal data in the literature[52,97,107], in several types of experiments we have found a significantly lower defecation rate for individually housed rats than that seen in group-housed controls[40,42], as well as a more moderate increase in plasma corticosterone levels following a 10-min openfield trial[40].

4.4 Additional comments: "Of rats . . .

Locomotor activity, defecation and corticosterone levels exhibited by a rat in response to a novel environment have been said to depend upon its 'emotionality'. We agree with Gray[54] that "whatever else anxiety is, it is undoubtedly an emotion" and that "it is a moot point whether 'anxiety' or 'emotion' is the more opaque concept." We therefore hesitate to consider fear and emotionality as two independent entities. Two strains of rats, the Maudsley Reactive (MR) and Maudsley Non-reactive (MNR) rats, have been selectively bred for differences in emotionality[17,19], i.e., selected

Inter- and intra-pair comparisons for emotionality, assessed in the openfield.

	RHA/Verh vs RLA/Verh		SHR vs WKY		Isolated vs grouped Wistar	
	Our data	Reference data	Our data	Reference data	Our data	Reference data
Locomotor activity	>	>: 7, 29, 74	>	>: 24, 66, 72 (64, 88)	>	>: 36, 107 (22)
Defecation score (openfield test)	<	<: 29	<	<: 24, 66, 93	<	<: 97 =: 107 >: 52
Corticosterone (post openfield)	<		<	>: 94 =: 66, 73, 110 <: 25, 73, 77	<	
Emotionality	<		<		<	

RHA/Verh: Roman high-avoidance rats; RLA/Verh: Roman low-avoidance rats; SHR: Spontaneously hypertensive rats; WKY: Wistar-Kyoto rats (normotensive rats); Isolated Wistar: individually housed Wistar rats; Grouped Wistar: group-housed Wistar rats.

and bred for extreme differences in defecation scores when in an openfield. Concomitant differences in locomotor activity and corticosterone levels have also been described. According to openfield data, RHA/Verh, SHR and individually housed Wistar rats strikingly resemble the (less emotional) MNR rats, when all are compared to their respective counterparts. Therefore, despite different starting points (selection for extremes in avoidance behavior or blood pressure, or manipulation of a single environmental parameter), the behavioral and hormonal data presented lead us to conclude that all of these divergent rat pairings are comparable with regard to fear and emotionality (see table).

Another interesting comparison concerning these rat models, which may be a direct consequence of the similarities in their hormonal response to stress, concerns thymus weight. We have seen a prominent difference between the two Roman rat lines (both males and females), with the thymus weights of RHA/Verh rats being as much as 60% higher than those of RLA/Verh rats. We have similarly observed that SHR exceed WKY rats with respect to thymus weight (+ 18%, also unpublished data), and this confirms a previous report by Yamori[109]. A further (unpublished) result from our laboratory indicates that individually housed rats have heavier thymus glands after 12 weeks (+ 12%) and after 18 weeks (+ 28%) of isolation than do group-housed rats. These parallel differences observed in regard to thymus weights are very probably meaningful, as it is known[105] that increased levels of circulating adrenal hormones diminish the size of this organ. Such higher circulating hormone levels may well be present more frequently in RLA/Verh, WKY and group-housed Wistar rats, as these three groups have all been shown to react with an increased hormonal response to low-stress situations (see above). Whether the differences in thymus weight might have some further, immunological, consequences is unknown at present.

. . . and Men".

It might be questioned from the outset whether inhibited locomotor activity and defecation scores, as exhibited by a rat while exposed to a simple quadrangular test box (the openfield), could provide any useful information regarding the human situation. We believe that these signs of momentary fear are shared by both species. For example, becoming motionless and, especially, an urgent need to relieve the bowels can similarly be observed in humans upon being exposed to various stressful situations. Also, the more stressful such situations appear to an individual, be it animal or human (and probably depending upon genetic susceptibility to stress), the stronger will be this type of reaction.

