
2 Experimental Protocols for the Study of Stress in Animals and Humans

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KEY POINTS

- Stress is the response of an organism to a stressor of physical, chemical, or emotional nature.
- Exposure to stressors induces behavioral and neuroendocrine consequences in experimental animals as well as in humans, and this complex response can be adaptive or maladaptive.
- Experimentally, the exposure to different stressors is used in order to study the evoked responses and the mechanisms underlying them or to modify the behavior of animals in an attempt to reproduce reliable models of psychiatric symptoms with a stress-related component in humans.
- In animals of the same species, strain, sex, and age maintained in controlled environmental conditions, we can expect reproducible behavioral and neuroendocrine responses to stressful protocols, which are proportional to the intensity of the stressor and the duration of the exposure. The reproducibility of the response is crucially bound to the controlled experimental conditions used.
- In human experiments, the main difficulties in controlling experimental conditions are not related to the stressor (intensity and duration of exposure ethically acceptable), but are mainly related to the large interindividual variability in sensitivity to any kind of traumatic stimulus or event, which can sometimes be explained on the basis of genetic variables or particular personal histories.

1. INTRODUCTION

The term “stress” has several meanings; in behavioral research it is used in the sense of “a physical, chemical, or emotional factor (as trauma, histamine, or fear) to which an individual fails to make a satisfactory adaptation” or “the state or condition of strain (Webster’s dictionary, 1971).” Selye used “stress” to indicate the response of an organism to a “stressor” and made a distinction between adaptive and maladaptive responses (Selye, 1950, 1974). Emotional stimuli such as novelty, withholding of reward, and anticipation of punishment (rather than punishment itself) are the most frequent stressors and among the most efficacious activators of the neuroendocrine systems that play a role

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in stress responses (Mason, 1968, 1975). A more comprehensive definition of stress given by Goldstein (1987) is a condition when expectations, whether genetically programmed or established by a prior learning, do not match the current or anticipated perceptions of internal or external environment. This discrepancy causes a complex range of adaptive responses, whose pattern is dependent upon the type and duration of the provoking event (Chrousos, 1998; Pacak & Palkovits, 2001).

1.1. Modeling of Stress in Animals

Exposure to stressors induces behavioral and neuroendocrine sequelae that are often used to experimentally mimic in animals the symptoms that characterize specific human psychiatric disorders (Willner, 1995). This is possible because animals of the same species, strain, sex, and age maintained in controlled environmental conditions show a sufficiently homogeneous response to stressful conditions that is proportional to the intensity of the stressor and the duration of the exposure. Thus, a stress protocol can be calibrated and standardized in order to obtain reproducible behavioral and neuroendocrine modifications. These stress procedures should never exceed the rigid limits imposed by the international ethical committees, and their reproducibility is crucially bound to the controlled experimental conditions used. Within these limits we can design stressful procedures that may result in either adaptive or maladaptive reactions by acting more on the degree of control allowed to the animal on the stressor than on the stressor intensity.

1.2. Modeling of Stress in Humans

Exposure to comparable levels of stressful situations and controlled conditions is not possible in experiments on human beings, as common sense and stringent ethical rules absolutely limit the use of aversive stimuli. On the other hand, solving a simple arithmetical problem or participating in an easy game in front of one or more examiners may elicit an emotional reaction similar to that of a student undergoing examination, and different tests have been devised and validated in healthy voluntary subjects that induce psychological stress and measurable neuroendocrine responses. The main difficulties in controlling experimental conditions in human experiments are not primarily related to the stressor, but to the vast interindividual variability in sensitivity to any kind of traumatic stimulus or event (Kroll, 2003). This point seems to contradict the *Diagnostic and Statistical Manual of Mental Disorders*, which, when defining the causes of posttraumatic stress disorder (PTSD), states that “[the] severity, duration, and proximity of an individual exposure to the traumatic event are the most important factors affecting the likelihood of developing this disorder” (American Psychiatric Association [APA], 2000). There is no doubt that trauma is the necessary disease agent, but it is never a sufficient predictor of PTSD development. Other factors, both genetic (Bouchard, Lykken, McGue, Segal, & Tellegen, 1990) and acquired, such as previously experienced traumatic events (Breslau, Chilcoat, Kessler, & Davis, 1999) or strong cultural traditions (Welsaeth, 2002), may exert strong control over the individual acute reactivity to stressful events and the long-term consequences.

