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Defensive burying in rodents: ethology, neurobiology and psychopharmacology

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Abstract

Defensive burying refers to the typical rodent behavior of displacing bedding material with vigorous treading-like movements of their forepaws and shoveling movements of their heads directed towards a variety of noxious stimuli that pose a near and immediate threat, such as a wall-mounted electrified shock-prod. Since its introduction 25 years ago by Pinel and Treit [J. Comp. Physiol. Psychol. 92 (1978) 708], defensive (shock-prod) burying has been the focus of a considerable amount of research effort delineating the methodology/ethology, psychopharmacology and neurobiology of this robust and species-specific active avoidance or coping response. The present review gives a summary of this research with special reference to the behavioral (face and construct) and pharmacological (predictive) validity of the shock-prod burying test as an animal model for human anxiety. Emphasis is also placed on some recent modifications of the paradigm that may increase its utility and reliability as to individual differences in expressed emotional coping responses and sensitivity to pharmacological treatments. Overall, the behavioral and physiological responses displayed in the shock-prod paradigm are expressions of normal and functionally adaptive coping patterns and the extremes of either active (i.e., burying) or passive (i.e., freezing) forms of responding in this test cannot simply be regarded as inappropriate, maladaptive or pathological. For this reason, the shock-prod paradigm is not an animal model for anxiety disorder or for any other psychiatric disease, but instead possesses a high degree of face and construct validity for normal and functionally adaptive human fear and anxious apprehension. However, the apparent good pharmacological validation (predictive validity) of this test reinforces the view that normal and pathological anxiety involves, at least partly, common neurobiological substrates. Therefore, this paradigm is not only suitable for screening potential anxiolytic properties of new drugs, but seems to be especially valuable for unraveling the neural circuitry and neurochemical mechanisms underlying the generation of active and passive coping responses as different expressions of anxiety.

Keywords: Animal model; Defensive behavior; Fear; Anxiety; Coping, active; Coping, passive; Avoidance; Marble-burying; Shock-prod burying

1. Introduction

Defensive burying refers to the rodent behavior of displacing bedding material with typical alternating forward-pushing movements of their forepaws (paw-treading or thrusting) and shoveling movements of their heads directed at localized sources of aversive stimulation or threat. Harmful and noxious objects so buried include electrified prods (Pinel and Treit, 1978), rat chow pellets coated with quinine (Poling et al., 1981), spouts of bottles containing unpleasant tasting liquids such as pepper sauce and liquids to which they have developed taste aversion (Poling et al., 1981; Wilkie et al., 1979; Parker, 1988), mouse traps which strike (Terlecki et al., 1979; Gray et al., 1981), flashcubes which discharge near them (Davis and Rossheim, 1980), tubes which direct airbursts into their faces (Pinel and Treit, 1983; Pinel et al., 1994) or deliver noxious smells (Silverman, 1978) or predator odor (Holmes and Galea, 2002), dead conspecifics (Pinel and Treit, 1983) and predators (Calhoun, 1962; Coss and Owings, 1978; Londei et al., 1998). Although different in form, function and intensity, rodents may also bury seemingly harmless novel objects such as unelectrified prods (Pinel and Treit, 1983; Terlecki et al., 1979), flashcubes which do not flash (Terlecki et al., 1979), and marbles (Broekkamp et al., 1989; Poling et al., 1981; Njung'e and Handley, 1991b). By burying unfamiliar and/or harmful objects, individuals can successfully avoid (i.e., keep away from) or remove aversive and possibly life-threatening dangers from their habitat. Together with flight, freezing and fighting, defensive burying constitutes the behavioral

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repertoire of unconditioned species-specific defensive reactions that are readily and innately available to the animal. Combined with the demonstration that clinically effective anxiolytic drugs potently suppress this robust active avoidance response, burying behavior has been used extensively as an index of fear/anxiety in preclinical studies on the neurobiology and behavioral pharmacology of this human emotion. In this paper, we will review 25 years of research using the defensive burying test as an animal model of normal fear and anxiety.

2. The shock-prod defensive burying paradigm: history, methodology and ethology

2.1. History

In an early monograph based upon a dissertation entitled "One-trial learning: a study of the avoidance behavior of the rat", Hudson (1950) discovered that rats in their living chambers with wood shavings on the floor often pushed the shavings toward and over (i.e., buried) an object (metal food cup) that had shocked them. Claiming that ground squirrels bury traps that were ill concealed, he already suggested that this behavior was part of rodent's natural defensive behavioral repertoire to sources of aversive stimulation or predatory threat. Burrow-sealing behavior was subsequently described in the elegant and detailed studies of Calhoun (1962) reporting that low status wild rats would bury the entrance holes to their underground burrows when threatened by other, higher social rank conspecifics. Later, Silverman (1978) similarly reported that rats, mice and hamsters would plug the inlet pipes admitting cigarette smoke by flipping sawdust or faecal pellets with push-digging forward movements of their forepaws. In addition, Owings and Coss (1978) described that ground squirrels would spray and kick sand directly at approaching snakes ("snake-mobbing") so as to provoke hissing or rattling responses by snakes that allows the ground squirrels both to locate the predator in the darkened tunnels and to assess the dangerousness of the snake (Swaisgood et al., 1999), or by sealing themselves off from an approaching snake by burying the entrance holes to their burrows. MacClintock (1970) similarly described ground squirrels as using the burying response to construct walls in their burrows to create a wall around the predators. Furthermore, it was recently reported that mice emit defensive treading to bury a live scorpion (Londei et al., 1998). These observations already strongly support the contention that burying constitutes a naturally occurring, emotionally negative motivated, behavioral reaction of rodents to a variety of noxious stimuli that pose a near and immediate threat. However, it was Pinel et al. (1978), who originally designated this characteristic behavior as "defensive burying" and who introduced the so-called shock-probe defensive burying paradigm into the neuroscientific and psychopharmacological research fields. They comprehensively described that rats, when tested in a familiar environment with suitable bedding material on the floor, have a strong innate tendency to bury a wire-wrapped prod attached to the test chamber wall from which they have received an electric shock upon contacting it. Since this introduction, defensive (shock-prod) burying has been the focus of a considerable amount of research effort in the past 25 years delineating the methodology/ethology, psychopharmacology and neurobiology of this robust and species-specific active avoidance or coping response. The following sections summarize the major results obtained from these studies.