Various degrees of anxiety are frequently noted in certain psychiatric conditions, such as depression, schizophrenia and alcoholism. One of the major causes of anxiety is probably an inability to cope successfully with one's environment. In many patients there appears to be a permanent conflict between the vital desire to remain in contact with the environment and the fear of possible negative feedback coming from the same environment. Here we also see striking parallels between humans and experimental animals. In rats which are exposed to a novel environment (e.g., openfield), the conflict is between innate drives to explore the novel environment and, at the same time, to avoid threatening situations. The final behavioral pattern of the animal, resulting from these divergent drives is, as is the case for humans, specific for the individual, depending upon its subjective appraisal of the situation. The latter, in turn, depends upon the individual's previous experience and genetic predisposition. In both humans and experimental animals, anxiolytic drugs can alleviate anxiety leading, in humans, to 'relaxation' and, in rats, to an attenuated avoidance of adverse environments, reduced secretion of corticosterone, etc.

5. *Receptor binding studies*

Receptor binding studies provide important information relating to the chemical nature, the localisation and the function of pre- and postsynaptic recognition sites. As access to the human brain is limited, clinical studies have generally been confined to the use of biological material obtained at autopsy. Recent methodological developments such as positron emission tomography, or nuclear magnetic resonance (which work under in vivo conditions), however, are devoid of such disadvantages as post-mortem artefacts but are, at the same time, very expensive to operate.

Determination of receptor binding in the periphery represents an alternative, clinically relevant, approach which has recently come into frequent use. Binding studies on several types of blood cells and skin cells have been undertaken on the assumption that peripheral and central binding sites may be similarly regulated, therefore making it possible to monitor central events in the (more easily accessible) peripheral cells. For example, the density of ^{3}H-spiroperidol binding sites (D_2-receptors) on lymphocytes has been proposed to represent a possible marker for schizophrenia[12], and ^{3}H-imipramine binding sites and alpha$_2$-adrenergic binding sites on platelets have been put forward as markers for depression[31].

In addition to the expense and/or methodological problems involved, clinical binding studies are also difficult because drug-free patients or homogeneous patient-populations are rare. Thus, when one is interested in the relationship between specific binding sites and behavioral abnormalities, comparative studies between behaviorally-distinct groups of experimental animals can be carried out. Such receptor analyses (as well as other neurochemical determinations) in experimental animals have the following main advantages, when compared to clinical studies: a) environmental variables, such as the time of day, nutrition and life experiences are easier to control, b) experimental groups can be larger and more homogeneous with regard to genetic background and age and, most important, c) brain and peripheral samples are readily

and simultaneously available, thus making direct comparisons between the two different tissues possible.

Of the many binding sites which are determined today, some have been postulated to be particularly relevant to psychiatric questions, when it became evident that clinically effective drugs affected either their number or affinity. We will concentrate here on two distinct receptor types, namely benzodiazepine and imipramine binding sites. These binding sites represent relevant recognition sites for anxiolytics (benzodiazepine site) or antidepressants (imipramine site). It should, none the less, be emphasized that other receptors (e.g. serotonergic, dopaminergic, cholinergic, alpha$_1$-, alpha$_2$- and beta-adrenergic) have also been shown to be affected by clinically effective drugs.

5.1 Benzodiazepine binding

Benzodiazepines were introduced into medicine as tranquilizers some 25 years ago and soon became the most widely prescribed drugs. Their anti-anxiety effect is well documented in various species, and there is no sign of quantitative or qualitative changes in the nature of anti-anxiety drug action as one approaches, phylogenetically speaking, our own species [54]. In rats, benzodiazepines increase the number of punished responses in various conflict situations [37, 106], and effectively reduce the fear of entering adverse environments (e.g., the open arms of an elevated plus maze [50, 82]) or a well-illuminated arena [74]. While exposed to a novel environment (e.g., an openfield), drug-treated rats also show increased locomotor activity and an attenuated secretion of corticosterone [34, 50].