1.3. Uses of Stress Models

To minimize confusion we will use the terms “stressor” to define a stimulus or event that perturbs the equilibrium in an organism and “stress” to define the response of the

organism to such a stimulus or event. Experimentally, the effects of different stressors are useful to study the evoked responses and the mechanisms underlying them or to modify the behavior of animals in an attempt to reproduce reliable models of psychiatric symptoms that may have a stress-related component in humans. The two approaches differ only in the final aim, as both require very strictly controlled experimental conditions. An experimental model of a psychiatric disease must fulfill three basic requirements: face validity, as the behavioral modification should mimic a psychiatric symptom; predictive validity, as the behavioral modification should be reverted by the psychotropic drugs that control the spontaneous symptom; and construct validity, as the mechanisms underpinning the behavioral modification should be similar to those considered responsible, or associated with, the psychiatric symptom (Willner, 1995). These requirements are used to validate a model independently of the modality (pharmacological, genetic, or environmental) used to obtain it.

2. STRESSOR EXPOSURE AS A MODEL OF PSYCHIATRIC SYMPTOMS

2.1. *Depression*

We have devised two models for studying depression based on animal exposure to unavoidable stressors (Gambarana, Scheggi, Tagliamonte, Tolu, & De Montis, 2001). Rats exposed to a noxious avoidable stimulus quickly learn to avoid it; thus, when administered a sequence of threshold electric tail-shocks, a naive animal escapes from an average of 26 out of 30 shocks. This escape competence can be disrupted by previous exposure to an unavoidable stressor; in this condition, when tested for escape, the animal escapes from an average of 3–5 out of 30 consecutive tail-shocks (Gambarana et al., 2001). This escape deficit, which is a modification of the classical learned helplessness syndrome (Overmier & Seligman, 1967; Sherman, Sacquitine, & Petty, 1982), implies an *N*-methyl-D-aspartate (NMDA)-dependent neuronal plasticity process, since the acute administration of 0.1 mg/kg of dizocilpine, an NMDA receptor antagonist, 30 min before unavoidable stressor exposure completely prevents the development of escape deficit (Gambarana et al., 2001). The unavoidable stressor has been standardized (Maier, 1986) and consists of a series of 48 tail-shocks administered in 50 min in a condition of complete immobilization. It appears that the stressed rat “learns” that any effort to avoid the noxious stimulus is ineffective. This form of aversive memory is short-lived: it is maximal 24 h after unavoidable stressor exposure, and it rapidly declines within 48–72 h (Gambarana et al., 2001). This decline is dependent on μ -opioid receptor functionality, since the subcutaneous infusion of naloxone by osmotic minipump (1 mg/kg/24 h) postpones recovery as long as naloxone is infused (Fig. 1). The development of acute escape deficit is prevented by previous repeated administration of classical antidepressants. That is, imipramine, fluoxetine, clomipramine, phenelzine, mirtazapine, and reboxetine administered acutely before an unavoidable stressor show no protective effect; however, when they are administered for 14–21 d, depending on the compound, a preventive, protective effect is consistently observed (Gambarana et al., 1995; Raugg, et al., 2005). The experimental conditions must be strictly controlled, as adjunctive stressors may easily alter animals’ performance. For instance, experiments are always carried out on rats fed *ad libitum*, since the condition of fasting may completely protect them from the behavioral sequelae of unavoidable stressor exposure (Fig. 2).