2.2. Methodology/ethology

The procedure of the shock-prod defensive burying test is quite simple and basically unchanged since its original description by Pinel and Treit (1978). In a test chamber (either home-cage or familiarized test cage after several habituation trials) with enough and suitable bedding material on the floor, subjects are confronted with a wire-wrapped prod/probe (Ø = 1 cm; 6–7 cm long) inserted through a small hole 2 cm above the bedding in one of the test chamber walls. The uninsulated wires of the prod are connected to a shock source, and whenever the subject touches the prod with its forepaws or snout, it receives an electric shock (manually operated or automatically delivered, and the prod either remains electrified during the entire test period or is deactivated after the first contact). Following the first contact with the electrified prod, the animal's behavior is observed and/or recorded on video for a 10–15-min test session. During this observation period, some selected or all occurring behavioral postures and/or parameters can be quantified (e.g., see Table 1 and Fig. 1).

The repertoire of behavioral reactions is well delineated and catalogued in rats and mice (Tsuda et al., 1988b; De Boer et al., 1991) and methods for its reliable measurement have become standard equipment in behavioral–physiological and pharmacological laboratories. In general, the following behavioral categories and elements can be distinguished. (1) *Ambulation* consists of any horizontally locomotor activity, ranging from a single step to vigorously moving around in the cage, mostly accompanied by sniffing and exploratory activity directed at the floor, walls or in the air. (2) *Rearing* or raising the body on the hind limbs in a vertical position, mostly making sniffing movements with the nose up in the air. (3) *Immobility* consists of crouching, lying, sitting or standing still on at least three feet, with the body motionless except for small and slow lateral movements of the head (scanning). (4) *Burying* or pushing, shoveling, flicking and digging sawdust towards and around the prod with rapid movements of the snout and forepaws as originally described by Pinel and Treit (1978) (see Reynolds and Berridge, 2001 for a detailed description of forelimb motor parameters—bout duration, number of treading cycles, limb extension, etc.) in paw treading elicited by a shock-prod. (5) *Grooming* includes all activities directed toward various parts of the

Table 1
Behavioral–physiological parameters in the shock-prod burying test and its putative psychological index

Parameter	Effect of prod-shock	Putative psychological index
Behavioral		
Prod-directed burying (frequency/duration)	increase	Fear/anxiety, active avoidance (fight)
Burying latency (time)	decrease	Reactivity/anxiety
Height of pile at prod base (cm)	increase	Fear/anxiety
Prod-shock reactivity (intensity scale)	NA	Pain sensitivity/ reactivity
Prod contacts (number/duration)	decrease	Exploration/ avoidance
Prod-contact latency	increase	Exploration/ avoidance
Stretched prod-directed approach–withdrawal movements	increase	Risk assessment
Immobility/freezing (frequency/duration)	increase	Fear/anxiety, passive avoidance (freeze)
Grooming (frequency/duration)	decrease	Conflict
Rearing (frequency/duration)	increase	Explorative escape attempts (flight)
Ambulation	decrease	Exploration
Autonomic/endocrine physiological		
Heart rate	increase	Arousal/stress
Blood pressure	increase	Arousal/stress
Core temperature	increase	Arousal/stress
Plasma noradrenaline	increase	Arousal/stress
Plasma adrenaline	increase	Arousal/stress
Plasma corticosterone	increase	Arousal/stress
Plasma prolactin	increase	Arousal/stress
Plasma glucose	increase	Arousal/stress

body (i.e., face washing, scratching, tail biting and licking of the body. (6) *Prod explore* is defined as a rat's posture being oriented toward the shock-prod in a stretch/attend-like position and then suddenly withdrawing from it. (7) *Eating/ drinking* refers to all consummatory activities including pica (eating of bedding materials). In addition, the latency time to the first prod contact (shock) and the latency time to initiate burying behavior are recorded Traditionally, the frequency and duration of time spent on prod-directed burying behavior is scored (as well as an intimately related measure, the height of piled bedding material around the shock-prod) and these have been taken most commonly as the sole defensive behavior motivated by a state of fear/anxiety in this paradigm. However, the mean time engaged in burying varies considerably between studies and between individuals, and it typically represents only a fraction (3–30%) of the total observation time of the standard 10–15-min test. Thus, for the sake of a better understanding of the full defensive behavioral repertoire in rodents in this test situation, it is worthwhile, and in our view necessary, to observe and quantify (frequency and duration of occurrence) not only defensive burying but also other avoidance/defensive behaviors emitted in this test situation (i.e., reduced prod exploration, increased freezing/immobility) as well as general exploratory/self-care behaviors (ambulation, rearing, grooming). A complete ethogram, covering 100% of the observation time, allows for the measurement of several concurrent and putative competitive behavioral indices of fear/anxiety, avoidance, reactivity and exploration (see Fig. 1). These ethological analyses (e.g., Peacock and Wong, 1982; Tsuda et al., 1988b; Moser and Tait, 1983; Treit et al., 1986; De Boer et al., 1990) have clearly revealed that besides defensive prod-burying behavior, animals also display significant immobility/freezing postures in positions away from the prod, indicating a different behavioral expression of anxiety (see Fig. 1).