With the discovery of binding sites for benzodiazepines in the brain [75], a new dimension in research dealing with stress and anxiety was introduced. These binding sites were shown to be of high affinity, saturable and to have a distinct regional distribution, thus fulfilling the criteria necessary for relevant binding sites. Central benzodiazepine receptors are thought to form part of a GABA receptor, benzodiazepine receptor and chloride channel complex, and the benzodiazepines are thought to increase the affinity of the GABA receptor for GABA and its effect on the chloride channel [57]. It should be noted that anxiolytic efficacy observed in the clinic correlates well with the affinity of a drug for these benzodiazepine binding sites and, therefore, it is generally agreed that these central receptors are related to the anti-anxiety action of the benzodiazepines [75].

It was, therefore, not surprising when benzodiazepine binding was compared between two groups of rats known to differ in fear [86]. Using ^{3}H-diazepam as ligand, a higher binding was found in the less fearful MNR strain, as compared to the more fearful MR strain. Interstrain differences (maximum 20%) were present in all nine CNS-subregions studied. Subsequently, the same laboratory determined that 'emotional' mice (Balb b/cJ) had a significantly lower benzodiazepine binding than any of the three 'non-emotional' strains (AKR/J, C57BL/10J, C57BL/6J) studied [87]. Since, in this second study, Scatchard analyses had been carried out (using whole brain homogenates), it was further demonstrated that the difference in ^{3}H-diazepam binding was due to an altered maximal number of binding sites (B_{max}) rather than to a different affinity (K_d). With the behavioral similarities among MNR, RHA/Verh, SHR and individually housed Wistar rats in mind (higher locomotor activity and less defecation, when exposed to an openfield, than their respective counterparts), we decided to determine benzodiazepine binding in some of these animals.

5.1.1 RHA/Verh and RLA/Verh rats

For all 7 brain regions studied, a higher ^{3}H-diazepam binding (ligand concentration 3 nM) was established for the RHA/Verh rats, with the interline differences reaching significance in cortex, striatum and hippocampus [39]. These differences between the Roman rat lines, which were replicated, were in good agreement with the data obtained for the Maudsley strains and, therefore, may be added to the other parallels between RHA/Verh and MNR rats, as compared to RLA/Verh and MR rats, respectively [26].

Determinations of benzodiazepine binding in another stock of RHA and RLA rats have also been published [90]. Using 1.0 nM ^{3}H-flunitrazepam, those authors observed a sex-, but no line-difference between their RHA and RLA rats. The apparent discrepancy between that study and ours is probably due to experimental differences. We used naive animals and our rats were killed in the middle of the dark phase of an L12:D12 cycle, whereas the rats used in the other study had previously been used in behavioral tests (avoidance conditioning, food deprivation), and were killed 13 h after lights on, during an L18:D6 cycle (which is an unusual lighting regimen for this type of experiment). Furthermore, according to their description, those authors had apparently frozen whole brains in solid CO_2 and thawed them out, prior to dissection into subregions, at another laboratory (it did not appear that a punching system had been used on the still-frozen brains). In a subsequent paper, however, the same authors still found no support for an association between the number of benzodiazepine receptors and emotionality, in an investigation of genotypes derived from their Roman lines of rats [91].

5.1.2 SHR and WKY rats

We have not compared benzodiazepine binding between SHR and WKY rats, but the data presented in the literature can be summarized as follows: Thyagarajan et al. [101] found no significant difference between SHR and WKY rats for B_{max} and K_d of ^{3}H-flunitrazepam binding sites in cortex, cerebellum, pons-medulla and some peripheral organs. Even upon extending such comparisons to different ages (3, 10 and 20 weeks old) no significant strain differences could be established in cortical and cerebellar membranes [103]. In contrast to these reports, Hambley et al. [59], using ^{3}H-clonazepam, found a lowered binding in hypothalamic samples of SHR when compared to WKY rats. With the possible exception of the hypothalamus, therefore, there appears to be no noteworthy difference in benzodiazepine binding between SHR and WKY rats.