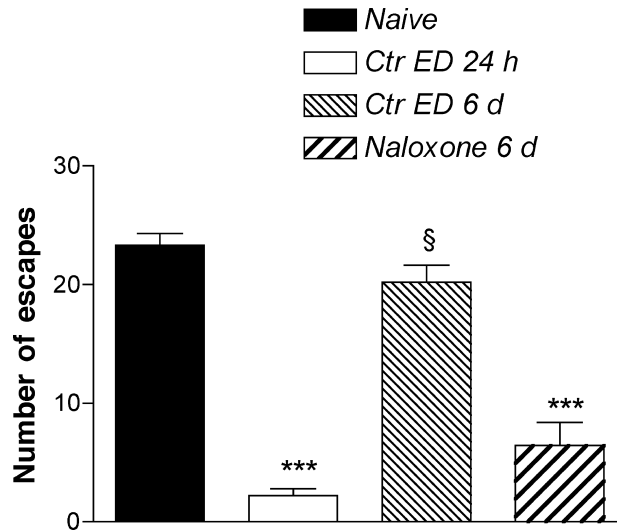


Fig. 1. Critical role of μ -opioid receptors in the extinction of unavoidable stressor-induced escape deficit. Twenty-four hours after exposure to unavoidable shocks, rats showed a clear-cut escape deficit (*Ctr ED 24 h*) compared to the performance of stress-naïve rats (*Naive*). This behavioral deficit rapidly declined within 48–72 h, and by d 6 rats had completely recovered (*Ctr ED 6 d*). Rats infused with naloxone subcutaneously by osmotic minipumps (1 mg/kg/24 h), exposed to unavoidable shocks under naloxone infusion and 6 d later to escape test (*Naloxone 6 d*), did not recover a normal escape response. Values represent mean \pm SEM of number of escapes ($n = 8$ in each group). *** $p < 0.001$ compared to the number of escapes of the *Naive* group, § $p < 0.001$ compared to the number of escapes of the *Ctr ED 24 h* group (one-way ANOVA followed by Bonferroni's test).

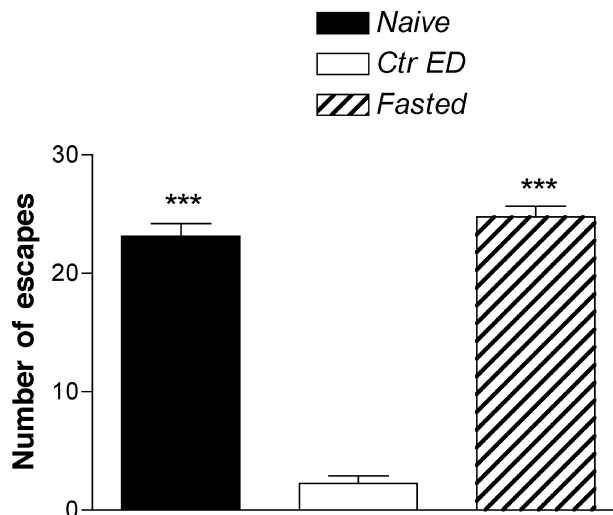


Fig. 2. Protective effect of a 24-h fast period on the development of escape deficit (ED). Rats were fasted for 24 h and then exposed to unavoidable shocks. Eight hours after stressor exposure, rats had unlimited access to food and they underwent the escape test 24 h after the unavoidable shocks. Values represent mean \pm SEM of number of escapes ($n = 9$ in each group). *** $p < 0.001$ compared to the number of escapes of the *Ctr ED* group (one-way ANOVA followed by Bonferroni's test).