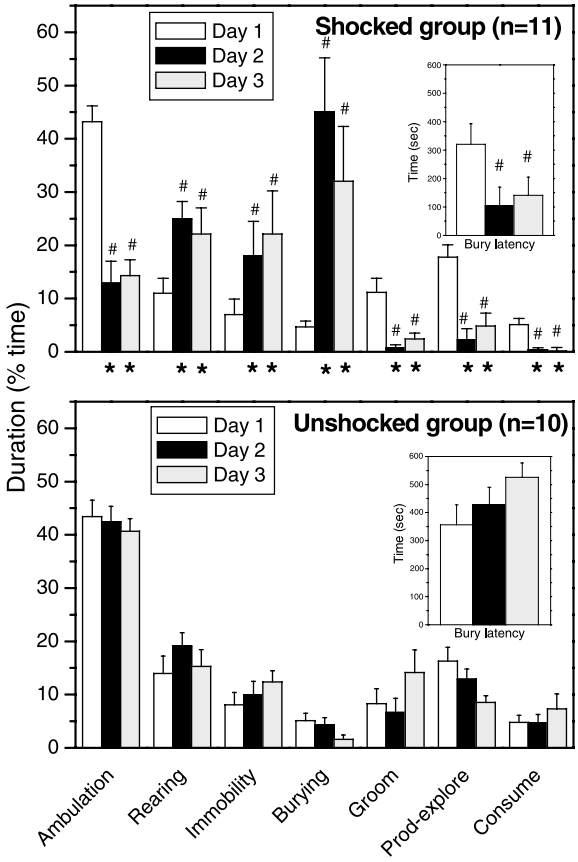

Fig. 1. Behavioral responses of wild-type rats to 10-min insertion of a wire-wrapped prod in their home-cage during three consecutive days. For the shocked group of animals (upper panel), the prod was electrified during the second trial on day 2 only; the prod was uncharged during trials 1 and 3. For the unshocked group of animals, the prod was uncharged during all three daily trials. Note the pronounced increases in defensive burying, immobility and rearing accompanied by reductions in ambulation, grooming, prod exploration and consummatory activities in the shocked group during trial 2 (conditioning electrified shock-prod session) and trial 3 (retention session) as compared to their preconditioning values in trial 1 (significant changes indicated by #), and as compared to the values of the unschocked group (significant differences indicated by *).

Table 2
Procedural and organismic variables influencing defensive burying behavior

Variable	Effect on burying	References
Procedural variables		
Type, size, shape and familiarity of test cage	Small>large	Pinel et al. (1980), Maggio and Harder (1983)
	One compartment>two compartments	Moser and Tait (1983), Tarte and Oberdieck (1982)
	Familiar cage>unfamiliar cage	Treit et al. (1986), Davis et al. (1982), Peacock et al. (1982)
Type and availability of bedding material	Wood shavings, corncob, sand, wooden blocks, sawdust	Pinel et al. (1980), Treit et al. (1986), Peacock et al. (1982), De Boer et al. (1990)
	Clean bedding>stress odor-contaminated bedding	Williams (1987)
Type and intensity of localized aversive stimulus	Electrified prod, electrified lever, flash bulb, airblasts, mouse trap, dead conspecifics, scorpions, rattlesnakes, predator odor TMT, noxious food/water spouts, marbles	Terlecki et al. (1979), Goldberg et al. (1983), Wilkie et al. (1979), Davis et al. (1981a,b,c, 1982), Owings and Coss (1978), Holmes and Galea (2002), Parker (1988), Poling et al. (1981)
	Shock intensity	Treit et al. (1980)
Time of the day/lighting conditions	Dark>light	Pinel et al. (1994), De Boer et al. (1991)
Organismic variables		
Between-species differences	Rats, mice, ground squirrels and gerbils show defensive burying	Whillans and Shettleworth (1981), Treit et al. (1980)
	Hamsters do not emit defensive burying	Davis and Rossheim (1980), Davis et al. (1981a,b,c)
	Rats>squirrels	Owings and Coss (1978), Heynen et al. (1989)
Within-species differences (between strain/genotype differences)	PVG>Wistar	Tsuda et al. (1988a,b)
	Fischer>Wistar	McKim and Lett (1979)
	Brattleboro = Lewis	Brito (1983)
	Long–Evans>Wistar	Tarte and Oberdieck (1982)
	Maudsly reactive = Maudsly nonreactive	Beardslee et al. (1989)
	Fischer>Long–Evans>Wistar	Treit et al. (1980)
	CF1>CB1>BALB/c	Maggio and Harder (1983)
	C57BL>DBA	Harder and Maggio (1983)
	SP = sNP	Richter et al. (2000)
	ANA>AA	Sandbak et al. (1998)
	WTG>Wistar	De Boer et al. (1991a,b)
	THE>TLE	Wada and Makino (1997)
	Wistar>WKY	Pare (1994)
	Aggressive SAL mice>nonaggressive LAL mice	Sluyter et al. (1996, 1999)
Within-strain/genotype differences (Inter-individual differences and relationship with other behaviors)		Pinel et al. (1978), Peacock and Wong (1982)
	Negative correlation with individual alcohol consumption	Sandbak et al. (1996)
	Positive correlation with individual aggressiveness	Sgoifo et al. (1996, this paper)
	No correlation with plus-maze performance	Overmier et al. (1997), Sandbak and Murison (2001) this paper
Age	Old>young	Treit et al. (1980)
	Old = young	Korte et al. (1992)
	11 weeks>21 weeks>3 weeks	Lopez-Rubalcava et al. (1996)
Sex	No sex difference	Treit et al. (1980), Maggio and Harder (1983), Sluyter et al. (1999), Frye et al. (2000)
Estrus cycle phase	Metestrous>proestrous	Fernandez-Guasti and Picazo (1990)
	Proestrous = estrus = diestrus	Frye et al. (2000)
Stage of pregnancy	First half>around parturition	Picazo and Fernandez-Guasti (1993)
Lactation	Lactating females>nonlactating	Pinel et al. (1990)
	Lactating = nonlactating	Picazo and Fernandez-Guasti (1993)
Metabolic/physiological condition	Satiated>food-deprived	Davis et al. (1981a,b,c)
	Water-deprived>nondeprived	Saldivar-Gonzalez et al. (1996)
	Normoglycemic>hypoglycemic	Davis and Rossheim (1980)
Previous ontogenetic experiences	Developmental experience with bedding substrate	Pinel et al. (1989)
	Postnatal anoxia = controls	Buwalda et al. (1995)
	Postnatal handling = nonhandled	Meerlo et al. (1999)
	Low maternal care>high maternal care	Menard and Meaney (2001)
	Control nursed>corticosterone nursed	Meerlo et al. (2001)
Prior adult experiences	Length of prod-shock test interval	Pinel et al. (1978)
	Number of habituation trials	Tarte and Oberdieck (1982), Davis et al. (1981a,b,c), Treit et al. (1988)
	Controllable prior tail-shock session>uncontrollable prior tail-shock session	Williams (1987)
	Control>prior defeat	Williams and Scott (1989)
	Uncontrollable foot-shock session>non-foot-shock control	Overmier et al. (1994)