5.1.3 Individually and group-housed Wistar rats

We did not find any significant difference in ^{3}H-diazepam binding between individually and group-housed Wistar rats, when employing the same experimental procedure as that previously used for the Roman rat lines (unpublished observations). Petkov's group, upon comparing their individually and group-housed rats, found a significantly reduced ^{3}H-flunitrazepam binding after isolation [84]. They further reported that in isolated-non-aggressive rats the reduction was significant in the hippocampus and cerebellum only, whereas in isolated-aggressive rats (21% of all isolated rats) a significantly reduced ^{3}H-flunitrazepam binding became apparent in the cortex, hippocampus, midbrain and cerebellum. Since such observations would indicate that alterations in benzodiazepine binding are more prominent in isolated-aggressive rats, it should be added that under our housing conditions muricidal behavior (used by Petkov et al. as a criterion for aggression) was extremely rare.

Comparative studies between individually and group-housed mice have also yielded contradictory results. Whereas Braestrup et al. [13] observed no change after individual housing, Essman and Valzelli [32] reported a reduction in the B_{max} of benzodiazepine binding sites in some brain structures of isolated mice. Most recently, Tyutyulkova et al. [104] de-

scribed a reduction in the B_{max} and K_d of benzodiazepine binding sites in the brain of isolated, aggressive mice and further showed that either a single, or chronic, treatment with medazepam had an anti-aggressive effect, leading to a restoration of the B_{max}.

5.1.4 Additional comments

Upon critically examining the benzodiazepine binding data for all of these groups of rodents, it becomes obvious that there is no absolute linkage between benzodiazepine binding and fearfulness (emotionality). Only those results obtained in RHA/Verh and RLA/Verh rats [39], and those obtained in the original studies [86, 87], support the hypothesis that benzodiazepine binding might be inversely related to fear. Recent results by Tamborska et al. [100], however, have also questioned the original results for the MR and MNR rats. Those authors, upon comparing central and peripheral benzodiazepine receptor binding between their MNR and MR rats, could not find any interstrain difference for cerebral cortex, cerebellum, hippocampus or medulla-pons. Furthermore, Drugan et al. [28], using a specific ligand for peripheral benzodiazepine binding sites (Ro-5-4864), found reduced binding in the heart and kidney of MR rats, but not in other peripheral tissues or in cortex, hippocampus and hypothalamus.

A difference in benzodiazepine binding between RHA/Verh and RLA/Verh rats might, on the other hand, be related to a differential sensitivity toward chlordiazepoxide [74], although an association between an animals' sensitivity toward benzodiazepines and the number of benzodiazepine binding sites has also been brought into question. In a recent study [81], 400 Sprague Dawley rats were trained in a two-lever operant chamber to respond only during the non-punished period. Each rat's number of positive responses during the punishment period, after oral chlordiazepoxide, was recorded as an index of drug sensitivity. Subsequently, those rats showing a low or high response to the drug, respectively, were compared with regard to diverse receptors. Both 'responders' and 'non-responders' showed similar ^{3}H-diazepam binding in cerebral cortex and cerebellum. In the hippocamus, however, 'non-responders' showed a higher ^{3}H-diazepam binding than 'responders', seemingly emphasizing the possible importance of this region in the action of the benzodiazepines.

5.2 Imipramine binding

High affinity binding sites for ^{3}H-imipramine have been detected in the brain of various species, including man. Pharmacological studies have indicated that these binding sites are related to the 5-HT-uptake mechanism [67], and that the regional distribution within the CNS parallels endogenous 5-HT-levels [80]. ^{3}H-imipramine also binds saturably, and with high affinity, to membranes prepared from blood platelets, coinciding with previous findings that platelets take up, store and metabolize serotonin in a similar manner to synaptosomes or brain slices [95]. It has been observed that ^{3}H-imipramine binding sites in thrombocytes are of the same kind as those detected in the CNS [14] and that both platelet- and CNS-sites can be concomitantly modified (e.g. chronic treatment with imipramine in cats has been found to down-regulate these binding sites to the same degree in both tissues [15]).

