Stress and Emotionality:
a Multidimensional and Genetic Approach

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RAMOS, A. AND P. MORMÈDE. *Stress and emotionality: A multidimensional and genetic approach.* NEUROSCI BIOBEHAV REV. **22**(1) 33–57, 1998—The use of behavioural tests aiming to assess the psychological components of stress in animals has led to divergent and sometimes arbitrary interpretations of animal behaviour. This paper presents a critical evaluation of behavioural methods currently used to investigate stress and emotionality. One of its main goals is to demonstrate, through experimental evidence, that emotionality may no longer be seen as a unidimensional construct. Accordingly, following a discussion about concepts, we propose a multiple-testing approach, paralleled by factor analyses, as a tool to dissociate and study the different dimensions of emotionality. Within this multidimensional context, genetic studies (illustrated here by different rat models) are shown to be particularly useful to investigate the neurobiology of stress/emotionality. A genetic approach can be used (i) to broaden and dissect the variability of responses within and between populations and (ii) to search for the molecular bases (i.e. genes and gene products) which underlie such a variability. © 1998 Elsevier Science Ltd. All rights reserved.

Stress   Emotionality   Anxiety   Factor analysis   Genetic models   Interstrain differences   Behavioural tests

1. INTRODUCTION

THE CONCEPT of stress has been the object of a countless number of studies involving a wide range of different approaches, each one presenting its own way of interpreting and assessing the phenomenon. Besides its great diversity, the literature on this subject often associates stress to concepts of psychological nature such as well-being, suffering, emotionality, fearfulness and anxiety. These terms are frequently applied as being interdependent and sometimes as describing the same phenomenon, even though their interpretation is not widely standardized. Since most of these concepts involve a subjective component, researchers are challenged by the lack of direct measures, being therefore obliged to infer such psychological states from their measurable manifestations (physiology, endocrinology, behaviour, etc). As a result, different research groups develop their studies on the basis of a number of assumptions that are often divergent and sometimes arbitrary. For instance, a given behavioural measure (e.g. locomotion in novel environments) can lead to different interpretations regarding its psychological significance, depending on who uses it and in what context it is being applied.

It is not the aim of this paper to present an exhaustive review of publications on this subject. Its main goal is to critically discuss, based on experimental evidence, the validity of some methods currently used to investigate stress. We shall first try to clarify the differences and overlaps among a few relevant concepts. Secondly, we shall analyse the consistency among different measures used to quantify stress or emotionality (with emphasis on behavioural tests for rats), regarding their respective significance. Moreover, we shall demonstrate the importance of revising an assumption that has prevailed (explicitly or not) for more than 60 years of research and which considers emotionality, fearfulness and anxiety as different denominations for the same unidimensional construct. Thus, the use of a multivariate approach paralleled by genetic studies in animals is proposed as a tool to reveal and dissociate different dimensions of emotionality. The dissection of this concept should help us to better understand the different biological systems as well as the molecular mechanisms which underlie stress and the interindividual variability of stress-related measures.

2. THE CONCEPTS

Before starting any discussion about stress, it is necessary first to define our interpretation of the term. In this paper, stress is considered as the response of an organism to environmental stimuli (stressors) which threaten its internal equilibrium, also called homeostasis. Such stimuli, which are perceived and evaluated by a cognitive/emotional system,

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may induce a variety of neuroendocrine, metabolic and behavioural changes in an attempt to maximise the probability of success over a demand. Once the intensity of the challenge reaches a level beyond which the specific homeostatic mechanisms (efficient under ordinary circumstances) may no longer ensure the maintenance of the internal equilibrium, a series of non-specific adjustments occur. The attainment of this non-specific stage of response may also result from a high emotional activation, produced by the interaction of environmental and psychological factors (e.g. stimulus intensity, perception/evaluation of the challenge and chances of adaptation). The intensity and the nature of the response depend on the characteristics of each individual and their influences on the individual's health and well-being depend on the efficiency of the coping process. Evidence pointing to harmful effects of stress on the immune system, the neuroendocrine balance, the well-being and several pathological states of humans and animals, has led scientists of different disciplines to investigate the biological processes underlying stress.

Submitting groups of subjects to different types of stressful conditions has revealed a great variability of response among individuals from the same species (for a review see (40)). This interindividual variation has been demonstrated for several species of animals and for humans, involving different aspects of the stress response (20,116,122,142, 149,164,201,209). Although it is generally accepted that such variability is caused by both genetic and environmental factors, little is known about its biological and molecular mechanisms of control. Studies providing this kind of knowledge will certainly improve the current methods of prevention and treatment of stress-related disorders.

Lazarus (143) has proposed that stress should be considered as part of a larger topic called emotions, but this approach, according to the author, is not adopted by most scientists. Terms such as ''emotion'', ''emotionality'' and ''emotional response'' are widely used in the literature, not only by psychologists but also by scientists working in neighbouring areas. The interpretation of these concepts, however, is far from being unequivocal.

From the Latin *emovere* (*e* = out, *movere* = to move), the word ''emotion'' evokes by its origin the idea of ''moving out'', in the sense of agitation or perturbation of the psychological state. The definition of ''emotion'' by Webster's Dictionary is closely associated with some definitions of stress itself: ''a psychic and physical reaction subjectively experienced as strong feeling and physiologically involving changes that prepare the body for immediate vigorous action''. Such an interpretation is not far from the one adopted by psychologists and neuroscientists, who consider emotion as a ''particular state of an organism facing well defined conditions (a so-called emotional situation) which is coupled with a subjective experience and with somatic and visceral manifestations'' (67). Hall (107), one of the first researchers to study emotionality in animals, considered the term as being related to the behavioural and peripheral changes hypothesized to accompany high sympathetic nervous system activity. It is interesting to note that the involvement of the sympathetic nervous system in the maintenance of the homeostasis played a central role in the primordial concepts of stress, as those elaborated by Walter Cannon (38). Since comparative psychologists started to develop behavioural models to measure the

emotionality of animals (mostly rodents), different interpretations of the term have appeared in the literature (see (6)). Nevertheless, most definitions of emotion and emotionality share the idea of a subjective experience (something in the field of the ''feelings'') associated to behavioural/physiological changes which are generated by non-ordinary situations.

The concepts of stress proposed by Cannon and Selye (see (38,200)) have evolved significantly in the past few decades. Rather than a unidimensional response profile, stress is currently seen by some authors as a multidimensional phenomenon. Lazarus (143) makes a clear-cut distinction between physiological and psychological stress and he classifies psychological stress in three categories: harm, threat and challenge. Regarding emotions, a variable number of classes are also recognized by different authors, such as fear, anger, anxiety, happiness, relief, sadness, shame, pride, etc. (67,143). The range of emotions experienced by non-primate animals, however, is thought to be much less complex than that, including only the states of fear, joy and anger (163). Even though different types of emotions are recognised, studies on animals have traditionally evaluated emotionality under experimental conditions where only some fear-like emotion is expected to be experienced (6,103). In this specific context, therefore, terms such as ''emotional responses'' shall be more specifically interpreted as ''fear responses''.

Anxiety is, as mentioned above, one of the classical types of emotion which has probably been experienced by all of us several times in life. By definition, anxiety is the emotional anticipation of an aversive situation, difficult to predict and control, which is likely to occur (67). In spite of fear—defined as the reaction to a dangerous situation which is already real and well defined (29)—being seen by some authors as independent from anxiety (9), the distinction between these two concepts is difficult. In the clinical field, a confusion between anxiety and fear may originate from some psychiatric classifications of mental disorders. The American Diagnostic and Statistical Manual of Mental Disorders (DSM), for example, considers that the different types of phobias (all fear-related) are subclasses of the so-called anxiety disorders (144).

It can be seen, through this brief discussion, that there is a remarkable overlap among the current notions of emotionality (which is often used as a synonym of fearfulness), stress and anxiety. For example, one of the three types of psychological stress defined by Lazarus (143), namely ''threat'', corresponds essentially to the emotion of anxiety, as defined above.

In this paper, for the sake of simplicity, the term anxiety will be used mostly with its pharmacological meaning (even though we recognise the limitations of this sole approach), that is, an emotional state behaviourally expressed under aversive environmental conditions, that can be attenuated or enhanced by the specific administration of anxiolytic or anxiogenic drugs, respectively. Also for the purpose of this paper, the emotional state of an individual will be considered as the central state of consciousness (involving a subjective component) during stress. Being a part of the stress mechanism, hence, the emotional state can simultaneously affect and be affected by all the behavioural and neuroendocrine changes. Note that a high level of stress assumes here an implicit idea of a high level of emotional activation. By presenting our personal (and probably not

definitive) interpretation of terms such as anxiety, emotional state and stress, we have defined a set of concepts that shall guide the further discussion in this paper.

3. SEARCHING FOR DIFFERENCES

The search for the biological bases of interindividual differences in stress implies, of course, that one must be able to reveal and quantify these differences. Nevertheless, quantifying stress is one of the common difficulties faced by researchers and it has been often an object of controversy. Historically, several approaches (e.g. neuroendocrinological, behavioural, pathological/physical, etc.) have been adopted in the attempt to measure stress levels (see (89,166,200)). Variables related to the activity of the hypothalamo–pituitary–adrenocortical (HPA) axis and to the autonomic nervous system may be changed, following the initial stages of stress, in a non-specific way (i.e. one response for different classes of stimuli). These neuroendocrinological parameters, therefore, measured in different conditions of acute or chronic stress are usually considered as adequate indicators of stress level. As far as behaviour is concerned, some applied ethologists (89) have suggested that certain behaviours displayed in the animal's habitual environment (e.g. feeding, general locomotion and occurrence of stereotypies) can give an idea of the state of chronic stress of an animal. In acute situations, on the other hand, animals are usually exposed to a stress-provoking situation and their behaviour is observed. However, this type of observation alone is meaningless if specific behavioural responses are not firmly associated with stress level and with the emotional state of the animals.

The task of establishing associations between ''peripheral'' outputs (neuroendocrine or behavioural responses) and ''central'' emotional states may raise a preliminary question: ''is it possible to assess (quantify/qualify) subjective experiences in animals?''. Indeed, even in the case of humans, the answer to that question is not an easy one. As pointed out by McFarland (163): ''How can we know what our feelings, themselves, really are? And, how can we know what another person's feelings are?''. Difficulties are pointed out by the author for each of three approaches considered (verbal expression, physiological and behavioural), leading him to suggest that it is wiser to study the animals' manifestations as such without trying to get to their underlying emotions. In the study of stress and emotionality, however, the measurement of behavioural and neuroendocrine variables which typically change in the presence of stressful situations, in spite of all difficulties, is the only tool available and it is the approach commonly adopted to assess the level of emotional activation of an individual. These variables should change, in an emotional situation, with a higher frequency and intensity than they would do in normal non-stressful conditions (29). Defining the variables to be used, how to measure them and how to interpret them is a crucial, though not simple, step. The validity and significance of some of the behavioural parameters currently used in the research on emotionality will be a major subject of discussion in this paper.

4. BEHAVIOURAL TESTS

A wide variety of behavioural tests have been developed throughout this century to characterize laboratory animals (mostly rodents) in relation to their responses to stressful or emotional situations. Several reviews on this subject can be found in the literature (6,78,79,103,143,147,175,184,216). Essentially, the different models involve the exposure of the animals (for a variable amount of time) to one or several aversive stimuli, with the simultaneous observation of their behaviour. The aversive stimuli may vary in nature from being physical (e.g. extreme temperatures, electric shocks, food deprivation, submersion in water, etc.) to those considered to be mainly psychological (novel environments, strongly illuminated areas, open spaces, heights, social instability, etc). An additional variable is the animal's ability to avoid the aversive stimulus. In some cases, no choice is offered, while in others, the animal can choose between approaching or avoiding the stimulus.

In the field of comparative psychology, the first animal models appeared in the early thirties and were named ''emotionality tests'' (107). Then, emotionality was often considered as a synonym of fearfulness. In 1957, the discovery of the first benzodiazepine which had strong anxiolytic properties opened a new area of research. In the pharmacology of anxiety, animal models became valuable (i) to study whether new compounds were endowed with anxiogenic/anxiolytic properties and, if so, (ii) to identify their mechanisms of action (79,216). In order to be validated as a model of anxiety, a behavioural test should allow the measurement of quantitative responses which vary in a consistent and predictable way in response to drugs with recognized anxiogenic/anxiolytic properties in humans. Such specific changes should not be similarly observed in response to other classes of drugs and should vary in opposite directions whether an anxiolytic or an anxiogenic substance is administered (79).