Table 2 (*continued*)

Variable	Effect on burying	References
Organismic variables		
Prior adult experiences	Prior copulatory behavior/no ejaculation> with ejaculation	Fernandez-Guasti et al. (1989)
	Progesterone withdrawal>control	Gallo and Smith (1993),
	Opiate withdrawal phase>saline control	Harris and Aston-Jones (1993), Harris et al. (2001), Basso et al. (1999)
	Prior IP saline injection (effects bidirectional temporal pattern)	Saldivar-Gonzalez et al. (1997)
	Prior 1.5 min social interaction>no experience>prior 15 min social interaction	Saldivar-Gonzalez et al. (1996, 2000)

>: more burying behavior. =: same level of burying.

Basically, there is a reliable temporal pattern or hierarchy in the rat's behavioral repertoire upon contacting an electrified prod and receiving a shock i.e., reflexive startle and withdrawing immediately to the far end of the chamber followed by brief alternating episodes of approaching toward and retracting from the prod (stretch-attend posture), freezing/scanning and rearing in that location away from the prod before emitting bouts of burying behavior. These bursts of burying behavior are separated by periods of immobility/freezing, rearing and stretch/attend-like prod exploration. The burying and immobility/freezing responses to a shock-prod can be characterized as active and passive forms of shock-prod avoidance behavior, respectively (De Boer et al., 1990; Tsuda et al., 1992; Sandbak and Murison, 1996). In terms of coping (an individual's behavioral and physiological response repertoire to master a stressful situation), both forms of avoidance behavior results in effective stress management, namely, avoiding further contacts with the shock-prod and, hence, receiving a painful shock. Moreover, under natural conditions, freezing is a highly selected response because movement makes the rat more detectable to predators and because predators are much more likely to attack moving than still prey (see Bolles and Fanselow, 1980 for a review).

Defensive shock-prod burying is very sensitive to a wide range of procedural and organismic variables; a summary of these is presented in Table 2.

The results of these studies convincingly show that the degree of defensive burying is dependent on environmental/procedural factors like: (1) type and intensity of the aversive stimulus objects, (2) type and availability of bedding material, (3) type, size, shape and familiarity of the test cage, (4) lighting levels. In addition, a number of organismic variables are importantly determining the intensity of burying behavior: (1) species, (2) strain/genotype, (3) type of individual, (4) sex, (5) age, (6) metabolic/physiological state, (7) prior experiences. Unfortunately, in most of these studies, only burying behavior has been scored; therefore, the effects of these parameters on other competing avoidance behaviors (immobility) and/or compensatory reactions are largely unknown. However, it is believed that the relative expression of these two major ways of coping may be a function of these organismic and procedural variables as well. Since it is beyond the scope of this review to discuss all these factors in detail, we only will focus on one important organismic variable namely individual variation because of its correlation with other indices of fear/anxiety and its importance as a variable for drug action in this paradigm.

2.3. Defensive burying: individual differences and correlations with other behavioral measures

Within any batch of animals, there is considerable variance as to the latency to initiate burying and the amount of burying. Some animals exhibit hardly any burying behavior at all but will rather stay away from the prod by remaining in a freezing/immobile posture throughout the observation period. This individual difference in expressed defensive burying was already noted in the initial studies of both Hudson (1950) and Pinel and Treit (1978), and has been observed in virtually every study on defensive burying since then. Fig. 2A shows an example of the broad individual differences in defensive shock-prod burying as observed in a large group of a (originally wild-trapped) feral strain of rats (*Rattus norvegicus*; wild-type Groningen strain) used in our laboratory. This particular strain is known for its wide variation in levels of aggressive behavior (De Boer et al., 2000; Koolhaas et al., 1999). However, these individual male resident WTG rats also differ widely in their level of species-typical defensive burying expressed towards an electrified prod in their home-cage, ranging from no burying at all to very high levels of intense burying behavior.

Furthermore, Fig. 2B shows that there is a strong reciprocal relationship (i.e., response competition) between the levels of burying and the amount of immobility/freezing behavior animals display in this test, as reported previously by others as well (Treit et al., 1986; Tsuda et al., 1992; De Boer et al., 1990). As already mentioned in the previous section, it, thus, appears that individual WTG rats differ widely in their propensity to engage in either an active behavioral strategy (defensive burying) or a passive strategy (immobility/freezing) to cope with the electrified prod.

Within the framework of (pro)active and passive/reactive coping strategies, as originally put forward by the late Jim Henry (1977), the degree to which animals react with an aggressive response to a stressor is regarded as an important discriminating factor of the two coping styles. These ideas led to the hypothesis that the individual level of aggressive behavior, that is the tendency to defend the home territory against intruders, is related to the way individuals react to environmental challenges in general. The results of a series of experiments in rats and mice in our laboratory have indeed