The stressor-induced models of depression most often used in rats or mice are the forced swim test and the tail suspension test. The forced swim test, also known as the Porsolt test, involves placing the animal in a tank filled with tepid water, where both rats and mice will struggle in the attempt to jump out, and measuring the latency for the animal to become immobile (Porsolt, LePichon, & Jalfre, 1977). Acute or short-term (3–4 administrations within 24 h) treatment with most antidepressants prolongs this latency and decreases the duration of immobility. A skilled experimenter can distinguish the class of antidepressant used from the pattern of movements of the animals; thus, norepinephrine reuptake blockers may increase climbing behavior, whereas selective serotonin reuptake inhibitors (SSRIs) increase swimming (Detke, Rickels, & Lucki, 1995). An interpretation of the test as a model of depression is that immobility time is a symptom of reduced reactivity to an aversive environment, and the fact that the administration of an antidepressant prolongs the struggling or swimming time should give predictive validity to the model. A main criticism of this interpretation is that a very short-term treatment is sufficient for shortening immobility time, and this fact conflicts with the delay necessary for an antidepressant compound to develop its therapeutic activity. This discrepancy limits the validity of Porsolt's test as a model of depression, but does not reduce its utility as a relatively rapid testing protocol for detecting agents with antidepressant-like activity. The tail suspension test, a variant of the forced swim test, is used in mice (Steru, Chermat, Thierry, & Simon, 1985; Steru et al., 1987). The mouse is suspended by its tail and the time it takes to become immobile (i.e., to hang passively upside down) is measured. Acute administration of most antidepressants decreases immobility. Thus, this model presents the same limits as the forced swim test, but the same adaptability as a quick screening tool.

False positives in the three described acute tests include drugs that are stimulants (and hence decrease immobility) but not antidepressants. In the acute escape deficit model, central stimulants increase the number of escapes when administered before the escape test but show no protective activity when given before the unavoidable stressor (Gambarana, Ghiglieri, Taddei, Tagliamonte, & De Montis, 1995).

2.2. Anxiety

The spontaneous capacity of animals to avoid aversive stimuli is often used to model anxiety. Both mice and rats prefer a protected to an open environment, and when placed in an elevated-plus maze (i.e., a maze with two closed arms and two open arms elevated above the floor level) they explore it but spend most of the time in the arms of the maze protected by walls. Since the acute administration of benzodiazepines or other anxiolytic drugs at a dose that does not modify spontaneous motility prolongs the time spent by animals in the open arms, the elevated-plus maze is used as a model of anxiety (Lister, 1987; Pellow, Chopin, File, & Briley, 1985). A mild physical stressor such as a continuous electric current in the metal tip of a water bottle does not completely prevent rats from drinking, but it reduces water consumption. Again, acute benzodiazepine administration reinstates basal water consumption, and this behavioral paradigm is used as a model of anxiety (Vogel, Beer, & Clody, 1971).

2.3. Chronic Models

The models so far described allow almost exclusively the study of the preventive effects of drugs on experimentally modified behaviors. When exposure to unavoidable stressors is repeated, the condition of reduced reactivity to environmental stimuli may be pro-

longed, and it may outlast by several days the end of stressor exposure. Different kinds of stressors, such as immobilization, repeated electric shocks, etc., have been used. Repeated episodes of immobilization are frequently used to study the neuroendocrinological and metabolic stress responses, rather than to induce reliable behavioral modifications. One of the most elegant long-term models of depression is the chronic mild stress (CMS) procedure devised by Willner (Papp, Moryl, & Willner, 1996; Papp, Willner, & Muscat, 1991). In the CMS model, chronic sequential exposure of rats to a variety of mild stressors has been shown to decrease the drinking of a sweetened solution, a condition that could be reversed by the chronic administration of classical antidepressant drugs as well as dopaminergic agonists (Muscat, Papp, & Willner, 1992a, 1992b; Muscat, Sampson, & Willner, 1990; Papp et al., 1996). Exposure to chronic mild stress also impairs the acquisition of place preference conditioning, in parallel with sucrose consumption (Papp et al., 1991). All of these deficits are reversed by chronic treatment with clinically effective antidepressant drugs (Moreau, Jenck, Martin, Mortas, & Haefely, 1992; Muscat et al., 1990, 1992b; Papp et al., 1991, 1996; Willner, Towell, Sampson, Sophokleous, & Muscat, 1987).