Treit (216) recognises three types of anxiety tests: (i) those based on unconditioned responses (e.g. exploratory, consummatory and social behaviours); (ii) those based on animal learning paradigms (e.g. conditioned active avoidance); and finally, (iii) those based on ''phylogenetically prepared forms of aversive learning'' (e.g. conditioned taste aversion). Tests based on learning or conditioning often involve the use of physically noxious stimulation, such as electric shocks and food/water deprivation. Such approaches have been frequently criticized in recent years. The need for long periods of animal training; the contaminating effects of pain threshold, appetite or thirst; the lack of behavioural and physiological validation and the growing ethical concerns in research are important drawbacks of this class of animal models (56,78,184).

In this paper, we will limit the discussion to some of the models based on unconditioned behaviours. Firstly, each test will be described briefly and the significance of its measures will be discussed on the basis of intra-test experimental evidence. In the next section, we will try, through a broader approach, to compare and correlate behavioural measures from a series of different tests.

4.1. The open field

This is one of the most widely used tests in behavioural research. Since 1934, when Calvin Hall published his work on emotionality in the rat (107), a countless number of studies have used the open field test to evaluate the effects of

environmental manipulations and genetic factors on the emotionality of rodents. The apparatus consists of a large arena (much larger than the home cage), where the animal is placed for a fixed amount of time, without having the chance to escape, since the area is surrounded by a wall. A number of different behaviours (see below) are quantified during the test and the floor of the apparatus is usually marked with lines (or equipped with photocells) to allow the quantification of locomotion. Although the open field is often considered as a standardized and reliable test (32), the literature shows a great variability in the testing conditions used by different authors. Differences in the form, colour, illumination level and recording methods (40,177,209,218,224) should be considered when comparing results from different laboratories. From its origins in comparative psychology, the open field test has gradually spread out to other areas of research and nowadays it is used not only for laboratory animals but also for pigs (170), chickens (227,228), quail (130) sheep and cattle (see (29)).

The classes of behavioural measures vary among studies, but they can include: ambulation, defecation, urination, freezing (resting immobile), grooming, jumping, rearing, time in the center, time to leave the center to the periphery, escape attempts, vocalization, etc. (6,224). Of all these variables, the two most commonly used and accepted as emotionality measures are ambulation (or locomotion) and defecation (103,115,147,177). The original view proposed by Hall (107) is that the fear response of an animal exposed to a novel, potentially dangerous environment is characterized by a high defecation rate caused by an activation of the autonomic nervous system. This initial view has evolved in the sense that a low ambulation also appeared as a main fear response of animals exposed to novelty (32,108,96,103). Therefore, according to this view, the level of emotionality of a rat would be positively related to the defecation scores and negatively related to the amount of ambulation during the open field test which, typically, is novel and brightly illuminated (147).

Some of the arguments supporting the use of these two measures as indices of emotionality are that: (i) several studies have shown negative correlations between defecation and ambulation for rats and mice (50,103,108,115,176); (ii) defecation increases by increasing the aversiveness (light or novelty) of the situation (127,165,177) and (iii) rats genetically selected for high defecation in the open field also show signs of high emotionality in other experimental situations (34). Moreover, several studies have shown that rats selected for emotionality-related measures from other types of tests show, concomitantly, the expected differences in defecation and/or ambulation in the open field test (35,76,93,94,96,97).

Some authors, however, have criticized the validity of the two aforementioned measures of emotionality. One of the main criticisms arose from a review by John Archer (6), who observed the following. (i) Behaviours not associated to emotionality, but rather to exploration, show the same response as defecation, that is, they decrease with repeated exposure to the same test situation (which is thought to be a sign of emotionality). Indeed, since ambulation is proposed as being negatively related to emotionality, this variable should increase with repeated exposure to the open field (inversely to what happens with defecation). Some studies, however, have shown the opposite, that is, both defecation

and ambulation decrease with habituation (127,179,221). (ii) Some selected strains differing in ambulation do not differ in their open field defecation (35), suggesting that the inverse correlation between defecation and ambulation is affected by strain factors. (iii) Animals may show active escape responses to novel environments (210), which makes high ambulation in the open field an inadequate measure of low emotionality or ''tranquillity''. To the main criticisms by Archer, it could be added that, whereas defecation can be increased by intensifying the aversiveness of the environment, ambulation has been shown either not to change or to increase with increasing light and noise levels (127). Moreover, previous electric shock, which is expected to increase the emotionality of rats and mice, actually increased the latency to ambulate without affecting total ambulation (both species) and defecation (rats) in the open field (210).

The effects of different types of drugs on the behaviour of rats in an open field test were assessed by Cunha and Masur (60). They found that ambulation, whereas increased by stimulants such as d-amphetamine and caffeine, was not changed by the anxiolytic diazepam which, in turn, significantly decreased rearings and increased immobility in some (but not all) experimental conditions. That some drugs may increase locomotion by mechanisms not related to the state of anxiety has led neuropharmacologists to consider ambulation in the open field as an unsuitable measure of anxiety (147,216). Indeed, whereas the anxiolytic chlordiazepoxide increased total locomotion (96), diazepam either did not change (low doses) or decreased (high doses) locomotion in the open field (87). Regarding defecation, it has been shown that chlordiazepoxide had no effect on this measure (96), whereas diazepam (for males only) and the adenosine analogue cyclohexyladenosine (for both sexes) decreased it (87). In this last study, perinatal exposure to caffeine, which has been suggested to increase emotionality, had no effect on open field defecation. Differences in testing conditions, previous experiences and/or genetic background may be responsible for some of these discrepant results. Nevertheless, such a variability of findings suggests that these open field measures, in spite of being seemingly affected by the emotional state of the animals, are not reliable indices of anxiety.

Another open field measure, which is not always considered in studies of emotionality, is the degree of approach/avoidance of the central area. As the arena's floor is normally divided in segments, some being in touch with the wall and some not, the general ambulation can be usually divided in ''central'' and ''peripheral''. It has been demonstrated (217) that rodents tend to avoid open spaces, probably because such environments prevent the animals from performing thigmotaxic behaviour (*thigma* = touch; *taxis* = orientational movement in relation to a stimulus). Consequently, in an novel open field test, animals tend to concentrate their ambulation in the peripheral area, where they can physically touch the walls, thus avoiding the more aversive central arena. Gentsch et al. (96) found that injecting chlordiazepoxide, a well established anxiolytic drug, in spontaneous hypertensive rats (SHR) and Wistar Kyoto (WKY) rats 60 min before testing, increased for both strains the central locomotion in the open field. In another study, a putative anxiolytic agent acting on adenosine receptors increased the occupancy of the central area in Wistar rats perinatally exposed to caffeine. The anxiolytic

diazepam, on the other hand, had decreasing effects on this behavioural measure (87). Using Long–Evans rats, Ossenkopp et al. (177) showed that repeated open field testing induced a decrease in defecation and an increase in the central activity, which was thought to reflect the lowering of emotional activation due to habituation. Thus, in spite of some contradictory results, a partition of the open-field locomotion into two categories (i.e. central and peripheral) may be useful to distinguish between a general type of locomotion from a locomotion with a higher emotional component.

4.2. The black and white box

Crawley (56) described an animal model for testing anxiety in mice which consists of a two-chambered apparatus with one compartment (2/3 of the total area) highly illuminated and the other painted black and not illuminated. The two compartments were connected by a small passage through which the animals could move freely. Transitions between compartments and general locomotion were automatically measured for 10 min. In this study, pharmacological testing compared the behaviour of control mice with that of animals treated with each of five benzodiazepine anxiolytics, two non-benzodiazepine anxiolytics, two antidepressants and one neuroleptic. All but one anxiolytic (benzodiazepine agonist R05-4864) induced significant increases in both the number of transitions and general locomotion. Such increases did not appear for animals treated with the antidepressants or with the neuroleptic. Based on these results, the author concluded that the aforementioned model meets the main criteria for an ideal test of anxiety: it is simple, efficient, rapid, reproducible and drug-specific.

Since the number of crossings between chambers was significantly correlated to general locomotion ($r = 0.71$) and frequency of rearings ($r = 0.72$), all three measures seemed to reflect the animal's emotional state (56). Crawley and Goodwin (57) have shown that the increase in locomotion following anxiolytic treatments in the black and white box did not appear for animals tested in an undivided open-field-like environment, which suggests that this test measures anxiety rather than general activity. Modifications in the apparatus and in the experimental conditions have been introduced in some studies (13,14,54). Even with a modified apparatus (two equal sized compartments connected by a tunnel) Belzung et al. (14) have shown in mice that a cholecystokinin type B receptor antagonist, with previously demonstrated anxiolytic properties, significantly increased the time spent in the white compartment and the number of transitions. On the other hand, in an apparatus consisting of two dark-colour compartments, one being illuminated and the other being in the dark, the time spent in the bright compartment and the number of transitions between compartments were found not to correlate (106).

Besides the variability in the experimental conditions, the major indices of anxiety considered in this test may also vary among studies. Whereas Crawley (56) has found the number of transitions and the total locomotion to be adequate measures of anxiety, Costall et al. (54) considered the time spent, the locomotion and the rearings in the white compartment to be the most reliable anxiety indices. It should be noted, however, that in the latter study, not only

were the apparatus and the mouse strains different from those utilised by Crawley, but also the animals were being tested during their dark cycle. Since rodents are typically more active during the night period, one might expect the biological meaning of locomotion and rearings in the white compartment to be affected by this particular experimental procedure.

Although this model is predominantly used for mice, a study on rats, which was carried out in our laboratory, has shown that the anxiolytic diazepam significantly increased the number of transitions and the duration of visits to the white compartment, for rats initially placed in the white compartment. Anxiolytic effects of chlordiazepoxide on the aforementioned measures, however, did not reach significance. Interestingly, when rats were initially placed in the black compartment, no anxiolytic effects of either drug could be detected (48). Therefore, the two testing conditions (white/black and black/white) seem to be unequal in their sensitivity to anxiolytics in rats. Moreover, both conditions have shown to be less sensitive to these drugs than the elevated plus-maze test (48).

Some care should be taken in the comparison of pigmented and albino strains, since pigmented animals appear to be less sensitive to strongly illuminated areas than albino ones (64,162). In our laboratory, rats from the Brown Norway (BN) strain have shown the lowest degree of aversion for the white compartment compared with five other strains. Interestingly, BN rats were the only ones with pigmented eyes, skin and hair (188). Therefore, interpretation of the approach to the white compartment should seriously consider possible differences in the visual systems.

4.3. The elevated plus-maze

Behavioural studies reported by Montgomery (168) have shown that rats display higher avoidance and lower exploratory behaviour in open elevated alleys than in enclosed alleys. Such a difference could be detected with or without offering the animal the choice between the two types of environment. In that study, the author interpreted the avoidance of the open alleys as being generated by the fear of novelty. Subsequently, Treit et al. (217) found that exposing animals to an apparatus with open and closed alleys for 18 consecutive days did not decrease their avoidance of open alleys and that previously confining them to an open alley for 30 min periods during 3 days resulted in a higher open-arm avoidance in the first test after treatment. These results indicate that the aversiveness of open alleys is not owing to their novelty. Further investigation by the same authors suggested that it is the aversiveness of open spaces, rather than that of heights or novelty, that creates the marked preference of rodents for enclosed rather than open alleys (217).

The work of Montgomery served as a basis for the development of one of the most popular anxiety models of the present decade: the elevated plus-maze test. Briley et al. (30) described the plus maze as an apparatus with four elevated arms, 50 cm long and 10 cm wide, arranged in a cross, two opposite arms being enclosed and two open, having at their intersection a central platform that gives access to any of the four arms. Rats are placed in the central platform and, for 5 min, total locomotion is measured through the total number of arm entries, whereas the

percentage of entries in the open arms is used as a measure of fear response. In this study, rats from four strains showed a marked preference for the enclosed arms, confirming the results presented by Montgomery (168). Pharmacological treatments showed that two benzodiazepine anxiolytics and one anxiogenic respectively increased and decreased the approach to the open arms. Treatments with seven anti-depressants with different pharmacological profiles had no effect on arm preferences. Treit et al. (217) also found diazepam to diminish open-arm avoidance.