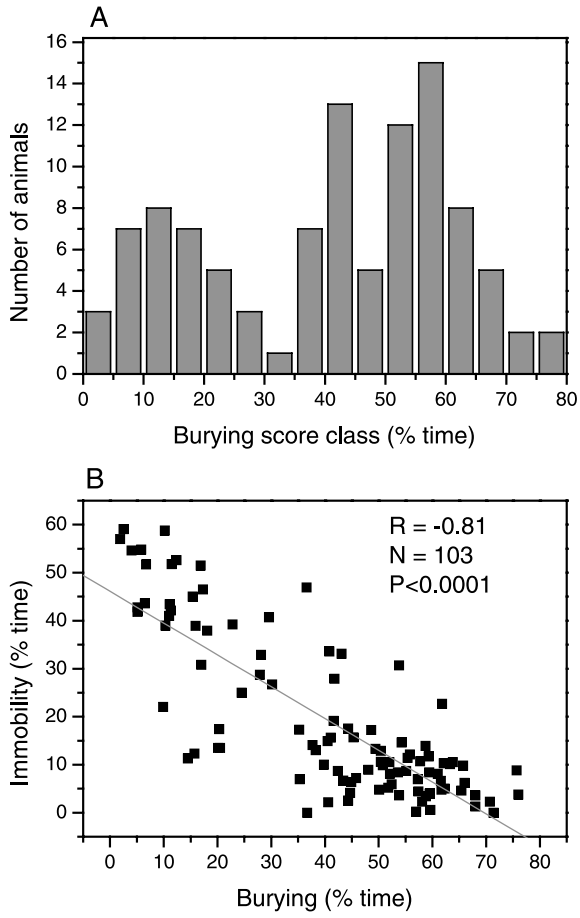

Fig. 2. Panel A: Defensive burying scores distribution of male wild-type rats ($n = 103$). Data are grouped in classes per 5% time spent in burying behavior. Although all classes are represented, it is obvious that the individual behavioral scores are certainly not normally distributed (Kolmogorov–Smirnov test for normality revealed significant deviation from a normal distribution). Panel B: Inverse correlation between active (burying) and passive (immobility) avoidance behavior displayed by wild-type rats during a 10-min encounter with an electrified prod within their home-cage.

clearly supported this view by demonstrating that the individual tendency to initiate offensive aggressive behavior is predictive for the individual reaction to other, nonsocial environmental challenges including exposure to a shock-prod (Koolhaas et al., 1999; Sgoifo et al., 1996; Everts et al., 1997; Koolhaas et al., 2001). Fig. 3A convincingly shows that the individual level of burying in WTG rats is highly correlated with the individual's offensive aggressiveness as measured in a prior resident–intruder test. Similar results have been found in artificially selected high (SAL) and low (SAL) aggressive house mice (Sluyter et al., 1996; Sluyter et al., 1999).

Thus, the individual level of defensive burying in the shock-prod test can be regarded as an expression of an individual's tendency for using a (pro)active coping style, whereas the level of immobility/freezing behavior is an expression of its passive/reactive coping tendencies. In many animal species including humans, the existence of different phenotypes in behavioral and physiological response patterns is well recognized (Koolhaas et al., 1999), and different terms have been used to characterize them, such as shyness and boldness, types A and B personality, active and passive emotional coping, etc. Despite this different terminology, they all seem to discriminate between similar basic behavioral characteristics namely response initiation and behavioral flexibility. The difference in response initiation forms the basis of the terminology we currently use for the different coping strategies in rats and mice, i.e., proactive and passive/reactive (see Koolhaas et al., 2001 for review on different coping strategies).

An important aspect as to the interpretation of defensive burying as a behavioral index of fear/anxiety in this test concerns its relationship with behavioral expressions of fear/anxiety in other well-validated tests like for example the widely used elevated plus-maze. In this test, animals are allowed to freely explore two open arms and two closed arms, and the animal's avoidance to enter and explore the two open platforms is generally taken as the behavioral measure of fear/anxiety. Surprisingly, however, there appears

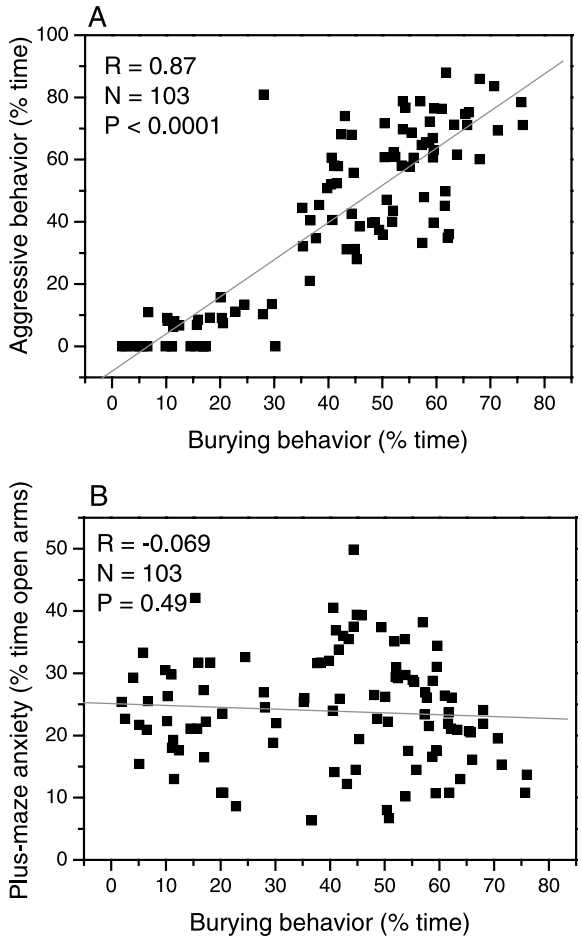

Fig. 3. Panel A: Strong positive relationship between the amount of burying behavior in the shock-prod test and the level of offensive aggression as measured in the resident–intruder test. Panel B: Absence of a correlation between the amount of burying behavior in the shock-prod test and the main anxiety score (percent time open arms) as measured in the elevated plus-maze.

to be no relationship between the levels of burying, and this plus-maze anxiety-like measure as can be seen in Fig. 3B. An absence of a correlation and/or dissociations in manipulation effects between behavioral measures of anxiety in the shock-prod test and the plus-maze test have been reported in several studies (Beardslee et al., 1989; Sandbak and Murison, 2001; Pare, 1994; Basso et al., 1999; Treit et al., 1993d; Treit and Menard, 1997). This discrepancy is usually explained by the suggestion that these different behavioral tests induce either different types of anxiety (e.g., state or trait anxiety, fear of a proximate stimulus/aversive object and fear of a distal threat/environmental openness) or different types of behavioral expressions of anxiety (e.g., active vs. passive avoidance responses) and that the neural systems controlling these are more or less independent of one another (Treit et al., 1993b, 1998).