The condition of escape deficit induced by a single exposure to unavoidable shocks can be maintained indefinitely by exposing rats that have developed the deficit to a sequence of milder stressors on alternate days, such as a brief immobilization period, a few tail-shocks, or exposure to the room in which shocks were previously administered (Gambarana et al., 2001). Moreover, 14 d after the last stressor exposure, chronically stressed rats still present a clear-cut escape deficit (Mangiavacchi et al., 2001). No significant differences in the amount of daily food and water consumption or in the curve of body weight increase is observed between control rats and rats exposed to this procedure for 3 wk (Mangiavacchi et al., 2001). Daily administration of a classical antidepressant, beginning 24 h after initial exposure to unavoidable shocks and continuing during chronic stressor exposure, results in reversal of the escape deficit after ≥ 3 wk of treatment (Gambarana et al., 2001). Thus, repeated administration of antidepressant compounds not only prevents the development of escape deficit, but also reverts it once established. This is strong proof of predictive validity in favor of the escape deficit as a model of depression.

After a 3-wk exposure to chronic stressors, rats show a decreased output of dopamine (DA) and serotonin (5-HT) in the medial prefrontal cortex (mPFC) and in the shell of the nucleus accumbens (NAcS), as detected by microdialysis procedure, which may last for days after the last stressor exposure (Mangiavacchi et al., 2001). Moreover, these rats also show a steady increase in plasma corticosterone levels and a decrease in glucocorticoid receptors in discrete brain areas such as the hippocampus and mPFC (De Montis, Rauggi, Scheggi, & Tagliamonte, 2004). These intense, long-lasting monoaminergic deficits, associated with a condition of increased hypothalamic–pituitary–adrenal (HPA) axis activity, are reminiscent of the serotonergic and catecholaminergic theories of depression and of the enhanced HPA axis activity observed in depressed patients (Halbreich, Asnis, Schindldecker, Zurnoff, & Nathan, 1985; Meltzer & Lowy, 1987; Schildkraut, 1965; Willner, 1983). Thus, altogether they constitute a strong neurobiological and endocrinological construct validity proof for defining chronic escape deficit as a model of depression.

Exposure to chronic stressful procedures also reduces spontaneous reactivity to rewarding stimuli in rats, as already mentioned for the CMS model. Rats are very fond

of vanilla sugar (VS), and they still consume it when fed *ad libitum* on a standard diet. Indeed, VS maintains its reinforcing property in satiated rats that consistently learn to choose between the two divergent arms of a Y-maze, the one contingently baited with VS (Ghiglieri et al., 1997). The disruption of the VS-sustained appetitive behavior (VAB) by chronic exposure to unavoidable stressors was developed as a model of anhedonia, which is a core symptom of depression, since rats exposed to the chronic stressor procedure during the training phase show reduced interest in the bait and never acquire the appetitive behavior (Ghiglieri et al., 1997). Interestingly, both the escape deficit and the impairment of VAB learning in the Y-maze are reversed by a chronic treatment with antidepressant drugs such as imipramine or fluoxetine. These findings further strengthen the face validity of the chronic stressor procedure, which is able to induce a reduced reactivity to both aversive and pleasurable stimuli. In addition, they also strengthen its predictive validity, as the administration of classical antidepressants antagonizes both its behavioral and neurochemical effects (Ghiglieri et al., 1997).

The effect of chronic exposure to an unavoidable stressor on VAB acquisition was crucial for clarifying the mechanism that underlies the chronic stress-reduced enticement toward palatable food. In fact, we observed that the dopaminergic response to VS consumption in satiated rats presented with VS for the first time is predictive of VS-reinforcing activity (Gambarana et al., 2003). Only rats that show a consistent increase in dopamine release in the mPFC and NAcS in response to VS consumption are able to learn VAB (Gambarana et al., 2003). Accordingly, rats that underwent CMS also showed a decreased DA response to palatable food consumption in the mesolimbic areas (Di Chiara & Tanda, 1997).