It has therefore been postulated that centrally occurring alterations in the B_{max} or K_d of ^{3}H-imipramine binding sites might be mirrored in (the more easily accessible) blood platelets. Given that disturbances in the 5-HT-system are thought to be involved in depression, and that 5-HT-uptake blockers are clinically effective in alleviating depressive symptoms, the B_{max} of these binding sites in depressed patients and healthy controls has been compared in several studies. The few studies comparing ^{3}H-imipramine binding sites in the brain (post-mortem tissue) suggest a reduced binding for depressed partients [83] and for suicides (96, as opposed to 6), when compared to controls. For platelets, some of the initial studies reported a reduced B_{max} of ^{3}H-imipramine binding sites in depressed patients. However, other, more recent reports, including our own [47, 51], could not confirm such a reduction. Surveying the data published until now, Bech et al. [8] have recently stated that "14 of the 26 studies found a significant decrease (10–50%) in depressed patients compared with normal control persons, whereas 12 of the studies found non-significant changes or slightly elevated values." So it remains an open question at present as to whether the B_{max} of platelet ^{3}H-imipramine binding sites indeed represents a valuable marker of depression. For more extensive overviews on the relationship between ^{3}H-imipramine binding, 5-HT-uptake and depression we refer the reader to two recent reviews [16, 69].

Other questions regarding ^{3}H-imipramine binding concern a) whether the number of these binding sites represents a state-dependent parameter (e.g. a lowered density in depressive states with a subsequent normalization while recovering), and b) whether the density of these binding sites could be genetically determined (possibly indicating a vulnerability to depressive episodes). Clinical data here are, once again, controversial [10, 68, 98], and the few twin studies carried out so far are contradictory [5, 35]. Animal studies could be of particular help in clarifying whether ^{3}H-imipramine binding could be under some genetic control. Comparative studies between genetically distinct groups of experimental animals have so far been neglected, however, with few exceptions. ^{3}H-imipramine binding has been compared between Fawn-Hooded rats (which have a hereditary platelet 5-HT-storage pool deficiency) and some more common strains (Sprague-Dawley, Long Evans). One laboratory was unable to detect any platelet binding [30], another found a reduction in density [4] and a third was unable to establish any strain-specific difference in brain ^{3}H-imipramine binding between Long Evans and Fawn-Hooded rats [62]. Hoping to find models which differ in ^{3}H-imipramine binding sites, we have compared our groups of rats using brain and, in two instances, platelet samples.

5.2.1 RHA/Verh and RLA/Verh rats

Scatchard analysis revealed, for RHA/Verh rats, a significantly higher B_{max} of ^{3}H-imipramine binding sites (+ 32%) than for RLA/Verh rats, and no significant interline difference in K_d, when using whole brain homogenates [44]. In a subsequent analysis, a higher ^{3}H-imipramine binding (at 2.5 nM ^{3}H-imipramine) in RHA/Verh rats was found in all central subregions tested, with the exception of the hypothalamus, which showed the opposite results. The interline differences, between 8% and 18%, reached a significant level in cortex, striatum and hypothalamus [45]. Using six different ligand concentrations in an additional set of rats, cortical, striatal and hippocampal densities of ^{3}H-imipramine binding sites were, once again, higher in RHA/Verh rats, with no differences in K_d; the hypothalamus, once again, was the exception (RLA/Verh > RHA/Verh) (unpublished observations). Scatchard analyses in platelets revealed absolutely no difference between the two Roman rat lines (unpublished observations).

5.2.2 SHR and WKY rats

Previous reports have indicated that SHR and WKY rats differ in some serotonergic parameters such as brain 5-HT-levels [88] and platelet 5-HT-uptake [85]. The latter difference has also been described in blood samples of hypertensive patients vs normotensive controls [11]. Based on the proposed relationship between 5-HT-uptake and ^{3}H-imipramine bind-

ing, on the one hand, and between the endogenous level of 5-HT and the density of ^{3}H-imipramine binding sites, on the other hand [80], we hypothesized that SHR and WKY rats might differ in the B_{max} of these binding sites. Our results for ^{3}H-imipramine binding in whole brain homogenates and blood platelets of adult SHR and WKY rats were identical with respect to both B_{max}- and K_d-values [46]. However, since no regional analysis was included, differences restricted to certain CNS-subregions, especially if they went in opposite directions, would not have been detected in that experiment.