One of the most extensive studies on the validity of the elevated plus-maze test was carried out by Pellow et al. (184). This study showed that rats consistently avoided the open arms and preferred the closed arms and that changing the light level in the closed arms did not alter the rats' behavioural responses. This finding is in agreement with previous results (109) which showed that the approach of open arms was not different between illuminated (170 lux) and dark conditions. Animals confined to the open arms for 20 min showed more behavioural and physio-logical signs of fear (decreased locomotion, higher immobility and freezing, higher defecation and higher concentrations of plasma corticosterone) than animals con-fined to the closed arms. Rats confined to the closed arms, however, also showed elevated levels of corticosterone when compared with the home-cage control group. These results confirm that open arms are more aversive than closed arms but they also show that a certain degree of aversion is present even in the closed arms, which is possibly owing to the novelty of this environment.

The pharmacological investigation in this study also confirmed previous data. Approach of the open arms was specifically increased by classical anxiolytics such as chlor-diazepoxide and diazepam and decreased by anxiogenic substances such as yohimbine, caffeine and amphetamine. Sedative drugs as well as antidepressants did not change the relative preference for the open arms. Further data on the pharmacological validation of this test have been reviewed by Handley and McBlane (109). The authors point out that, whereas some classical anxiolytic agents such as the benzo-diazepines consistently increase open-arm exploration, serotonin-related drugs have produced highly variable results (see also (123)). This lack of consistency is not seen as a drawback by the authors. Rather, they suggest that several mechanisms of anxiety may be differentially modu-lated by this type of compound. Such a multiplicity of processes, most often undetected by other anxiety models, might be expressed in a model like the elevated plus-maze, which comprises at the same time elements of conflict, avoidance and escape (109).

Additional ethological measures have been included in some plus-maze studies (see (59,74,191,193)) as an attempt to obtain a more subtle and discriminant interpretation of the psychological elements present in this test. Measures of hesitation (or risk assessment) to enter an open arm or to exit an enclosed arm (191,192) have been proposed as additional measures of anxiety, sensitive to anxiolytic treatments (63,203). In a study by Cruz et al. (59), risk assessment in rats appeared to be ambiguous, but genetic and/or metho-dological differences may be responsible for some of the discrepant results (see further discussion on the significance of different plus-maze measures in the next section).

As for the open field and the black and white box, some

inter-study variation has also been observed regarding the experimental set up of the elevated plus-maze test (109,123). A variation in the aversiveness of the test con-ditions (e.g. illumination) is likely to affect the baseline anxiety levels and/or the sensitivity of animals to anxiolytic compounds (123). Moreover, differences in the construction of the maze (e.g. with/without open arm ledges) have been shown to alter the response of rats to benzodiazepines (74,123). Nevertheless, in spite of some studies having demonstrated the importance of the experimental set up, Falter et al. (73) have found the fear responses in the plus maze to be fairly resistant to experimental conditions. In this study, several kinds of environmental manipulations, such as changing the light intensity, the height of the apparatus and the physical disposition of the arms, were ineffective in changing fear-motivated behaviours in the plus maze (73). Studies on the effect of previous stress on the plus maze behaviours have given variable results, making it difficult to propose a general behavioural response to previous stress of various types (73,102,158,191,192). However, it has been proposed that animals that are stressed before testing should be more anxious (193) and, hence, more responsive to anxiolytic treatments when tested in the plus maze (123). Finally, early handling and environmental enrichment seem to decrease the fearfulness of animals exposed to the elevated plus-maze (4,198) which may decrease their anxiolytic responses to drugs (123).

4.4. The social interaction test

The social interaction test, developed by File and Hyde (83), is based on the observation that the time spent by pairs of male rats in performing social behaviours varies with the aversiveness of the environmental conditions. High light level and the novelty of the environment, two naturally aversive stimuli, inhibit social interactions. This inhibition, which can be assessed through the manipulation of both environmental factors, was found to be correlated with other signs of emotionality, such as defecation, freezing and displacement activity (78,83). The test consists of placing two male rats, which had been isolated for 5 days and which had never seen each other before, in a large square arena. Four test conditions are classically utilized by combining two light levels (30 or 300 lux) and two levels of novelty (familiar and unfamiliar arena). Locomotor activity can be measured automatically by photocells, whereas the time spent in social interactions must be interpreted and mea-sured by a trained observer. The following behaviours are registered and classified as active social interaction: sniff-ing, following, grooming, kicking, mounting, jumping on, wrestling and boxing. These categories can be divided in two general classes: aggressive and non-aggressive behav-iours. The measures of locomotor activity, which may indicate sedative or stimulant effects of drugs, can be used in the final analysis as covariates, thus providing a more specific assessment of the changes on the social interaction behaviours (81).

Groups of rats exhibiting low scores of social interaction also presented elevated plasma corticosterone levels, enlarged adrenals and higher concentrations of noradrena-line in the hypothalamus (78). Similarly, rats tested under bright light showed higher corticosterone levels (81) and the administration of ACTH in low light familiar conditions

decreased the time of social interaction. The anxiogenic effects of ACTH were abolished by chronic administration of chlordiazepoxide and by acute administration of ethanol (84).

Data from other pharmacological experiments are not so clear-cut. Whereas chronic (5 days) administration of some benzodiazepines (diazepam and chlordiazepoxide) increased social interaction without altering locomotion in the most aversive situation (high light and unfamiliar environment), their acute administration reduced both measures in all test conditions (78,83). Acute treatment with ethanol at low doses produces anxiolytic-like effects, that is, an increase in social interaction (85). On the contrary, propanolol, meprobamate and sodium pentobarbitone, also thought to be anxiolytic agents, did not produce the expected effects over the different test conditions when given acutely or chronically. Peripheral injections of morphine reduced social interaction without affecting locomotor activity and, lastly, two benzodiazepines (lorazepam and triazolam) did not increase social interactions in any of the test conditions (78). The author proposed that drug treatments that caused a decrease in locomotion and in social interaction presented sedative effects, therefore not being suitable anxiolytic treatments. Nevertheless, considering that aversive stimuli (light and novelty) are present in the social interaction test, locomotion in the arena may be affected, at least in part, by the animal's emotional state (which is the basic assumption of the open field test of emotionality).

4.5. Other tests

There are certainly many other tests of stress/emotionality/anxiety that would deserve attention in a more thorough review, but, as already mentioned, this is not the aim of this paper. It is worthwhile, however, to mention briefly some other current behavioural tests, for their references may help interested readers to carry out further investigation.

Conflict tests involving punished drinking (Vogel test) or feeding (Geller–Seifter test) by electric shocks have been reviewed (79,216). Conditioned emotional responses and conditioned active avoidance, which involve either avoidable or unavoidable electric shocks, have also been reviewed (216). The use of a chronic social stress based on social instability to assess changes in the neuroendocrine system and in weight gain is proposed by Mormède et al. (169), whereas physical restraint in water is used to assess the effects of stress on the frequency of stomach ulcers (181,189).

Hyponeophagia is a conflict test of emotionality where hungry rats are exposed to food placed in the center of a brightly lit novel environment. Highly emotional rats are expected to show both a longer latency to approach the food and a lower food consumption (182,218). Among the so-called ethologically based models we can still mention the following: ultrasonic vocalizations emitted by rat pups following separation from their mothers (147,175); muscle contractions (startle reflex) in response to an intense acoustic stimulus (98,218); neophobic responses in a situation where both familiar and unfamiliar stimuli are simultaneously accessible (13); defensive burying and defensive withdrawal (181); stress-induced hyperthermia following removal from the home cage (232) and behavioural responses to exposure to cat or cat odor (24,124).

Another ultrasonic vocalization test, but this one using adult rats, has also been used as an anxiety model. In this test, following a learning period of four daily sessions of inescapable footshocks, ultrasonic vocalizations are recorded for 5 min during the intervals of three series of shocks. The total duration of these vocalizations is then taken as an index of anxiety (129).

All of the tests described in this chapter have been validated somehow, either pharmacologically or by means of correlations between a behavioural response and other stress-related measures (physiological, neuroendocrine or behavioural). Manipulating the level of aversiveness of a test to verify how this will influence a putative measure of emotionality has also been used as a validation method. Moreover, theoretical attempts of explaining why a fearful animal will respond to a stressor in one way and not in another (i.e. what is the adaptive value of a given behaviour) are sometimes used as arguments of validation. In no case, however, will one find ''the perfect test''», one that will always respond in a consistent and sensible manner to all attempts of validation. Most tests described herein utilize as indices of emotionality/anxiety behavioural responses that depend on body activity and/or locomotion. Therefore, a pure measure of emotionality, which is totally devoid of non-emotional components (e.g. activity or exploration) does not seem to be available. On this matter, comparative studies involving different tests may (or may not) reveal a number of major psychological/behavioural constructs which are common to different test conditions. This kind of approach will be discussed in the following section.

5. BREAKING DOWN THE UNIDIMENSIONAL APPROACH

5.1. The complexity of emotionality

Since the first animal models of emotionality were developed, the assumption that emotionality corresponded to a single general trait (being, though, expressed in various ways) was implicitly or explicitly present in the conclusions of many important studies (see (6,33,103,107,108)). Nevertheless, different authors have suggested that stress, emotionality and anxiety are not simple unidimensional entities (6,14,80,104,109,143,147,150,196,197). Despite a growing body of evidence giving support to such a hypothesis, the unidimensional approach still prevails among old and recent publications.

It has been proposed that emotionality, not being a single construct, can only be assessed by a series of different tests, involving a variety of behavioural and physiological measures (6,197). Such a proposition has not been widely accepted (103) and, even in recent years, the use of single variables (e.g. defecation) to evaluate the emotionality of animals is frequently observed (76,94,97,177). As pointed out by Archer (6), correlational studies involving ambulation/defecation in the open field (two classical measures of emotionality) and emotionality measures from other behavioural models often fail to support the concept of emotionality as a unitary trait. For example, the defecation scores do not show a consistent correlation with the heart rate measured in stressful environments and in mice, some studies show no correlation among defecation scores assessed under different test situations (see (6)).

Even if one considers only the open field test, experimental

evidence shows that measures of ambulation and defecation are not always correlated (3,40,94,179). Yet, when these two measures do show significant correlations, such values can be widely variable, being sometimes negative and sometimes positive (210,226). Environmental and genetic factors, which vary among studies, are likely to be responsible for part of the variability regarding the correlation indices. However, although one might expect that the most aversive testing conditions would provide the largest correlations between ambulation and defecation (since the former would be influenced more by emotionality and less by general motor activity), this does not seem to be the case. Whereas an open-field test under dim light produced moderate correlations between these two variables, the same test applied under bright light revealed no correlation at all (212). Analytical methods may also account for some of the inter-study variability, since it has been shown that correlation analyses based on individual measures may provide lower correlation coefficients than those based on the sum of repeated measures (e.g. over days) (176).

The possibility of dissociating ambulation and defecation, however, is demonstrated by a series of studies aiming at the distinction, within one heterogeneous population, of groups of rats displaying extreme performances regarding either exploratory activity or open-field defecation (15–17,19,215). A population of Wistar rats was tested in both an open field (to measure defecation) and an activity cage (which measured the number of rearings in 6 min trials). Following this behavioural screening, four groups of rats were obtained, i.e. high/low activity and high/low defecation. Since no correlation was found between defecation scores and exploratory/locomotor activity and as different neurophysiological profiles were associated to these traits, it was suggested that these two measures represented ''two independent qualities of higher nervous activity'' (18)

The comparison of anxiety tests for pharmacological purposes has shown that some drugs (or previous stress) may produce contradictory results when tested in different anxiety models (11,102,109,110). Similarly, the study of behavioural responses of undrugged animals often reveal a lack of consistency among anxiety measures from different tests (46,80). In addition, exposure to different tests of anxiety may produce different profiles of neurochemical changes in rodents (86). Such inconsistencies indicate that, either one (or all) of the tests considered is unable to produce a pure and reliable measure of anxiety, or else that the different tests assess different forms of anxiety.

The elucidation of this issue can be more easily achieved by the use of multivariate statistical analyses (e.g. factor analyses) (72,151). The application of this approach to emotionality-related traits usually consists of testing each individual in a series of different experimental settings and then, through a correlation or covariance matrix, extracting a few main factors which are formed by specific combinations of variables.

It should be noted that the term ''factor analysis'', in spite of being applied somewhat broadly in the non-specialised literature, is in fact only one method of multivariate analysis. This one may also include, for example, principal component analysis (PCA), discriminant analysis, cluster analysis, etc. Both PCA and factor analysis have in common the objective of reducing the number of variables considered for a group of individuals, to a smaller number of new indices that are linear combinations of the original variables.