The administration of antidepressant drugs antagonizes the disrupting effect of chronic stressors on VAB acquisition to the limited extent that the treatment is initiated long before stress exposure (Gambarana et al., 2001). When rats begin imipramine or fluoxetine treatment soon after exposure to unavoidable shocks and escape test and they begin Y-maze training after a 7-d exposure to chronic stressors and antidepressant treatment, they never acquire VAB (Gambarana et al., unpublished results). Interestingly, VS consumption at the end of the first week of combined stressor exposure and antidepressant treatment does not induce a dopaminergic response in the NAcS (Table 1). Rats exposed to this experimental protocol began Y-maze training in a condition of decreased or absent competence to perceive VS as a palatable food, and they probably perceived Y-maze training as an adjunctive stressor. Accordingly, after 3 wk of daily antidepressant treatment, the escape deficit was reverted, but they had not learned the appetitive behavior. Thus, while imipramine and fluoxetine can reverse a state of stressor-induced escape deficit, they seem to have no effect on stressor-induced devaluation of food palatability.

2.4. Models of Adaptive Stress

We try, experimentally, to calibrate stressor intensity and length of exposure on the basis of the stress response of an organism, since it is impossible to predict *a priori* the limit between an adaptive and a maladaptive stress. The experimental protocols described so far are designed to induce a maladaptive stress response in the organism, and thus mimic some of the core symptoms of psychiatric diseases. A very common stressor used to induce adaptive stress is food restriction of different degrees; fasted animals show a very low response threshold to different environmental stimuli and appear to be in a latent state

Table 1

Dopamine Accumulation in Response to Palatable Food Consumption in the Nucleus Accumbens Shell of Rats Fed *Ad Libitum*

<i>Group</i>	<i>Maximum (pg/10 μL) increase in DA levels</i>
<i>Ctrl</i>	37.3 \pm 1.1
<i>Stress</i>	5.4 \pm 3.3***
<i>Stress + IMI</i>	3.3 \pm 2.3***
<i>Stress + FLX</i>	4.6 \pm 2.2***

Rats were exposed to unavoidable shocks and escape test, then to chronic stressor protocol for 7 d. Imipramine (IMI) (5 mg/kg i p, twice a day) and fluoxetine (FLX) treatments (5 mg/kg/day, ip) began soon after the escape test. Twenty-four hours after the last stressor exposure and drug treatment, rats were implanted with microdialysis probes in the nucleus accumbens shell, and microdialysis was performed the following day. When consistent baseline dopamine (DA) levels were attained ($\geq 10\%$ between sample variation), rats were presented with five vanilla sugar (VS) pellets. Rats in the *Ctrl* group ate all the pellets in less than 5 min; only 5 rats out of 12 in the *Stress* group, 6 out of 12 in the *Stress + IMI* group, and 5 out of 11 in the *Stress + FLX* group ate the VS pellets. Values represent the mean \pm SEM of maximum increases in extraneuronal DA levels after VS consumption minus baseline levels in each rat ($n = 10$ in the *Ctrl* group, 5 in the *Stress* and *Stress + FLX* group, 6 in the *Stress + IMI* group). *** $p < 0.001$ compared to the maximum increase in the *Ctrl* group (one-way ANOVA followed by Bonferroni's test).

of alert. Basal glucose consumption in the brain is significantly higher than that in any other organ, and this consumption further increases in a condition of stress. Thus, the stress response includes a cascade of metabolic events aimed at decreasing glucose consumption by peripheral organs in order to spare it for brain function (Peters et al., 2004). Exposure to a condition of caloric restriction is considered to be the easiest method of causing stress in animals, because eating is entirely instinctive, natural, and the most desirable behavior for animals, particularly for fasting animals (Martin & Seneviratne, 1997; Rodeck, 1969). A condition of partial, well-controlled food restriction produces gastric stress ulcers in rats (Yi & Stephan, 1998) and it is a widely used model of stressor. Moreover, chronic food restriction enhances sensitivity to the rewarding and motor-activating effects of amphetamine, cocaine, and other drugs of abuse (Bell, Stewart, Thompson, & Meisch, 1997; Cabeza de Vaca & Carr, 1998; Cabib, Orsini, Le Moal, & Piazza, 2000; Carr, Kim, & Cabeza de Vaca, 2000; Carroll & Meisch, 1984; Deroche et al., 1995), as many other stressors do (Antelman, Eichler, Black, & Kocan, 1980; Bozarth, Murray, & Wise, 1989; Deminihre et al., 1992; Deroche et al., 1992; Deroche, Piazza, Le Moal, & Simon, 1994; Pacchioni, Gioino, Assis, & Cancela, 2002).