2.4. Autonomic and endocrine physiological correlates of defensive burying

By instrumenting the experimental animals with chronic vascular catheters for repetitive collection of blood samples, and/or radio-telemetry sensors for continuous recording of physiological indices, it is possible to obtain additional autonomic and endocrine measures of fear/anxiety in this test (see Table 1). It has been proposed (De Boer et al., 1991) that the combined assessment of behavioral and autonome/endocrine indices of the animal's fear/anxiety state within the same experimental setting may facilitate the interpretation of manipulation effects (e.g., anxiolytic drug action). Studies employing such an endocrine approach have demonstrated that prod-shocked rats show increases in plasma catecholamine, corticosterone, prolactin and glucose concentrations (De Boer et al., 1991, 1990; Sgoifo et al., 1996; Korte et al., 1992; Groenink et al., 1995). Physiologically, a shock-prod burying session is also associated with sympathetically driven increases in heart rate, blood pressure and core body temperature (Korte and Bohus, 1990; Diamant et al., 1991; own unpublished data). However, the magnitude of the classical endocrine (i.e., catecholamine, corticosterone and prolactin) stress responses is rather small compared to several other stressful situations frequently employed within biomedical laboratory stress research (e.g., foot shock, restraint, social defeat, etc) (Koolhaas et al., 1997; Sgoifo et al., 1996). In fact, rats engaged in defensive burying and/or passive avoidance of the electrified prod show no greater corticosterone responses and only slightly higher adrenaline increases than rats merely exposed to a nonelectrified prod (De Boer et al., 1990, 1991). This is probably due to the high degree of controllability and, hence, reduced fear/anxiety, experienced over the situation by either actively or passively avoiding the shock-prod. Indeed, in an environment without bedding material on the floor where the burying option is eliminated and all rats are "forced" to display passive avoidance, relatively low plasma noradrenaline and high plasma corticosterone and adrenaline responses are seen (De Boer et al., 1991; Korte et al., 1992). Within our strain of wild-type rats, plasma noradrenaline (reflecting neurosympathetic reactivity) correlates with the amount of burying, whereas similar (low) plasma corticosterone responses are seen in both actively and passively coping animals (Sgoifo et al., 1996). These neuroendocrine data provide empirical support for the view that the activity of sympathetic nerves, as reflected in plasma noradrenaline content, is tightly related to conditions involving actual skeletal muscle exertion regardless of the emotional connotations of the challenge, whereas adrenomedullary and adrenocortical stimulation occurs primarily during emotional stress or fear/anxiety-provoking situations in which coping capabilities are limited or abolished.

2.5. The shock-prod burying paradigm as a one-trial learning and memory test

The shock-prod paradigm also allows investigating the conditioned (one-trial learned) response to a control (non-electrified) shock-prod. A certain period (ranging from several seconds within one trial to days or weeks between trials) after the experience of a shock from the electrified prod (conditioning), the behavioral reaction of rats to an uncharged control prod can be assessed (retention). An example of this experimental design is also shown in Fig. 1. It nicely demonstrates that 1 day after the electrified shock-prod trial, animals still actively (burying) and/or passively (immobility) avoid a similar, but now unelectrified, shock-prod. Obviously, animals show a good retention of the acquired aversion to the prod established by the touch–prod-shock contiguity in the learning trial, i.e., animals have a memory for the aversive event. It has been shown that this memory endures for at least 20 days (Pinel and Treit, 1978). Some studies have used this shock-prod avoidance retention paradigm to assess the cognitive effects of amygdala manipulations (Roozendaal et al., 1991; Lehmann et al., 2000) or traumatic experiences (Overmier et al., 1994). Note, however, that most studies that label defensive burying as a conditioned response (conditioned defensive burying paradigm) refer to the burying observed in only a single trial after animals have received one (manually delivered) shock upon first contact with the prod. Immediately after delivery of this shock, the shock source is turned off and the prod remains uncharged during the remaining test session. The objective of these studies is principally not aimed for unravelling cognitive processes.

3. Psychopharmacology

Since defensive burying, both as an unconditioned and conditioned response, is widely regarded as a behavioral expression of animal's fear/anxiety state, numerous pharmacological studies have employed this well-distinguishable behavior as the dependent variable for screening potential therapeutic (e.g., anxiolytic) agents (Treit, 1985a; Treit et