A PCA, when applied to a set of correlated variables, produces a number of indices (principal components) that are not correlated among themselves and that represent the underlying dimensions of the data. The lack of correlation between these indices suggests that the different dimensions are independent from each other. The factor analysis, more complex, may start with a PCA and then after ignoring those components (now called factors) accounting for only a small part of the total variability, can perform a rotation of the retained factors. The rotated factors, in contrast to the unrotated ones, should have very high and/or very low correlations with each of the original variables, in such a way that their significance can be interpreted more easily. If an orthogonal rotation (e.g. varimax) is chosen, then the factors will continue to be uncorrelated and, consequently, we can still consider the underlying dimensions as being independent. Finally, after the rotation, one can perform the calculation of the factor scores, i.e. the scores of the different factors for each of the individuals (151).

5.2. Dissecting emotionality

In spite of still being unfamiliar for many, multivariate analyses have been employed in behavioural studies for several decades. In human psychology, factor analyses have been used in studies of personality structure. Based on questionnaires, peer ratings and objective tests, psychologists have analysed large sets of variables under different test conditions for almost 50 years. The most evident conclusion is that human personality is a complex construct which comprises different dimensions. Although only two factors (with a third one being added later) had originally been proposed by Eysenck (45,66), numerous studies carried out in different cultures and populations agree about the existence of five major factors, namely: extraversion/introversion; friendliness/hostility; conscientiousness/will; neuroticism/emotional stability, and intellect (66). A factor analysis of primate social behaviour has indicated that monkeys, under a stable situation, present three main personality factors: affiliative; hostile, and fearful, which are suggested to be respectively similar to extraversion, psychoticism (hostility) and emotionality in humans (45). The finding that factor analyses on different behaviours of octopuses also revealed three independent factors (which were termed as ''activity'', ''reactivity'' and ''avoidance'') has led to the suggestion that some general traits of personality may have been conserved across phyla (156).

As far as the emotionality tests for rodents are concerned, evidence suggests that different dimensions can be detected even among the measures of one single test. Cruz et al. (59), for example, doing a factor analysis of 13 behavioural variables from the rat elevated plus-maze test, have shown that four independent factors could be identified. The first two factors represented variables of anxiety and locomotion, respectively. Percentage of open arm entries, time in the open arms and time in the closed arms (negatively related) were the variables with the highest loadings on the first factor. The second factor was associated mainly to the number of entries in the closed arms. Total number of arm entries, which is normally used as the main index of locomotion in the plus maze (30), has proven to be ambiguous,

loading simultaneously on both anxiety and locomotion factors. That this measure is affected by anxiety has also been suggested by other studies, indicating that the number of closed-arm entries is a better measure of locomotion than the total number of arm entries (5,74,80,194). The time spent in the central area loaded on the third factor, thought to reflect waiting capacity or decision-making. The fourth factor (difficult to interpret, according to the authors) had the highest loading from grooming behaviour. The existence of two main axes corresponding to anxiety and locomotion in the elevated plus-maze has also been shown in mice (146,194).

As further behavioural measures are added to the analysis, more complex factor solutions are obtained. A series of factor analyses of mice behaviour in the elevated plus-maze, involving a range of standard as well as other ethological measures, produced a two-, three- or six-factor solution, depending on the set of variables included (194). An analysis of the standard variables resulted in two factors, reflecting anxiety and locomotor activity. When time in the central platform was added, a third axis (thought to reflex decision-making) appeared. Finally, an analysis of 19 plus-maze variables produced six independent factors. Factor 1 included all standard indices of anxiety as well as a series of risk-assessment measures (most of them performed in the protected areas of the maze). Factor 2 comprised locomotion-related variables and Factor 3 comprised two variables of risk assessment. The three remaining factors represented groups of variables thought to reflect decision-making, vertical activity and exploration, respectively (194).

In another study on rats, besides the anxiety and locomotion factors described above, it has been found that the decision-making dimension (central platform) was in fact dissociated in two factors, one seemingly associated to the ''openness'' and the other to the ''height'' of the plus-maze open arms (74).

A number of factor analyses has revealed the existence of distinct behavioural dimensions also for the open field test. A study by Whimbey and Denenberg (226) has shown that defecation and locomotion form two independent axes, thought to reflect emotionality and exploration, respectively. A two-factor resolution has also been found by Tachibana (212), who proposed the existence of a ''gross bodily activity'' factor (ambulation, approach to the center and rearings) and an ''elimination'' factor (defecation and urination). The structure of these two factors were stable across five days of testing and were very similar in both dim- and bright-light conditions. The same two factors, which were also resistant to illumination conditions, have been found by Maier et al. (150). A more sophisticated method of factor analysis (three-way PARAFAC model) has also revealed two general factors, one thought to measure emotional reactivity (involving defecation, urination and avoidance of the center) and the other thought to measure exploratory activity (mostly activity in the central area) (177). A factor analysis involving a large number (twenty two) of less-classical open-field measures has produced three factors accounting for 60% of the total variance. These were called: ''exploration'' (variables of motor activity); ''fear'' (mostly defecation) and ''shifted activity'' (mostly grooming) (152). According to a review by Royce (196), different factor studies on open field measures for

different species allowed the recognition of three invariant factors. These factors were termed by the author as: (i) motor discharge (latency to move, general activity and central activity); (ii) autonomic balance (defecation) and (iii) territorial marking (urination). In addition, the author recognises a higher order factor of emotional stability, common to these three factors.

Thus, most factor studies on open-field measures identify at least two independent factors which represent, in general, activity and elimination under a novel and stressful environment. The evidence provided by this multifactorial approach, therefore, indicates that the two most classical open-field measures (ambulation and defecation) do not pertain to the same psychological dimension. Nevertheless, some of these studies show that ambulation does not load exclusively on the exploration factor, but it can also appear moderately associated to defecation (226). It has been suggested that activity in the open field has in fact a two factor basis and that the activity during the first minutes of the open field is motivated by fear more than by exploration and vice-versa (152). Activity during the first 2 min of an open-field test has, in fact, been found to be moderately correlated to the approach of the open arms in an elevated plus-maze (141). This may explain why the correlations between open-field ambulation and defecation may be positive in the first day of test and negative in the following days (226). Hence, according to this view, activity in the open field would be influenced by both fear and exploration, with the influences of the first drive decreasing and those of the second one increasing with habituation. Such a concept, however, remains theoretical and still awaits further corroboration, but the concept that activity in rats has a multidimensional basis has also been found by Paulus and Geyer (183).

Studies adopting a multiple-testing approach (the characterization of each animal in a set of different tests), followed by factor analyses, can be particularly useful to test the concept of multidimensionality as well as the hypothesis that a given psychological phenomenon can be assessed by different experimental paradigms. Different studies involving behavioural measures of emotionality have produced a variable number of factors (6). An extreme example of multidimensionality in this type of study is the factor analysis of 32 measures supposedly related to emotionality, which produced 12 factors (197). Different terms, such as: ''freezing'', ''timidity'', ''reactivity to light'', ''approach–withdrawal'', etc., were used in this study to name the factors. Archer's conclusion was that ''in general, no factor corresponding to a general emotionality construct was found''.

One factor analysis (150) on the behaviour of rats submitted to different tests of emotionality, general activity and exploration has produced four independent factors. The first one, comprising variables from a running-wheel test, was thought to measure voluntary activity. The second one, formed by open-field exploration variables such as ambulation and rearing, was thought to measure the exploratory activity. A third factor represented variables related to the emotional reactivity of rats during handling and capture in the cage [rodent emotionality rating scale (RERS)], which includes defecation and urination. Finally, the fourth factor had high loadings only from defecation scores in the open field. Therefore, according to this study, ambulation in a

novel open field (Factor 2) is associated neither with the spontaneous activity in a familiar running wheel (Factor 1) nor with the emotional reactivity to human intervention (Factor 3). Open-field defecation, in addition, is not correlated either with defecation in the RERS test or with any of the activity measures from the other tests.

The first factor analysis involving elevated plus-maze variables was the one performed by Richard Lister (146). By analysing the behaviour of mice tested in the holeboard first and in the plus maze immediately after, Lister found three orthogonal factors. The first one (anxiety) reflected the approach towards the plus-maze open arms, the second one was associated with the exploration (head-dipping) in the holeboard and the third one had loadings from the locomotor activity in the holeboard and the total arm entries in the plus maze (number of closed-arm entries was not included in the analysis).

A study by Trullas and Skolnick (218) on inbred strains of mice showed significant genetic correlations between fear-related behaviours measured in the elevated plus-maze and those measured in two other putative models of anxiety, the acoustic startle response and the hyponeophagia paradigm. Interestingly, no relationship was found between the plus-maze measures and the ambulation scores in a brightly illuminated open field, which is classically seen as a model of emotionality. Furthermore, these ambulation scores were not related to ambulation in a dimly lit open field. The multidimensionality of emotional reactivity is strongly supported by these results. For example, the two mouse strains (BALB/cJ and A/J) obtaining the two lowest open field ambulation scores (in bright and dim light conditions) showed, among all strains, extreme opposite responses in the elevated plus-maze. In spite of showing little activity in the open field, BALB/cJ mice spent 90% of their time in the open arms of the plus maze, whereas A/J mice spent only 2%. Similarly, C57BL/6J mice, which had an ambulation score (bright-light open field) three times higher than CBA/J mice, spent only 6% of their time in the open arms of the plus maze, whereas mice from the latter strain spent 69% of their time in the open arms. A factor analysis involving plus maze and open field (dim light) variables showed that ambulation measured in both tests formed one single factor, which was thought to measure general activity, whereas variables of open arm avoidance formed another axis thought to represent fear or anxiety (218). Therefore, a growing body of evidence suggests that the elevated plus-maze, as well as most other behavioural tests, may assess different dimensions of the emotional responses.

Another factor analysis performed with mice involved variables from five behavioural tests (13). Two independent factors emerged, the first one comprising measures of neophobic responses (towards an object or a place) and the second one, variables from the holeboard test and the elevated plus-maze. The criteria used to determine the number of factors to be retained, however, are not clear in this study. This is an important point since variables from the elevated plus-maze test, which in other studies are usually dissociated into two factors (anxiety and locomotion), are placed here on one unique axis, termed ''general activity or exploration'' by the authors. Such a factor presents high loadings for activity variables and only a weak loading (0.30) for the percentage of entries in the

open arms of the plus maze. Indeed, this suggests the possible existence of a third axis (not presented) on which the percentage of open-arm entries would have a greater weight. It was clear in this study, however, that the plus-maze measure of anxiety was not related to measures of neophobia. These results suggest that neophobia tests, which offer a free choice between familiarity and novelty, and the plus maze test assess two different forms of the emotional response.

In a multiple-test study carried out in our laboratory (188), males and females of six inbred strains of rats were successively tested in an open field (novel environment/dim light), an elevated plus-maze, a black and white box and a social interaction test. A factor analysis based on a genetic correlation matrix produced three independent axes accounting for 85.1% of the total variation. The first factor received high loadings from anxiety measures in the elevated plus-maze and the black and white box as well as the central locomotion in the open field. The second factor had high loadings from variables related to locomotion in novel environments, like total and peripheral locomotion in the open field, number of closed arm entries in the plus maze and locomotion in the social interaction test. The general structure of the first two factors is represented in Fig. 1 (variables with redundant meanings are not included here for the sake of simplicity).

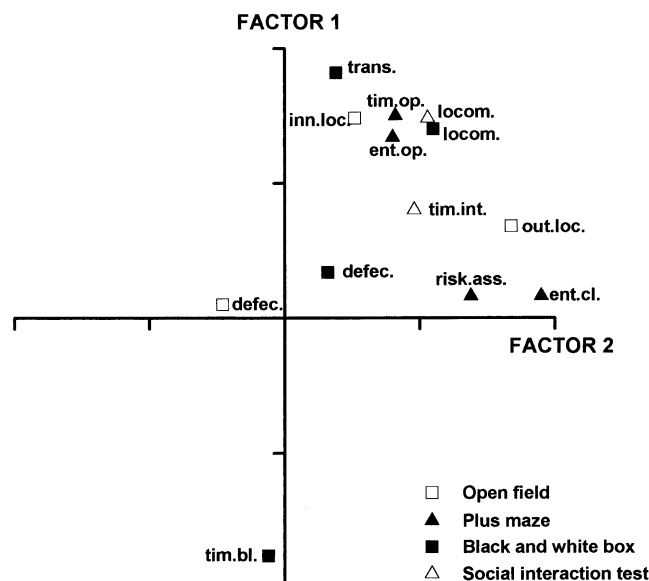The loadings on Factor 1 indicate that rats tending to

FIG. 1. Principal component analysis with varimax rotation performed on the behavioural measures of 12 genetic groups of rats (mean values of males and females of six inbred strains): distribution of the variables along the first two factors. The coordinates of the two axes represent the factor loadings (−1 to 1) of the variables from four behavioural tests (redundant measures are not represented here). Open field test (novel environment/dim light): OUT.LOC. (outer locomotion); INN.LOC. (inner locomotion) and DEFEC. (defecation scores). Elevated plus-maze: TIM.OP. (time in the open arms); ENT.OP. (% entries in the open arms); ENT.CL. (number of entries in the closed arms) and RISK.ASS. (risk assessment measured as the number of hesitations to exit an enclosed arm). Black and white box: TRANS. (number of transitions between compartments); LOCOM. (locomotion in the black compartment); DEFEC. (defecation scores) and TIM.BL. (time spent in the black compartment). Social interaction test: LOCOM. (locomotion) and TIM.INT. (time of social interaction).

approach the center of the open field, also approached the open arms of the plus maze and the white compartment of the black and white box. The structure of Factor 2, on the other hand, shows that groups that were highly active in the open field periphery were also highly active in the elevated plus maze, as measured by the number of total and closed arm entries. The independence of these two factors suggests that approach/avoidance towards aversive stimuli (putatively related to anxiety) and locomotion in novel environments represent two dimensions of the emotional response.