5.2.3 Individually and group-housed Wistar rats

Using whole brain homogenates, Scatchard analysis for individually and group-housed Wistar rats did not reveal any significant housing effect [44]. Once again, potential differences in only one or a few subregions might have remained undetected. We know of only one other report which has compared ^{3}H-imipramine binding between individually and group-housed rats. Guisado et al. found, for isolated rats after 10 months of social deprivation, a higher ^{3}H-imipramine binding than in group-housed controls [56]. To the best of our knowledge, platelet ^{3}H-imipramine binding has not been compared between differentially housed rats.

5.2.4 Additional comments

The lack of difference in platelet ^{3}H-imipramine binding between SHR and WKY rats is somewhat puzzling in the light of the known interstrain difference for 5-HT-uptake [85]. Our results in experimental animals, however, are in line with a clinical study by Kamal et al. Those authors were unable to detect any difference in ^{3}H-imipramine binding when comparing platelet samples of hypertensive patients and those of normotensive controls [63].

The absence of a difference in platelet ^{3}H-imipramine binding between the two Roman rat lines, seen in the light of the dissimilar central binding repeatedly observed, casts doubt upon whether there is always an interrelation between the two tissues with regard to ^{3}H-imipramine binding. Discrepancies between central and peripheral ^{3}H-imipramine binding have already been observed by others. Ieni et al. [62], upon finding a similar brain ^{3}H-imipramine binding in Fawn-Hooded and Long Evans rats, questioned the usefulness of blood platelets as a model system reflective of CNS serotonergic activity. Also, Abel et al. [1], after treating rats with electroconvulsive shocks, observed an increase in platelet, but not cortical, ^{3}H-imipramine binding. They also concluded that the platelet and brain binding sites for ^{3}H-imipramine are not regulated in an identical way.

The opposite direction for the difference in ^{3}H-imipramine binding between the two Roman rat lines, which was observed in the hypothalamus when compared to other brain areas, needs some further consideration. Such a finding might be linked to the divergent emotional responses expressed by differential activation of the hypothalamic-pituitary-adrenal axis, as described previously [41], or it might be related to the particularly different serotonergic responses in the hypothalamus of these two lines of rats following shock stress [27], or both.

6. General discussion

Upon reviewing various behavioral and hormonal parameters in rats, comparable differences have been observed between RHA/Verh, SHR and individually housed Wistar rats, versus RLA/Verh, WKY and group-housed Wistar rats, respectively. The divergent locomotor activity, defecation and plasma corticosterone levels seen upon being exposed to a novel environment point to intrapair differences in emotionality (fear) in all three cases. Therefore, despite their various origins, namely a genetic selection for extremes in shuttlebox performance or blood pressure, or a variation in housing conditions, similar intrapair differences have been induced. Such findings for experimental animals are remarkably consistent with clinical observations, where genetic, as well as environmental (social), pressures are known to be triggering factors in psychopathology. Therefore, notwithstanding the common psychiatric practice of diagnostically differentiating endogenous (genetic) and reactive (environmental) forms, clinical symptoms shown by either classification may indeed be identical.

The search for concomitant differences in receptor binding, however, has yielded less impressive results. Differences, if present at all, have been only marginal, and neither of the binding sites reviewed here systematically differed in all pairings of rats. Thus, no absolute relationship between the binding sites and emotionality was found. In pharmacological studies, on the other hand, one has become accustomed to larger differences in receptor binding. After the administration of drugs or neurotoxins, or after lesioning distinct brain regions, marked alterations in different receptors have been observed. However, a loss in neurons is induced by some of these interventions, and the animal is often seriously affected behaviorally. The changes possibly underlying emotional disturbances are, however, unlikely to be accompanied by such dramatic neurochemical alterations, and it is certainly inappropriate to assume that only a large difference in the density of receptors might be meaningful. Hirsch, exemplifying this for the benzodiazepine receptors [61], showed that only 10–20% of the sites needed to be occupied by diazepam in order to prevent pentylenetetrazol-induced convulsions, or to exhibit anticonflict activity. Thus, small changes in diazepam binding could have a marked effect on the action of the drug.