Table 3
Summary of drug effects on defensive shock-prod burying behavior

Drug	Dose range (mg/kg)	MED (mg/kg)	References
Drug treatments that decrease burying			
8-OHDPAT	0.0625–0.5	0.125	Fernandez-Guasti and Hong (1989)
	0.25, 0.5	0.5	Lopez-Rubalcava et al. (1996)
	0.05–0.2	0.05	Treit et al. (1993a,b,c,d)
	0.0625–0.5	0.125	De Boer (this paper)
R-(+)-8-OSO2CF3-PAT	1.0, 3.0	3.0	Barf et al. (1996)
Allopregnanolone	0.125–1.0	0.25	Picazo and Fernandez-Guasti (1995)
Alnespirone	0.25–4.0	0.25	Munoz et al. (1997)
Alpha-helical CRF	1.0–25 μg, i.c.v.	5.0	Korte et al. (1994)
D-Phe CRF	0.04–1.0 μg, i.c.v.	1.0	Basso et al. (1999)
Angiotensin II	0.1–1.0 μg, i.c.v.	0.1	Tsuda et al. (1992)
Buspirone	1.0–4.0	1.0	Treit et al. (1981), Davis et al. (1981a,b,c)
	0.2, 0.5	0.2	Rohmer et al. (1990)
	2.5–10	5.0	Lopez-Rubalcava et al. (1996)
	0.25–4.0	0.25	Munoz et al. (1997)
Chlordiazepoxide	1.0–6.0	3.0	Treit et al. (1981), Treit and Pesold (1990)
	2.5–10.0	2.5	Treit and Fundytus (1988)
	10–32	4.0	Craft et al. (1988)
	2.0–7.1	5.0	Beardslee et al. (1990)
	3.0–27	9.0 oral	De Boer et al. (1990)
	2.5–10	2.5	Czech and Quock (1993)
Chlorpromazine	2.0		Davis et al. (1981b)
	1.0–3.0	1.0	Treit et al. (1981)
Desipramine	2.5 for 21–26 days		Fernandez-Guasti et al. (1999)
Diazepam	0.1–6.0	0.5	Treit et al. (1981)
	0.5–1.0	0.5	Blampied and Kirk (1983)
	0.5, 2.0	0.5	Tsuda et al. (1987, 1988a,b)
	0.25–1.0	0.25	Fernandez-Guasti et al. (1989, 1992a,b)
	0.2, 0.5	0.2	Rohmer et al. (1990)
	2.0		Treit et al. (1993a,b,c,d)
	0.5–2.0	1.5 oral	Sakamoto et al. (1998)
	2.5		Treit et al. (2001)
Diazepam + naloxone	1/10		Treit (1985a,b,c)
EMD 68843	10–40	20	Treit et al. (2001)
Ethanol	0–2000	2000	Treit (1990)
FG 7142	5, 10	5	Rohmer et al. (1990)
Flesinoxan	1.0, 3.0	1	Groenink et al. (1995, 1997)
Haloperidol	0.5, 2.0	0.5	Tsuda et al. (1987)
Imipramine	4.0–6.0	8.0	Craft et al. (1988)
Ipsapirone	0.065–10	2.5	Korte and Bohus (1990), Korte et al. (1992)
	0.625–10.0	2.5	Fernandez-Guasti et al. (1989, 1992a,b)
	2.5, 5.0	5.0	Lopez-Rubalcava et al. (1996)
Indorenate	3.1–10.0	10	Fernandez-Guasti et al. (1989, 1992a,b)
	5.0, 10.0	10	Lopez-Rubalcava et al. (1996)
Methiotepin	0.31		Fernandez-Guasti et al. (1992a,b)
Methylphenidate	3, 30	3.0	Tsuda et al. (1987)
Midazolam	1.0–3.0	1.0	Treit (1990)
Morphine	2.0–8.0	2.0	Craft et al. (1988)
Meprobamate	75–125	75	Craft et al. (1988)
Nitrous oxide	10–40%	30%	Czech and Quock (1993)
Oxprenolol	10–20	10	Blampied and Kirk (1983)
p-CPA	130 (4 days)		Treit et al. (1993a,b,c,d)
Pentobarbital	1.0–6.0	3.0	Treit et al. (1981)
	10–20	15	Treit (1990)
Pentylenetetrazol	20		Treit (1987)
Perospirone	0.03–1.0	0.3	Sakamoto et al. (1998)
Picrotoxine	0.5		Treit et al. (1981)
	2		Treit (1987)
Pimozide	1		Beninger et al. (1980)
Progesterone	0.5–4	1.0	Picazo and Fernandez-Guasti (1995)
R 121919	0.63–20	10 oral	Heinrichs et al. (2002)
Ro 16-6028	0.1, 1.0	0.1	Rohmer et al. (1990)
RU 24969	0.125–0.5	0.25	Fernandez-Guasti and Hong (1989)
RU 28318 + RU 38486	50/50 ng, i.c.v.		Korte et al. (1996)
S-15535	0.25–4.0	0.25	Munoz et al. (1997)
Toluene	1000–4000 ppm	2000	Lopez-Rubalcava et al. (2000)
Drug treatments that do not change burying			
Alprenolol	5.0		Fernandez-Guasti et al. (1992a,b)
Amitriptyline	0.25, 2.5		Tsuda et al. (1987)
Barakol	0–20		Fiorino et al. (1998)
Bicuculline	1.25, 2.5		Fernandez-Guasti and Picazo (1990)
Buspirone	8.0–64		Craft et al. (1988)
CDP + flumazenil	5/5 and 5/10		Treit (1987)
CDP + pentylenetetrazol	5/5		
CDP + picrotoxine	5/1		
CDP + CGS 8216	5/10		
CGS 2816	5.0–10		Treit (1987)
Chlorpromazine	1.0–16.0		Craft et al. (1988)
	0.5–5.0		Treit (1990)
Desipramine (chronic)	2.5 b.i.d., 8 weeks		Beardslee et al. (1990)
Diazepam + picrotoxine	1/1		Treit et al. (1982)
5,7-DHT	75 μg, i.c.v.		Lopez-Rubalcava et al. (1996)
Flumazenil	5.0–10.0		Treit (1987)
	5, 10		Rohmer et al. (1990)
	5, 10		Fernandez-Guasti and Picazo (1990)
	10		De Boer (this paper)
Haloperidol	0.03–1.0		Sakamoto et al. (1998)

Table 3 (*continued*)

Drug	Dose range (mg/kg)	MED (mg/kg)	References
Drug treatments that do not change burying			
Imipramine (chronic)	2.5 b.i.d., 7 weeks		Beardslee et al. (1990)
Morphine	1.5		Treit et al. (1981), Treit (1985a,b,c)
Naloxone	3.0		Whiteside and Devenport (1985)
	10.0		Treit (1985a,b,c)
Pargyline (chronic)	2.5 b.i.d., 12 weeks		Beardslee et al. (1990)
p-CPA	600 (3 days)		Lopez-Rubalcava et al. (1996)
Pentylenetetrazol	1.0–6.0		Treit et al. (1981)
	5.0–20		Treit (1990)
Physostigmine	2.0		
Picrotoxine	0.5		Treit et al. (1981)
	0.5, 1.0		Fernandez-Guasti and Picazo (1990)
Pindolol	3.1		Fernandez-Guasti et al. (1992a,b)
Practolol	0.5		Fernandez-Guasti et al. (1992a,b)
Pregnenolone sulfate	1.0–4.0		Picazo and Fernandez-Guasti (1995)
Scopolamine	0.05–1.25		Treit (1990)
Drug treatments that increase burying			
Amitriptyline	2.0		Davis et al. (1981b)
B-CCE	0.1–5.0	0.5	Tsuda et al. (1988b)
D-Amphetamine	1.0		Treit et al. (1981)
CRF	0.3		Diamant et al. (1992)
	0.1–3.0 μg, i.c.v.	0.1	De Boer (this paper)
DMCM	10–100 μg, i.c.v.	30	De Boer (this paper)
Yohimbine	0.5–2.0	0.5	Treit and Fundytus (1988)

al., 1981; Broekkamp et al., 1986; De Boer et al., 1991b). Table 3 summarizes the results from studies, performed during the past 25 years, of drug effects on burying behavior in the shock-prod paradigm.