Measures of risk assessment (hesitation to exit an enclosed arm or to enter an open arm) in the plus maze were not associated with most of the classical measures of anxiety (Factor 1), being, instead, associated with locomotion in novel environments (Factor 2). Cruz et al. (59) have found for rats, measures of plus-maze risk assessment to be ambiguous, since they loaded on three different factors and failed to respond to anxiogenic compounds. In mice, on the other hand, different measures of risk assessment have been shown to load exclusively on an anxiety factor (194). Such measures (inversely related to the approach of the open arms) were those displayed in the protected areas of the maze (closed arms and central platform). This negative association between open arm approach and protected risk assessment is not really surprising, since a higher exploration of the open arms might result in a lower occupancy of the protected areas (and hence a lower frequency of protected behaviours). Such an association also suggests that measures of protected risk assessment may be seen, in this case, as indices of anxiety. In this same study (194), other measures of risk assessment (protected + unprotected) formed a separate axis (risk assessment) and were not related to the locomotion factor (differently from the study on rats carried out in our laboratory). It should be emphasised that the criteria for defining risk assessment behaviours are far from being homogeneous among different laboratories. This and the fact that different species (rat vs mice) or strains may vary in terms of the respective significance of their risk-assessment behaviours should be considered before any generalisation is made regarding the validity of these measures. Nevertheless, that measures of risk assessment, even when not associated with an anxiety factor (194), have been shown to respond to some anxiolytic treatments (63,194,203) seems to support the growing idea that different forms of anxiety may be present in a given model of anxiety (74,80,109,194).

The fact that in our study (188), locomotion in the black compartment of the black and white box also loaded on the first factor is not surprising, since it has been shown that this measure is likely to be associated to anxiety (48,56,57). On the other hand, the fact that locomotion in the social interaction test loaded higher on Factor 1 than on Factor 2, may be owing to the higher aversiveness of this test (bright light/unknown social partner) in comparison with the open field test (both tests performed in the same apparatus).

The highest loadings on Factor 3 (not shown here) were those from the defecation scores (in both the open field and the black and white box) and the time of social interaction. Accordingly, rats displaying little social interaction, which should be considered as highly anxious (78), tended to defecate less than rats that were highly interactive. Due to its complexity, however, one may suppose that the manifestation of social behaviours can be affected by many factors not related to emotionality. According to File (81), for example, hooded Lister strains are recommended because of their higher tendency to interact socially. Moreover, neither mice nor female rats, owing to the particularities of their social behaviour, are recommended for this type of model (78,80,128). Because of these restrictions, the social interaction test may not be the most adequate model for inter-strain studies of anxiety, since genetic differences in sociability may be responsible for differences in the time of social interaction.

A factor analysis by File (80) gives further support to the multidimensional concept of emotional response. Behavioural variables from three different models of anxiety in rats (social interaction test, elevated plus-maze and Vogel test) produced a five-factor solution. At least three out of the five axes could be seen as anxiety factors, since each one integrated a different group of variables corresponding to each of the three tests of anxiety. The author suggests, therefore, that the three tests measure three different types of anxiety. Regarding the elevated plus-maze, more recent factor analyses from the same laboratory still suggest that repeated testing alters the neurobiological state of the animals (74). Since the anxiety measures from trial 1 (naive animals) and trial 2 (experienced animals) produce two independent factors, the authors propose that the two repeated plus-maze trials detect, in fact, two different forms of anxiety.

In conclusion, the results discussed above suggest that emotionality is a highly complex trait to explore and that different forms of it may be independently displayed under different conditions. The two factors, corresponding to locomotion and anxiety, provided by several multivariate studies, indicate that locomotion in novel stressful environments and measures of anxiety represent two different dimensions of emotionality. Moreover, we saw that anxiety itself may be further dissociated and that this concept may comprise different psychological constructs. The multidimensional nature of emotionality suggests that distinct biological substrates might be involved in the control of this phenotypic myriad.

5.3. A psychobiological interpretation of the multidimensionality

Going back to our initial concept of stress, where environmental stimuli elicit a series of biological adjustments as well as changes in the central state of subjective experience, we may hypothesize a system which comprises: (i) an external input; (ii) a central emotional state, and (iii) a measurable output. This last component would include physiological, neuroendocrine and behavioural manifestations with a potential feedback action on the other two levels. The multiple dimensions of the emotional response might reflect a variability within each of the three levels of this model and this should be considered when two or more individuals are compared in relation to these dimensions.

The three anxiety factors corresponding to the three tests analysed in the aforementioned study (80), for example, seem to reflect different dimensions at the input level. The emotional susceptibility to each of three types of stimuli (electric shock, elevated open spaces and environmental/social novelty) appears to vary independently among individuals. Consequently, a rat that is highly anxious in a social

situation might not experience a high anxiety when exposed to a non-social challenge (e.g. open arms of a plus maze). Such a distinction has in fact been supported by factor studies carried out in our laboratory involving non-social anxiety models as well as different situations of social stress (21). A dissociation between responses to social and non-social challenges has also been revealed in pigs (142). Similarly, mice that highly avoid a novel cage compartment or a novel object do not necessarily display a high avoidance of the plus-maze open arms (13). In other words, two individuals may differ, not only in their general fearfulness (central state) or in their fear responses (output), but also in the type of stimulus which is capable of eliciting their fear. The multiple dimensions observed at this level may reflect, to some extent, an interindividual variation in the perception and/or in the cognitive evaluation of the different types of environmental stimuli (input).

Alternatively, different dimensions may exist at the central level of emotional experience. Hence, factor analyses may be able to reveal different subsets of emotionality which are independently controlled and experienced, in an analogy with the various classes of emotions (as already mentioned in this paper) and the different types of human anxiety disorders (80,144,147). Pharmacological studies using drugs with recognised effects on different kinds of emotional human disorders might be particularly helpful with this approach.

Finally, that two putative measures of emotionality from the same test load on two independent axes suggests that different dimensions may exist at the output level, even when the input stimulus is the same. Since an animal may respond to the same open field test with a seemingly paradoxical profile (high ambulation and high defecation, for example) it can be suggested that for a given emotional state, the external manifestations of stress may vary among individuals. Accordingly, BN rats respond to novelty with high defecation in spite of not displaying an inhibited locomotion (typical response of WKY rats) either in the periphery or in the center of the open field. Lewis (LEW) rats, on the contrary, display high avoidance of the center without showing other signs of fear, such as high defecation and low ambulation (188). On this basis, it may be supposed that not all individuals from the same species express their emotionality in the same way, which may be related more to the sensitivity of peripheral mechanisms of adaptation than to the central state of emotional arousal. Therefore, in the search for the biological mechanisms which control a specific emotional response (e.g. locomotion in novel environments), one should be aware that the results might apply, not to emotionality as a central state, but only to that particular fashion of coping with environmental challenges.

5.4. The multiple dimensions of anxiety

It has been proposed that the multidimensionality revealed by animal models of anxiety can be associated with the heterogeneity found in human anxiety disorders (80,82). A major difficulty, however, is to determine a specific form of clinical anxiety which can be associated with a particular animal model. Regarding the different forms of anxiety, Lister (147) has brought to discussion in the psychopharmacological research two concepts originated in the clinical field: state anxiety and trait anxiety.

The former is defined as the anxiety a subject experiences at a particular moment, in the presence of an anxiety-provoking situation. The latter, conversely, would be constant throughout the time as a permanent feature of the individual. The animal models used in anxiety research were seen by the author as measures of state anxiety. They may not correspond, therefore, to the human generalized anxiety disorder, which is characterized by a chronic state of anxiety and that would presumably be associated with the notion of trait anxiety. Lister's conclusions pointed out the importance of attempting to develop animal models which would involve chronic forms of anxiety.

Griebel et al. (104) are likely to be the first authors to claim to have developed such a type of model. A free-exploratory paradigm has been developed, where mice are given a 24 h period of familiarization with one half of a test box prior to testing. During the 10 min test period, the partitions which prevented the contact of the animal with the other unfamiliar half of the apparatus are removed and the signs of approach/avoidance of the novel environment are registered under red light. In such conditions, where the animals can freely choose between the familiar and the novel compartment, BALB/c mice exhibited a preference for the familiar compartment whereas C57BL/6 mice preferred the novel one. The neophobia showed by BALB/c mice was abolished by diazepam, chlordiazepoxide and the quinolizinone Ro 19-8022. Adding fresh sawdust or rubbing mouse urine in the novel compartment also completely reversed the neophobic responses of BALB/c mice. These results indicate that novelty was the major factor in determining the avoidance response in this strain and they suggest that this test is a valid model of anxiety. Misslin and Cigrang (165), using the same paradigm, have shown that animals that freely choose to explore the novel environment do not show signs of fear as measured by corticosterone levels, defecation and urination. In contrast, animals which are forced into the novel environment clearly showed the above mentioned stress-related responses. Griebel et al. (104) concluded that this test is "devoid of intrinsic stressful elements" and that, therefore, the neophobia of BALB/c mice would reflect what Lister (147) called trait anxiety (see also (13,14)). Considering the results reported in the aforementioned study (104), however, little evidence can be found to suggest that the neophobia displayed by BALB/c mice reflects a constant state of anxiety or fear. The avoidance of novelty, shown in this study, indicates that BALB/c mice are particularly fearful in this test situation. Whether these animals experience a chronic state of anxiety when they are left undisturbed in their home cages, in the absence of the aversive stimulus of novelty, cannot be concluded from the results reported therein.

Some confusion appears on the assumptions about the nature of the two types of anxiety. State anxiety is often seen as being environmentally determined, whereas trait anxiety is considered to be influenced by genetic components (80). Yet, a considerable amount of recent data has shown that different strains of rats and mice can be highly different in their responses to anxiety tests. Therefore, if the models thought to measure state anxiety can easily detect genetic differences among populations, it becomes clear that these fear responses are influenced by genetic factors. Whether such responses are specific to a particular moment or are constant throughout long periods of time, they should

always be affected by the genes and their products. In conclusion, the two types of anxiety discussed by Lister are, very likely, both under genetic influences.

In summary, we have seen in this section that the different emotional responses do not vary along a single axis. Factor analyses represent a useful tool which simplifies complex data and reveals the different psychological dimensions assessed by a set of behavioural variables. Moreover, the different dimensions of emotionality in rodents have been considered as potentially related to different forms of psychological disorders in humans. Only through the recognition and the fine investigation of these dimensions will we be able to significantly advance in this matter.

6. THE GENETIC MODELS

The observation that different animal strains can respond differently to environmental challenges, indicates that genetic factors are partially responsible for the interindividual variation observed in stress-related responses (29,40,214). It has been proposed, therefore, that such groups of animals may represent a useful tool in the study of the biological mechanisms involved in stress-related disorders (40,80,140,190,218).

The study of contrasting genetic groups can improve the knowledge on the biology of stress in at least two different ways. On the one hand, the phenotypic characterization of different strains, regarding a number of stress-related measures, may reveal correlations between two or more of these measures, suggesting that a given set of traits may share a common biological substrate. On the other hand, the coupling of molecular and interstrain studies, in the search for an association between genes and phenotypic traits (50,88,213), can provide a different perspective on the relationships among genes, gene products, neurobiological systems and stress-related phenotypes (186). Regarding the first approach, it should be noted that the simple correlation between phenotypic traits among a series of parental strains (genetic correlation) does not prove that two related parameters share a common biological substrate. In fact, two correlated traits may appear simultaneously in one or more strains by chance only and not owing to a genetic link (120). The reliability of such an approach, however, can be increased by augmenting the number of strains studied.