In addition, receptors are unevenly distributed throughout the CNS. Thus, assuming that an alteration in the B_{max} or K_d of a receptor is restricted to only some brain subregions (or nucleii), one risks overlooking such small, but possibly relevant, changes when studying larger brain areas. Furthermore, receptors represent only one of the many elements responsible for the maintenance of neurotransmission. It is, therefore, not imperative that any change in neurotransmission needs to be reflected in an alteration at the receptor level, since a malfunctioning system could already have been counterbalanced by up- and down-regulation of other parts of the system (synthesis, release, degradation etc.), or even by another system. Finally, circadian peculiarities should also be considered because, as has been recently emphasized, differences in receptor binding between strains may vary depending upon the time of day [108].

Although many methodological and statistical problems will be encountered when trying to elaborate small differences in receptor binding between non-pharmacologically manipulated groups of experimental animals, we feel that such differences are more applicable to the clinical experience. To mention ^{3}H-imipramine binding as an example, the differences between depressed patients and healthy controls, if present at all, have been around 10–20% in most studies [8]. Behaviorally speaking, however, these groups show pronounced differences. As a consequence, animal models revealing small differences in that parameter, e.g. learned helplessness in rats which produces a reduction of 25% [92], are superior to those describing large differences, e.g. administration of a neurotoxin (5,7-DHT) which produces a reduction of up to 80%, depending upon the brain subregion [20]. An alternative approach to those discussed in this review would proceed in the opposite direction and determine, after selective breeding for extremes in a neurochemical parameter, behavioral correlates. Obviously, for such an approach, the animals would have to survive the chemical analysis, which has become practicable only recently with the intro-

duction of new techniques such as brain dialysis, positron emission tomography and nuclear magnetic resonance. Secondly, when peripheral and central binding sites are known to be identical and similarly regulated, it should become possible to select animals according to the density of some peripheral binding sites (e.g. ^{3}H-imipramine sites on blood platelets or D_2-binding sites on lymphocytes) prior to breeding. Observation of later generations might then point out which behavioral peculiarities may be attributable to a given neurochemical parameter.

A further approach may be illustrated by work recently done with SHR and WKY rats. As these two strains have been bred for many generations for differences in blood pressure, it might be questioned whether the subsequent behavioral and neurochemical peculiarities noted between SHR and WKY rats are not merely a coincidence. Hendley et al. [60], using an elegant experimental design, have provided the information that hypertension and hyperactivity are not directly interrelated, genetically. Starting with an original cross of SHR and WKY rats, those authors produced a gene-segregating F_2-generation out of which, by inbreeding selected brother/sister pairs, a new strain of hyperactive rats with normal blood pressure was produced [76]. By additionally incorporating such a strain into neurochemical and further behavioral (directed toward emotionality/fear) comparisons between SHR and WKY rats, it could be determined which neurochemical alterations in SHR rats are linked to the high blood pressure and which are more likely to be related to the locomotor activity. A similar breeding technique with the Roman rat lines (and other selectively bred stocks) would, obviously, also be of much interest.

As a final remark, we would like to emphasize that mental disturbances in humans, under most circumstances, are induced by non-pharmacological causes and that psychopharmacological agents exert their beneficial action in patients, but not in healthy controls. For example, antidepressants, when taken by a healthy person, do nothing more than to induce fatigue. In depressed patients, however, the same drug and dose alleviates depressive symptomatology. For animal experimentation this has the following consequences: Genetically- and/or environmentally-manipulated models should be preferred to pharmacological models, i.e. it is closer to the clinical situation when a pharmacological effect is observed in animal models with the appropriate 'abnormal' initial behavioral patterns, such as the examples described in this review.

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