Chronic food restriction activates complex endocrine and autonomic mechanisms that control food intake and energy metabolism (Berthoud, 2002). These mechanisms may lead to the initiation of ingestion, or they may simply lead to an adjustment in autonomic and/or endocrine outflow. In experimental chronic food restriction, the ingestive response is by definition partial, and the resulting changes in energy balance are orchestrated by a central neural network centered in the hypothalamus that receives and

integrates neural, metabolic, and endocrine signals and organizes appropriate responses of energy resources allocation and expenditure (Peters et al., 2004). The neocortex and the limbic–hypothalamus–pituitary–adrenal system control both energy resource allocation and intake. Brain neurons utilize glucose almost exclusively, and ATP-sensitive potassium channels are considered the detectors of glucose concentration in neurons of the neocortex that control the HPA axis and the autonomic sympathetic system (Peters et al., 2004). HPA axis and sympathetic system activation results in reduced glucose utilization in skeletal muscles and increased brain glucose availability, among other effects. High-affinity mineralocorticoid and low-affinity glucocorticoid receptors, located in the neurons of limbic areas such as the hippocampus and hypothalamus, determine the setpoint of the HPA system (Herman et al., 2003; Jacobson & Sapolsky, 1991). This setpoint can be modified by exposure to a chronic stressor, such as partial food restriction or decreased environmental temperature, and it can reach a new functional equilibrium characterized by increased reactivity to stressful stimuli. On the other hand, it can be permanently and pathologically disrupted by extreme chronic metabolic, psychological, and physical stressors such as starvation, infectious diseases, hormones, drugs, substances of abuse, or chemicals disrupting the endocrine system.

In this context, we may define the response to chronic food restriction as adaptive stress and the response to the chronic escape deficit procedure as maladaptive stress. Accordingly, diet-restricted rats and mice have improved cardiovascular stress responses (Wan, Camandola, & Mattson, 2003) and increased resistance to high temperature (Hall et al., 2000) and to a number of different toxins (Bruce-Keller, Umberger, McFall, & Mattson, 1999; Duan & Mattson, 1999), as well as to unavoidable stressor exposure (Fig. 2). Conversely, exposure to the chronic stress procedure results in a condition of chronic escape deficit and anhedonia that is used to model mental depression.

2.5. Models of Stress in Humans

Dietary restriction in humans can increase life span and reduce the incidence of age-related diseases including cancer, diabetes, and kidney disease (Weindruch & Sohal, 1997), and it is the basis for the treatment of several metabolic and cardiovascular disorders. Dietary restriction, particularly in overweight subjects, is a stressful experience, and compliance with a rigid diet can be as poor as spontaneous abstinence is in drug abusers. On the other hand, dieting is just one of the many stressful tools employed in treating patients.

Returning to experimental stress, the use of stressors on humans is limited to the evaluation of the emotional threshold in healthy subjects and in well-defined forms of psychopathology. The stressors used are light physical stimuli, such as a brief foot immersion in cold water (Oshima et al., 2001), or a protocol of simple tasks to be solved, such as the Trier Social Stress Test (TSST) (Kirschbaum, Pirke, & Hellhammer, 1993), which induces a psychological stress. TSST consists mainly of a free speech and a mental arithmetic task in front of an audience. Including introduction to the free speech and a preparation phase, the total procedure takes approx 15 min. The most frequently measured variables are plasma or salivary cortisol, plasma adrenocorticotrophic hormone (ACTH), and cardiovascular parameters such as heart rate and blood pressure. Salivary cortisol has been shown to be a reliable, noninvasive method of assessing plasma cortisol levels (Kirschbaum & Hellhammer, 1989; Reid, Intrieri, Susman, & Beard, 1992) and has