Alternatively, the search for biological associations among phenotypic traits may be rendered more reliable by performing correlational analyses on segregating populations (e.g. F_2 or backcrosses derived from two contrasting strains) (40,50). In this case, two measures that are eventually associated in the parental strains but that are not influenced by the same genomic regions (or the same neurobiological mechanism) are expected to be dissociated in such a segregating population (120). In addition, means and variances from different generations (P_1, P_2, F_1, F_2 and backcrosses) can be used to estimate several parameters of quantitative genetics, such as heritability, additivity, dominance and the number of genes involved (32,44). In spite of their widespread use, these parameters should be carefully interpreted since they can vary among different populations and environments (186,214). A more sophisticated method of genetic analysis, the diallel cross, is based on the comparison of several inbred strains and all possible F_1

hybrid crosses. The advantages of this method are discussed elsewhere (32,186).

Interstrain differences used in stress research can result either (i) from a planned genetic selection upon specific stress-related responses or (ii) from random effects of independent breeding carried out on different strains or populations. The latter approach is certainly faster, since one works with strains which are already established, but it may be less informative than the former one.

In the first situation, bidirectional selection (genetic selection of the two extremes of a trait) upon a given population has the advantage of maximising the differences between groups (several examples are discussed below). After many generations of selective breeding, it is expected that one of the resulting lines will contain almost all the genes that affect positively the trait of selection, whereas the other will have most genes with negative influences on the same trait. Hence, their phenotypic differences should tend to a maximal contrast (22,31,93,174). However, a limiting factor that should not be ignored is that the genetic selection can only act upon those genes that are polymorphic (different alleles for one gene) in the original population. For those genes that are already homozygous at the beginning, nothing will change during the process of selection. Consequently, the lower the genetic variability of the original population, the lower is the probability of obtaining two strains which differ in all the existing genes for a specific trait.

With the second approach, where one searches for differences that have been produced ''by chance'', the probability of obtaining an extreme contrast between strains is lower. In addition, if one works with outbred populations, the phenotypic differences eventually found may vary with time and with sub-samples of the groups, since the genes of interest may not be fixed within each population (i.e. a major gene may present two or more alleles which segregate within a genetic group). Such an internal variability may be responsible for contradictory results sometimes observed among interstrain studies (see (40), for example), but it can be eliminated (or minimised) if one chooses to work with inbred strains (see discussion below), which are theoretically homozygous for all loci. Since the animals within each of these strains are genetically identical, their use allows an easier control of environmental influences on the phenotype and it favours molecular studies of segregating populations (e.g. (88)). Nevertheless, whatever strategy is chosen, when working with two-strain models one must be aware that many important genes, which are polymorphic for the species as a whole, may not be polymorphic (hence, not informative) for the strains of one single model.

Several recent studies have shown that inbred strains of rodents, which have not been selected for emotionality, may show striking differences regarding a range of different emotional responses (8,58,98,106,140,157,182,190,218). Such differences, although not intentionally produced, may constitute a useful resource for the study of stress. As an example, we may cite the Lewis (LEW) and Fisher (F344) inbred rat strains. Female LEW rats have been shown to rapidly develop arthritis following a single injection of group A streptococcal cell wall peptidoglycan polysaccharide (SCW), whereas F344 females are resistant to the same stimulus (see (229)). Studies have shown that this susceptibility of LEW rats is related to the hyporesponsiveness of

their HPA axis to inflammatory mediators (37,207,208). Moreover, in response to other types of stressors (open field exposure and acoustic stimuli), LEW females also showed lower corticosterone responses than F344s (99,100). Differences in corticosterone response to stressful conditions have also been observed in males (47). In addition, LEW rats were shown to have lower serotonin (5-HT) levels and lower density of 5-HT$_{1A}$ receptors in the hippocampus than F344 rats (36,47), which may be related to the hyporesponsiveness of the HPA axis. It is likely that, just as the Lewis/Fisher pair of strains has been found to be a useful model for the study of the HPA axis and its implication in stress, other pairs of inbred strains will reveal in the future their usefulness for the study of other dimensions of stress.

In the search for genes associated with stress, it is reasonable to think that different genes may act at different levels of the response mechanism (see the three levels hypothesised above: input/central state/output). Theoretically, thus, a given gene can affect an output response (e.g. defecation) by acting at the perception level (input), at the central level (subjective experience) or at a peripheral level (e.g. reaction of the autonomic nervous system to stressors). Therefore, the higher the level of integration (central state) affected by a gene, the more likely this gene will influence a wide range of emotional responses instead of a single one (and vice-versa). In addition, since most manifestations of stress are not pure measures of the emotional response, it should be considered that a given gene can affect a specific response by means of a system not associated to emotionality.

A considerable amount of interstrain studies using a great number of mouse and rat strains have been reported throughout this century. Nevertheless, the present discussion will be limited to the general profile of a few genetic rat models, product of intentional genetic selection, which are most relevant for illustrating the usefulness of a genetic approach.

6.1. The Maudsley Reactive and Non-reactive strains

One of the classical models for the genetic study of emotionality was developed in the 1950s and 1960s in London. Following selection procedures similar to those already utilised by Hall (32) and Billingslea (23), in 1954 Broadhurst started a two-way selection program on rats for high and low defecation rate in an open field test. The result was the establishment of two contrasting populations called the Maudsley Reactive and Non-reactive lines (MR and MNR, respectively). After 15 generations of selection, the lines presented marked differences in their defecation scores in the open field, with the Non-reactive strain displaying scores close to zero and the Reactive strain scores close to four, for both males and females (31). As a secondary result of selection, the two strains also differed for ambulation scores in the open field test, with MR rats being less active than MNR ones. Although less marked than the differences in defecation (less than twofold in the 15th generation), differences in ambulation were also observed in both sexes (31).

Two other behavioural differences, possibly related to the selection trait, were found in conditioning experiments (34). In the Skinner box, a decrease in the learned behaviour of bar pressing to obtain a water reward is normally observed following the sole presentation of a flashing light which had previously been associated with a painful shock. The decrement of bar pressing owing to the conditioned fear is called the conditioned emotional response. In this test, MR (high defecating) rats showed a greater decrease in bar pressing than did MNR (low defecating) rats. Similar results concerning conditioned suppression of drinking were obtained by Commissaris et al. (51). In a second test, called escape-avoidance conditioning, rats learn to escape from an electric shock by crossing from one side to the other of a shuttle box. Afterwards, the rats learn to avoid the shock by doing the same behaviour, but now in response to a sound signal that precedes the shock. In this test, MR rats were slower than MNR rats in acquiring the avoidance response as well as in executing it. These two results suggested to the authors that MR rats, being more emotional, respond to both aversive situations with inactivity or freezing. Such an inactivity, in one case, would favour the conditioned emotional response as measured by the decrease in bar pressing, whereas in the second test, inactivity would lead to a lower performance in the conditioned response, which requires the animal to move as fast as possible (34). However, the relationship between active avoidance behaviour and classical indices of emotionality is far from being clear (see discussion on Roman rats below).

Many other studies comparing these two strains have been carried out. Crossbreeding experiments have produced high estimates of heritability for defecation (> 0.9) and medium to high estimates for ambulation (0.4 to 0.8) (32). Broadhurst (33) has reviewed over 100 studies on the Maudsley strains involving behavioural, physiological, and pharmacological measures. The differences reported therein have led to the proposition that the two strains differed in a generalized trait of ''emotional reactivity'', with the Reactive strain being more ''emotional'' than the Non-reactive strain. The Reactive strain, besides the traits already discussed, tended to take longer to emerge from a familiar to a novel environment; did more grooming behaviour; showed a higher inhibition of food and water intake under aversive situations (confirmed by Commissaris et al. (52,53)) and developed more stress-induced gastric ulcers. It should be noted, however, that out of the 280 results summarized in that review, almost half (i.e. 132) were not interpretable in terms of emotionality, whereas 42 of them (15%) opposed what should be expected according to the emotionality hypothesis.

Further experiments, carried out in the 27th generation of the Maudsley strains, showed that MR rats had greater defecation scores than MNR animals not only in the open field but also during handling. On the other hand, MR animals showed lower heart rates in both stressful situations when compared with MNR ones (113). Besides the differences in heart rate reactivity, the Maudsley strains also differed in other parameters of sympathetic activity (25). MNR rats had higher plasma noradrenaline levels as well as lower blood pressure and heart rate under resting conditions (27). MNR rats also displayed higher concentrations of noradrenaline in several tissues including the hypothalamus, the heart and the gastrointestinal system (28,204). In another study, differences in the noradrenergic function were not correlated to differences in the emotional behaviour in a conflict situation involving punishment (222). Some similarities appear, thus, between the Maudsley and the Wistar-Kyoto strains which, as discussed below, also present differences in open field

activity as well as in the sympathetic nervous system activity. Rats from the MR strain have also shown higher serum prolactin levels under basal conditions than MNR animals (26).

Additional studies have raised doubts about the idea that the Maudsley strains differ in emotionality in a broad sense. Beardslee et al. (12) found no difference between the two strains in their performance in the defensive burying paradigm, thought to be a model of anxiety. A study by Overstreet et al. (178) showed that MR rats were more immobile in the forced swimming test, a putative model of depression, than MNR rats, suggesting that the former were more emotional than the latter (the same responses are interpreted in the opposite direction elsewhere (1,2)). On the contrary, no differences were found between the two strains in the active avoidance in the shuttle box, which is not in agreement with results previously reported (34). When tested in the plus maze, MNR rats, as expected, spent more time in the open arms than MR rats (178). Abel (1) found that MR rats did not show any difference in the corticosteroid levels before and after two stressful tests (open field and forced swimming). The overall results suggest that the two Maudsley strains differ for some but not for all current measures of emotionality or anxiety, which further confirms the multidimensionality of these constructs.

6.2. The Roman rat lines

Selection of these strains began in 1961 from a Wistar population for high and low rates of active avoidance conditioning in a shuttle box similar to the one described above, the sole difference lying on the nature of the conditioned stimulus (light vs sound (22)). In contrast with the procedure used with the Maudsley rats, here, inbreeding was avoided during selection. After five generations, the two selected lines [Roman High Avoidance (RHA) and Roman Low Avoidance (RLA)] differed markedly (at least threefold differences) in the number of avoidances during the trials, with no effect of sex being detected. Further studies showed that the RHA strain was more active in the open field test than the RLA strain, without displaying any differences in the open-field defecation (35). Based on these results, the authors suggested that the similarities between Roman and Maudsley rats in terms of active avoidance performance are mediated by common features related to activity but not to defecation. A factor analysis involving measures in the open field and in an activity cage indicated that the two strains differed in relation to a factor reflecting activity and not to another factor thought to measure emotionality (125).

A different conclusion could be drawn from other studies on the Roman lines. Roman/Verh rats are the Swiss sublines of RHA and RLA rats which have been reselected by Driscoll (see (68)). Gentsch et al. (94) found that RHA/Verh rats were not only more active than RLA/Verh rats in the open field, but they also defecated less and showed a lower corticosterone increase following the test. A study by Ferré et al. (77) has shown that defecation was higher in RLA/Verh rats in six different test situations which involved either novelty or an approach/avoidance conflict. In the Vogel's conflict test, thought to measure anxiety, RLA/Verh rats showed a higher shock-induced suppression of drinking as well as higher defecation rates than RHA/Verh

rats. The hyponeophagia also indicated that RLA/Verh animals were more anxious than RHA/Verh rats.

Numerous studies have shown that the Roman lines differ in several neurobiological parameters which are possibly associated with emotionality. For example, differences have been reported on: the hypothalamus–pituitary–adrenocortical (HPA) axis (10,95,97,223); the cholinergic (153,230,231), dopaminergic (62,71,230,231) and serotonergic (49,139,230,231) systems; the biochemistry and morphology of the pineal gland (199); the levels of octopamine in the brain (105) and the oxytocin and vasopressin responses to stressors (39). RLA rats also respond to different types of stimuli with a higher increase in heart rate when compared with RHA rats (62). Moreover, differences in the sensitivity to several drugs have been reported (68,70,111,202). Such a wide range of differences has often led to the assumption that the differences between the Roman lines are related to emotionality or anxiety (62,69).

The assumption that the rate of avoidance conditioning (the trait of selection of the Roman lines) reflects emotionality is questionable. Among Sprague–Dawley rats, for example, which were highly heterogeneous regarding their behavioural reactivity, a group of animals displaying low avoidance responses in a shuttle-box showed high locomotion and low stress-induced arousal (ambulatory inhibition following a loud noise) in an open field test. Conversely, high-avoidance rats responded to the acoustic stimulus with high immobility (61). Moreover, the behaviour of the two Roman lines (differing in their avoidance responses) in three tests of anxiety revealed that RLA rats (usually considered to be more emotional) spent more time in the open arms of an elevated plus-maze and explored the white compartment of a black and white box more than the RHA rats did (46,139). Some studies on the effects of different anxiolytic and anxiogenic agents on the acquisition of shuttlebox avoidance, on the other hand, have supported the view that this test may be considered as an animal model of anxiety (see (75,187)). These controversial results indicate that the psychological meaning of the differences between the two Roman lines is not clear-cut.

RLA rats, differently from their RHA counterparts, show a general tendency to respond to stressful conditions in a passive way, that is, with freezing or immobility (195). Accordingly, the differences in activity already described for the open field were confirmed by measures of exploration in the shuttle box and in a circular corridor (41). In the same study, however, no differences were found for defecation scores in the open field. In addition, no differences were found for corticosterone and ACTH concentrations, before or after exposure to the open field, the circular corridor or to the CRF challenge. Conversely, significant prolactin reactivity to the novel environment was observed only for RLA rats. Castanon et al. (43) showed that no differences in the reactivity of the HPA axis (ACTH and corticosterone) to psychological (open field) or physiological (CRF) stimulation were found in 14-week old Roman rats. Differences in the ACTH (but not corticosterone) response to the open field were observed for rats more than 20-week old. A marked difference in prolactin reactivity, however, was found again at all ages, with RHA rats displaying little or no increase in prolactin concentrations following the open field test. In the same study, differences in open field defecation between the two strains did not appear before 42 weeks of age.

Correlational analysis in both segregating (F_2 and back-crosses) and nonsegregating (RHA, RLA and F_1) generations showed a clear association between the avoidance behaviour in the shuttle box and the prolactin reactivity to a novel environment, suggesting that both traits are affected by common neurobiological mechanisms. On the other hand, the corticosterone response to a stressful situation was not correlated with the avoidance behaviour in the shuttle box. A factor analysis confirmed the link between prolactin and avoidance behaviour and revealed no clear association between this behaviour and the weight of adrenal glands and thymus (44).

These seemingly contradictory results indicate that no generalization should be made regarding the level of emotionality of each of the two Roman lines.

6.3. The SHR and the Wistar Kyoto rats and their derived strains

Developed at the University of Kyoto, Japan, two inbred strains derived from Wistar rats were established as a model of human essential hypertension (174). One of these strains, called the Spontaneously Hypertensive Rat (SHR), was selectively bred for the tendency to develop arterial hypertension, whereas the control strain (WKY), from which the SHRs were derived, maintains normal blood pressure. Although this is neither the only nor the first rat model of hypertension (159), SHR and WKY rats represent one of the most accepted and widely used pair of strains for the study of hypertension. Besides a large amount of studies searching for the basis of this specific pathology, a considerable number of investigations have looked at other neurophysiological and behavioural differences between these strains.

It has been shown, for example, that in anticipation to electric shock, SHR rats show greater and longer lasting increases in plasma levels of adrenaline and noradrenaline and greater increases in heart rate and blood pressure, when compared with WKY rats (132,161). Picotti et al. (185) also found that SHR rats show a higher noradrenaline response to cold exposure than WKY rats. In a review by McCarty (159), the author concludes that the two strains do not differ in the basal plasma levels of catecholamines, but SHR rats show greater and longer-lasting increases in response to a variety of stressors (handling, footshock, immobilization, etc). An exacerbated response of the sympathetic system in SHR rats, as compared with WKY and three other inbred strains, has also been found after forced swimming (8). Synthesis, release and uptake of catecholamines in the hypothalamus have been shown to differ between WKY and SHR rats. The magnitude and the direction of such differences vary among studies and among different hypothalamic regions (7,148,220). Differences in the dopaminergic and serotonergic systems in different brain regions have also been observed (118,126,137,145,220). On the other hand, contradictory results have been found regarding the activity/reactivity of the HPA axis (8,42,65,114,131,138,206).

In a dim-light open field test, where the rats could leave their open home cages, SHR rats left their cages sooner, spent more time and were more active inside the open field than WKY rats (135). When exposed to electric footshock, SHR rats jumped more and were more active than their WKY counterparts and the latter responded with higher immobility than the former (160). Hence, SHR rats react actively whereas WKY rats react passively to different stressful stimuli. This observation has led many authors to consider SHR as "hyperreactive to stress" which, at least from the behavioural point of view, supposes that immobility would reflect a lower, rather than a higher, emotional reactivity. This supposition, however, is questionable.

Behavioural studies on the two strains carried out in the 1960s and 1970s were reviewed by Tucker and Johnson (219) and by McCarty (159). Most of the results discussed confirm that SHR rats are more active than WKYs in novel situations and that this difference is already found in young animals that have not yet developed hypertension. However, whereas SHR rats are often characterized as hyperactive or as "behaviourally abnormal" (117,121), some studies show that this strain is not hyperactive when compared with other unrelated rat strains. WKY rats showed lower ambulation in the open field test when compared with F344, SHR and Wistar rats, whereas SHR rats were not different from the other two groups (180). Results from our laboratory (188) also suggest that SHR rats have the same activity level as several other inbred strains and it is the WKY strain that is in general "hypoactive" under novelty.

Hård et al. (112) confirmed the higher activity of SHR rats in the open field and showed that this strain presented lower auditory startle response when compared with WKY rats, suggesting that the former are less fearful than the latter. Similar results, regarding the startle response, were obtained by Svensson et al. (211). A high tendency of WKY rats to develop ulcers following stress has been reported (180,181,189). Gentsch et al. (96) showed that SHR rats had more visits to the open arms of an elevated plus-maze and to the central area of an open field than WKY rats. Accordingly, Söderpalm (205) showed that SHR rats approached the open arms of a plus maze more than WKY rats, with no differences being detected in the Vogel's conflict test. SHR rats made more entries and spent more time in the open arms of a plus maze, showed higher central (but not total) locomotion in an open field and spent more time with social interaction in the social interaction test of anxiety than normotensive Wistar EPM-1 rats (101). In conclusion, for several indices of anxiety, SHR rats seem little anxious as compared with WKY as well as to several other strains.

In 1986, a new inbred strain was created combining the "hyperactivity" of SHR rats and the normotensive trait of WKY rats, which was designated as the Wistar-Kyoto hyperactive (WKHA) strain (119,120). The new strain was the product of a selection performed on a hybrid population resulting from a SHR × WKY cross. Starting with F_2 animals and continuing for seven generations, brother–sister pairs of rats were selected for high activity scores and low blood pressure. Following similar selection procedures (but now with brother–sister pairs being selected for low activity and high blood pressure), Hendley and Ohlsson (117) developed a fourth inbred strain called Wistar-Kyoto hypertensive (WKHT), with high blood pressure, similar to those found in SHR rats and low activity scores, similar to those found in WKY rats.

When submitted to electric footshocks, the two "hyperactive" strains, SHR and WKHA, responded with higher increases in plasma catecholamines when compared with the "hypoactive" WKY rats. When exposed to air-jets, SHR and WKHA rats also showed higher cardiovascular reactivity when compared with WKHT and WKY rats (136). These results suggest that high sympathetic and

cardiovascular responses to stressful conditions are associated with hyperactivity rather than with hypertension. WKHA (and to a lesser degree SHR and WKHT) rats had a reduced prolactin response to open field exposure, in contrast to WKY rats (42). In this sense, some similarity is found between two pairs of strains, WKY/WKHA and RLA/RHA which, as already discussed, present differences in activity as well as in prolactin response to stressors. No differences in prolactin were found between SHR and WKY rats following a forced swimming test (8). The study by Castanon et al. (42) confirmed that SHR and WKHA strains were more active and had higher heart rates than WKY and WKHT strains. It is interesting to note, however, that WKHA rats were even more active than SHR rats in the activity chamber and in the periphery of the open field. In contrast, SHR rats made more crossings and more rearings in the center of the open field (thought to be a sign of low fearfulness) than the WKHA rats. It seems, therefore, that WKHA did not ''inherit'' all behavioural traits from the SHR strain.

The SHR and WKY derived strains provide a remarkable illustration of how a set of phenotypic traits which are seemingly associated (for being simultaneously expressed in a single strain) can be genetically dissociated following adequate genetic experiments. For example (see earlier), through selection procedures, hypertension and hyperactivity (both present in SHR rats) have been shown to be genetically independent. Moreover, differences between WKHA and WKY rats regarding (i) the locomotion in novel environments and (ii) anxiety-related behaviours in the elevated plus maze, were shown to be dissociated within a segregating population (F_2) derived from these two strains (55).

6.4. The Tsukuba strains

The Tsukuba rat strains started to be selected in 1972 at Tokyo, being transferred later to the University of Tsukuba, Japan (90). In spite of their use in research having been somewhat restricted to their country of origin, a large amount of behavioural and physiological information on these strains has been produced since their selection (for a review see (93)). From an initial population of Wistar rats, individuals were selected in a long (125 cm) runway mildly illuminated (85 lux), connected through a hole to a non-illuminated start box. Rats were placed in the start box and 30 s later a door was open giving access to the runway. Tests lasted 5 min and were repeated for three consecutive days. Total ambulation scores were used as the selection criterion for 35 generations of brother/sister matings. Thereafter, selection was not continuous, being performed every five generations (154,155).

After 34 generations, two strains with large differences regarding the selected trait were established as the Tsukuba High-Emotional (THE) and the Tsukuba Low-Emotional (TLE) strains. Interestingly, across generations, defecation during testing increased for THE and decreased for TLE rats (171). In the runway test, THE rats (the less active ones) showed higher latencies to leave the start box and took longer to arrive to the end of the runway. In the open field (with or without shelter), THE rats were again less active (they stayed still in the corner of the open field) than TLE rats, a difference found in all other novel situations

(91,133,134,172). A general conclusion, according to the authors, is that ''TLE rats coped with novel stimulations in an active manner, while THE animals did it in an inactive or passive manner'' (93). Such a profile could be compared with those observed in the Maudsley, the Roman and the Wistar-Kyoto derived strains. It is interesting to notice that, in a test of spontaneous activity (10 days), TLE rats were more active than THE rats, not only during the first 3 h (novel environment) but also during the night periods once the activity was already stable (6th to 9th day of testing) (173). The same pattern was observed for males during 24 h in a burrow-available habitat, were the animals had the opportunity to dig holes in the ground (93). Therefore, differences in activity in novel environments may relate, to some extent, to differences in spontaneous activity, in spite of the former being of greater magnitude than the latter.

In the shuttle box, TLE rats showed higher active avoidance acquisition and less defecation than THE rats (92). Differences in the avoidance behaviour of these strains were, however, of much smaller magnitude than those observed between the Roman lines. THE also vocalized more than TLE during handling (171). In addition, TLE rats had higher concentrations of noradrenaline and adrenaline in many regions of the brain. The possibility of a highly active sympathetic nervous system associated with high activity levels in TLE rats parallels the behavioural and neuroendocrinological profile of SHR and WKHA rats. Factor analyses on different behaviours in novel environments produced one main factor with high loadings from all measures of ambulation. It is along this axis, called ''activity'', that the two Tsukuba strains are likely to have been selected (133,134).

6.5. Towards a molecular approach

We have indicated in the previous section how emotionality is likely to be a complex multidimensional construct. Accordingly, considering that all the genetic models just described have not been selected according to the same criterion, different dimensions of emotionality are possibly represented by the different pairs of strains. By comparing the phenotypic profiles of these models, one finds, indeed, that all the ''high emotional'' strains do not display the same patterns of emotional response, the same being true for the ''low emotional'' strains. On the other hand, several similarities can be found among the different models. For example, there is a consistency among models regarding their locomotor reactivity to aversive stimuli (and more specifically to novelty). In general, one of the strains tends to react actively (e.g. MNR, RHA, SHR, and TLE) and the other tends to react passively (e.g. MR, RLA, WKY and THE) when exposed to a stressful situation. As a result, pairs of strains generally contrast in their activity scores in the open field test. Differences in defecation, to a certain extent, parallel the differences in activity, being negatively related to it.