the further advantage of measuring plasma free cortisol levels, hypothesized to be the more biologically active form of plasma cortisol (Elkins, 1990). Salivary cortisol provides a measure of non-protein-bound, “free” cortisol levels. Salivary “free” cortisol levels track closely with plasma levels, showing a 1- to 2-min lag, and have been previously used to monitor cortisol activity in both ambulatory and laboratory challenge paradigms (Kirschbaum and Hellhammer, 1994).

The great number of studies conducted in healthy volunteers aimed at defining gender differences in cardiovascular and HPA axis responses to psychological stress in healthy men and women of different ages did not reach consistent results (Gaab et al., 2003; Kirschbaum, Klauer, Filipp, & Hellhammer, 1995; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Kudielka et al., 1998; Kudielka, Schmidt-Reinwald, Hellhammer, Schurmeyer, & Kirschbaum, 2000; Seeman, Singer, Wilkinson, & McEwen, 2001; Watamura, Donzella, Alwin, & Gunnar, 2003; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001).

Besides the different experimental conditions used, the main reason for the discrepancies among studies is the immense interindividual variability (Berger et al., 1987), which can sometimes be explained on the basis of genetic variables (Wust et al., 2004) or particular personal histories (Brody, 2002).

Controlled studies aimed at the assessment of psychological and physiological reactions to psychological stress provocation are less frequently carried out in psychiatric patients. This may be due to the complex interactions among the various factors that modulate the stress response and the dynamics of the disease under investigation. Thus, in different forms of psychiatric disorders the basal activity of the HPA axis is studied, rather than the susceptibility to standardized stressors. The better assessed alteration in the HPA axis is the decreased response to the dexamethasone suppression test (DST) in major depression (Carroll et al., 1981), a psychiatric disorder characterized by high plasma cortisol levels. A vast number of studies have established that the sensitivity of DST in major depression is no more than 40–50% (Arana, Baldessarini, & Ornstein, 1985). In this context, Holsboer, von Bardeleben, Wiedemann, Müller, and Stalla (1987) serially performed corticotropin-releasing hormone (CRH) challenge on major depressive episode cases after pretreatment with dexamethasone (DEX) and found that the hyperresponses of ACTH and cortisol during the episode were normalized with recovery. This phenomenon was confirmed in a large-scale study by Holsboer-Trachsler, Stohler, and Hatzinger (1991). Heuser, Yassouridis, and Holsboer (1994) concluded that the sensitivity of this combined DEX/CRH test in major depression is >80%, which far exceeds that of the standard DST. The DEX/CRH test has also been proposed as a predictor of medium-term outcome in patients with remitted depression (Zobel et al., 2001). On the other hand, a decreased response to the DST is not specific for major depression since it has also been observed in schizophrenic patients (Tandon et al., 1991).

Cortisol, ACTH plasma levels, and cortisol salivary concentration are all reliable markers of HPA axis activity in humans, but modifications of these parameters elicited by mild stressor exposure are bound to a number of variables that render any findings or conclusions impossible. Thus, experimental models based on stressor exposure have several useful applications in animal research but are still of limited clinical value in human research.

3. CONCLUSIONS

Exposure to stressors induces a complex behavioral and neuroendocrine response—stress—in experimental animals as well as in humans. This response can be adaptive or maladaptive.

In animals maintained under controlled experimental conditions, stress is reproducible and proportional to the intensity of the stressor and the duration of the exposure. This notion allows the study of the mechanisms underlying the responses to stressors and the use of stress-induced behavioral modifications as models of psychiatric symptoms.

The experimental study of stress is still of limited clinical value in human research because of ethical problems. However, it is the large interindividual variability in the sensitivity to any kind of traumatic stimulus or event that largely accounts for the low concordance in the conclusions of the existing studies.

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