An increased secretion of noradrenaline has been observed in Wistar rats displaying extreme high scores of activity and low scores of defecation (18). Indications of a higher sympathetic activity in the most behaviourally active (less ''emotional'') strains have been found in the Maudsley rats (MNR), in the WKY-derived rats (SHR and WKHA)

and in the Tsukuba rats (TLE). In addition, a low prolactin response displayed by the highly active rats when exposed to stressful stimuli has been observed in the Roman and WKHA strains. Any generalisation regarding a putative association of traits should certainly be avoided, since additional information about these and other genetic models can disprove any speculative assumption. Nevertheless, the behavioural similarities among the different models discussed above are worthy of attention.

In the previous section, it has been shown that locomotion in novel environments can be dissociated from other measures thought to reflect anxiety (13,80,177,218). The results presented above suggest that those strains considered as non-emotional on the basis on their high activity and low defecation scores from the open field (and from other tests) will not necessarily behave in a non-anxious way in tests of anxiety (e.g. the plus maze, the black and white box, the defensive burying paradigm and the social interaction test). Further testing of this hypothesis is essential, since the assumption that high emotionality (classically measured in the open field test) will naturally correspond to high anxiety is often observed in the literature. Additional information is also needed regarding the contribution of the various dimensions of emotional reactivity upon the measures obtained from the different behavioural tests. The multidimensional characterization of different strains of animals on the same set of tests may be particularly useful in this matter.

An illustration of the usefulness of genetic and multivariate approaches to shed some light on the multidimensionality of stress is represented by two series of experiences carried out in our laboratory. A behavioural and neuroendocrine characterization of WKY and WKHA rats has revealed (and confirmed) marked differences between these two strains regarding several measures of stress (55). As compared with WKY, WKHA rats displayed higher locomotion scores under novel and familiar environments; higher central locomotion in the open field test; higher approach of the open arms in the elevated plus-maze; lower defecation in the open field and lower neuroendocrine responses (corticosterone, prolactin and renin) to a 10-min exposure to a novel environment. These results might suggest that the higher activity in novel environments, the lower anxiety (as measured in the plus maze) and the lower neuroendocrine responses to a stressor are all genetically related traits, since all of them are present in the WKHA strain. Nevertheless, a correlational study of these phenotypes in an F_2 generation derived from the two parental strains has shown this not to be the case. Not only were the different neuroendocrine measures shown to segregate independently of each other, but no important correlation was found between neuroendocrine and behavioural traits. Moreover, a factor analysis of the behavioural measures in the F_2 generation has revealed that activity (measured in novel and familiar environments) and anxiety (measured in the elevated plus-maze) produce two independent axes which must represent two different dimensions of emotionality. In addition, defecation scores (open-field) loaded alone on a third factor, showing again that this classical measure of emotionality can be dissociated from other measures of activity and anxiety. This study thus further illustrates that the co-variation of traits among parental strains does not necessarily imply that a genetic link exists between these traits.

Besides this genetic and multiple-testing approach, molecular studies at the genomic level may provide additional information on the genetic association between phenotypic traits, as well as a novel perspective on the molecular mechanisms underlying the different dimensions of stress. By working with a large number of DNA markers, which are polymorphic for the parental strains and well spread along the whole genome, one can perform a genetic linkage analysis that shall reveal one or several QTL (quantitative trait loci, i.e. genomic regions containing one or more genes affecting a quantitative phenotype) involved in the determination of a trait. Since no a priori hypothesis is required and since the search is not based on candidate genomic regions (or candidate neurobiological systems), such a broad approach may reveal important genes and/or systems that would not be otherwise detected. Alternatively, the study of gene expression in different regions of the brain, without a priori assumptions (analysis of global mRNA and protein populations), has been proposed as another tool to investigate the biological bases of emotionality (225).

In recent years, different research groups have attempted to map QTL involved in the determination of emotionality-related behaviours in rodents. For example, by the use of four phenotypic markers (i.e. phenotypic traits), which allowed the determination of the corresponding genotypes of F_2 and backcrossed mice (derived from two inbred strains), two chromosomal regions were shown to be associated to the peripheral locomotion in a dim-light open field (50). Through a different approach (the use of molecular markers to characterise 22 recombinant inbred strains), several chromosomal regions likely to influence the open field behaviour (either baseline or following restraint) of mice have been revealed (213).

Another recent contribution to the field of behavioural genetics is a study by Flint et al. (88). Using the F_2 generation derived from two inbred mouse strains selected for high and low activity in the open field, this study revealed (by the use of 84 genome markers) six loci that significantly influenced open field activity. Interestingly, three out of the six loci were also associated with three other measures: open field defecation, activity in a Y maze and open arm activity in the elevated plus-maze. From these findings, it could be suggested that activity in novel environments and other anxiety measures do pertain to the same psychological construct. However, the plus maze measure used in this study was the absolute number of entries in the open arms rather than the classical measures of anxiety (percentage of entries in the open arms and time spent in the open arms). Entries in the open arms expressed as percentage of the total entries correspond to a preference index. This measure has been pharmacologically validated and it has been used typically in anxiety experiments (13,30,59,109,147,184,198). The absolute number of open arm entries, on the contrary, has been shown to be contaminated by locomotor levels, impeding its use as an index of anxiety (59).

The reason for not using the open/total ratio in the study by Flint et al. (88) was that this parameter was not correlated with the so-called measures of emotionality (open field activity and defecation). The lack of correlation between this classical measure of anxiety and the other measures of emotionality in a segregating population further indicates,
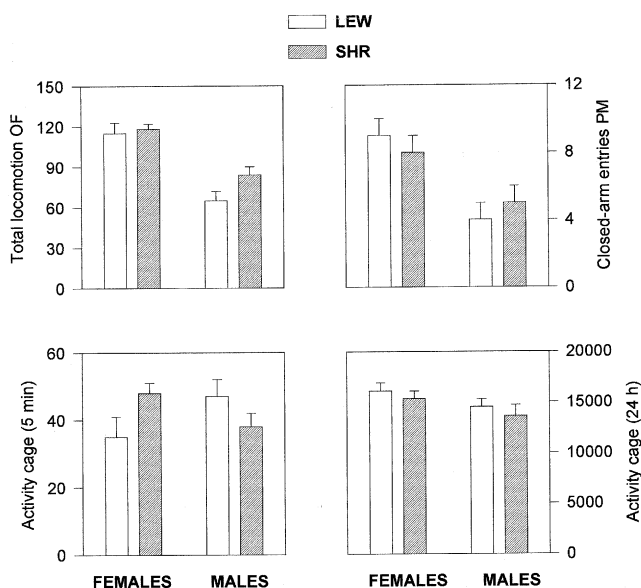
FIG. 2. Measures of locomotor activity of Lewis (LEW) and Spontaneously Hypertensive Rats (SHR) in different behavioural tests: the open field (OF) test (total number of squares crossed); the elevated plus maze (PM) (number of closed-arm entries) and the activity cage (number of beam breaks) in tests of either 5 min (individual animals in novel cage) or 24 h (grouped animals in familiar cage). Except for the activity cage in 24 h (where $N = 6$), all the other values are the means and SEM of $N = 12$. Within each sex, no significant differences ($P > 0.05$) were found between the two strains.
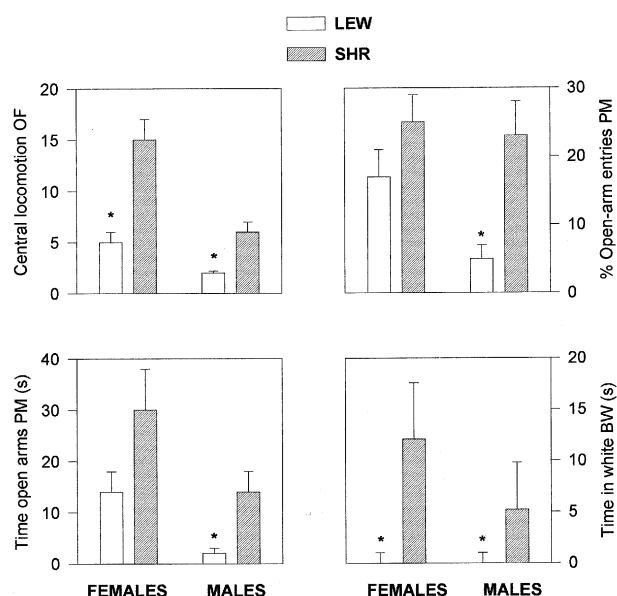
FIG. 3. Putative anxiety measures of Lewis (LEW) and Spontaneously Hypertensive Rats (SHR) in different behavioural tests: the open field (OF) test (number of inner squares crossed); the elevated plus maze (PM) (open-arm entries expressed as a percentage of the total number of entries and time spent in the open arms) and the black and white box (BW) (total time of visit to the white compartment). For all measures, the values are the means and SEM of $N = 12$. Significant interstrain differences ($P < 0.05$) within each sex are represented by *.

in fact, that the open field measures do not reflect the same trait as other behaviours known to respond to anxiolytic and anxiogenic drugs.

To further illustrate this point, we will briefly mention a genetic linkage study performed in our laboratory with the WKY and WKHA strains. Following the phenotypic characterization of 196 rats from the F_2 generation (as discussed earlier), each of these animals had its DNA genotyped for 67 polymorphic microsatellite markers. A linkage analysis was performed, detecting a major QTL on chromosome 8 which strongly influenced activity measures in both novel and familiar environments (167). Interestingly, this QTL was not associated either with measures of anxiety in the elevated plus-maze or with defecation scores in the open field. The fact that spontaneous motor activity and locomotion in a novel environment are both influenced by the same genomic region further suggests that much care should be taken when considering the latter measure as a reliable index of emotionality or anxiety.

As illustrated above, searching for QTL that influence emotionality in animals without recognising the multi-dimensionality of this construct can be misleading. Each single measure of emotionality (as already discussed) may be affected by different underlying mechanisms, some of which may have no association with emotionality as such (e.g. spontaneous locomotor activity). However, if a multivariate analysis is able to reveal a number of factors that represent the different psychobiological aspects affecting a given set of measures, then it may be useful to perform a QTL analysis using each factor as one phenotypic trait. As we saw in the last section, the factors are linear combinations of the original variables and, as such, they can be considered as new synthetic variables. Since one can easily

calculate the factor scores for each individual, it should be simple to use these scores (instead of/in addition to the original variables) in the QTL analysis. If such an approach proves successful, this might lower the risks of carrying out a long research program to study genes affecting a given behaviour, when these genes have no real influence on the psychological dimension of interest.

Once a QTL has been detected, the investigation on the biological bases underlying the phenotype can be further developed, either by the study of candidate genes mapped in the same chromosomic region, or by the positional cloning (i.e. localization and isolation) of the gene(s) associated to this specific QTL, as it has been previously performed for other non-psychological traits (e.g. the obesity in mice) (233).

Based on the experimental evidence just discussed, we have recently decided to further investigate the dissociation between locomotor activity and anxiety. To this end, we have searched for a new pair of strains which did not differ in novel-environment activity while displaying a maximal contrast in recognized models of anxiety. Following the behavioural characterization of six inbred rat strains in four models of anxiety/emotionality (open field, elevated plus-maze, black and white box and social interaction test), we have found that SHR and Lewis rats satisfied these criteria (188). As shown in Fig. 2, the two strains did not differ in several measures of locomotion, such as: (i) the total locomotion in the open field; (ii) the number of closed-arm entries in the plus maze; (iii) the activity scores (5 min) in activity cages (novel environment), and (iv) the activity scores (24 h) in activity cages (familiar environment). Similarly, no interstrain differences were found in the defecation scores in the open field. On the contrary, the two strains showed significant differences (Fig. 3) in: (i) the central locomotion in the open field; (ii) the number of entries and

(iii) the time spent in the white compartment of the black and white box and (iv) the time spent and (v) the percentage of entries in the open arms of the plus maze. In all of these situations, SHR rats displayed less avoidance of the aversive stimuli as compared with Lewis rats. Unexpected results were only obtained in the social interaction test, where the time of social interaction was not different for males and it was greater for Lewis rats among the females.

Further studies (similar to those applied for WKY/WKHA rats) are being carried out to better characterize these two strains and to investigate the genetic links among the different phenotypic traits. Nevertheless, these initial results already support the hypothesis of a dissociation between different dimensions of emotionality as well as the possibility of developing distinct genetic models for the investigation of distinct psychological phenomena.

In summary, the information presented and discussed in this paper supports the view of emotionality as a multidimensional construct and indicates that the different dimensions of it may be revealed by factor analyses on multivariate data sets. The use of genetic models with intercrosses of contrasting strains represents an interesting approach for the search of the genetic and neurobiological mechanisms underlying different aspects of emotionality. By the simultaneous analysis of different dimensions of emotional reactivity a better comprehension of this complex construct may be acquired.

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