For its predictive validity, animals models for anxiolytic drug effects have to fulfill a number of pharmacological criteria, i.e., (1) therapeutic class specificity, (2) dose-dependent sensitivity and correlation with clinical potency, (3) behavioral selectivity, (4) absence of tolerance. As can be deduced from Table 2, a variety of agents belonging to the clinically proven benzodiazepine (chlordiazepoxide, diazepam, midazolam, Ro 16-6028) and serotonergic class of anxiolytics (buspirone, ipsapirone, 8-OH-DPAT, *R*-(+)-8-OSO2CF3-PAT, alnespirone, EMD-68843, flesinoxan, indorenate, methiopentin, S-15535) potently suppress shock-prod burying behavior. This anxiolytic-induced suppression of burying occurs in a dose-related manner and, for the actual clinical compounds, with a relative potency that is roughly similar to that found in clinical settings. Within the clinically relevant dose range, the benzodiazepine and serotonergic-induced suppression of burying does not appear to be secondary to behavioral sedation or locomotor incapacitating effects (Blampied and Kirk, 1983; Treit et al., 1981; Treit, 1990; Groenink et al., 1995; Treit and Fundytus, 1988), analgesia (Treit, 1985c) or associative learning deficits (Blampied and Kirk, 1983; Korte and Bohus, 1990). Furthermore, the benzodiazepine-induced suppression of burying can be reversed by benzodiazepine/GABA-receptor antagonists (Treit, 1987), whereas serotonin$_{1A}$-receptor antagonists block the anxiolytic serotonin$_{1A}$-receptor agonist decreased burying (De Boer et al., unpublished findings). Upon chronic treatment with either diazepam (Treit, 1985b) or flesinoxan (Groenink et al., 1997), tolerance for the burying-suppressive effects has not been observed.

Several nonanxiolytic agents from other pharmacological drug classes like barbiturates (pentobarbital, pentylenetetrazol, picrotoxine) tricyclic antidepressants (amitryptiline, desipramine, imipramine, pargyline), antipsychotics (chlorpromazine, haloperidol), opiate analgesics (morphine), psychostimulants (methylphenidate, amphetamine), adrenergics (alprenolol, pindolol, practolol, yohimbine) and cholinergics (physostigmine, scopolamine) either do not significantly affect defensive shock-prod burying behavior, increase it, or cause a nonspecific (i.e., sedative, motor impairment) decrease.

Since chronically administered antidepressant drugs are clinically also very effective in the treatment of several anxiety disorders (Borsini et al., 2002), the suppression of burying after chronic desipramine (Fernandez-Guasti et al., 1999) cannot be regarded as a false positive in this test.

Additionally, suppression of burying has been reported for some "new" compounds with putative anxiolytic properties like the serotonin2/dopamine2 antagonist perospirone, corticotropin-releasing hormone (CRH)-receptor antagonists (alpha helical-CRH, D-Phe-CRH and R121919), neurosteroids (allopregnanolone), steroid receptor antagonists (RU28318, RU38486) angiotensin II and the gases nitrous oxide and toluene. On the other hand, it also has been shown that some anxiogenic agents like the α_2-adrenergic receptor antagonist yohimbine, the benzodiazepine receptor inverse-agonists β-CCE and DMCM and corticotropin-releasing hormone (CRH) enhance the amount of shock-prod burying in rats, indicating that the burying paradigm is also capable of detecting prostress or anxiogenic effects.

Although most pharmacological studies have employed defensive burying as the sole index of anxiety, some have used a more ethological approach by assessing the effects of anxiolytic compounds on additional and/or competitive behavioral indices of anxiety/fear as well. As already mentioned before, besides defensive burying as an active avoidance reaction to the shock-prod, enhanced immobility/freezing postures as a passive avoidance response are also displayed. In several other animal tests, this type of behavior is equally well considered to be true indicators of fear/anxiety (Bolles and Fanselow, 1980; Bolles, 1970; Rodgers, 1997), and does seem to be attenuated by anxiolytic drugs

(Conti et al., 1990; Treit, 1985a) Hence, this competing behavioral expression of defense can be used as an additional anxiety/fear index in the shock-prod paradigm. Paradoxically, benzodiazepine anxiolytics like chlordiazepoxide (CDP), diazepam and Ro 16-6028 seem, concurrent with a decrease of burying, to increase this immobility score (Treit et al., 1986; De Boer et al.., 1991; Tsuda et al., 1988a). Although some studies have reported a decrease of crouched/upright freezing postures after treatment with 5-HT_{1A} receptor agonists (Treit et al., 1993d; Groenink et al., 1995), our own studies show either no change [ipsapirone (Korte et al., 1992), alnespirone and S-15535] or an increase (buspirone and 8-OH-DPAT; see Fig. 4) in general immobility as a measure of passive avoidance behavior. Ipsapirone also failed to affect immobility behavior even if bedding material was not made available and, hence, animals were "forced" to passively avoid the prod (Korte et al., 1992).

Furthermore, the obviously pronounced suppression of prod-exploratory behavior can be employed as yet another index of rat's fear/anxiety state. Meert and Colpaert (1986) first demonstrated that anxiolytics reliably and dose-dependently block this inhibition, while most nonanxiolytics do not produce this effect. Several additional studies have indeed confirmed that benzodiazepine and 5-HT_{1A} anxiolytic-induced decreases in defensive burying are concurrent with increases in exploratory prod contacts (Craft et al., 1988; De Boer et al., 1990; Treit, 1990; Treit et al., 1993d). In addition to an anxiolytic-induced increase in prod exploration, this measure is even further decreased after treatment with putative anxiogenic agents like CRH and DMCM (Fig. 4).

Since it has been suggested that increased rearings of rats in their familiar home-cage may be related to attempts of escape (Rohmer et al., 1990), the enhanced rearing activity rats typically display (especially in the period shortly after receiving the prod-shock) during the test may be a fourth fear/anxiety index. In our studies, we have not observed reliable changes in this parameter after different anxiolytic/anxiogenic manipulations, although robust increases and/or decreases can be observed after various treatments (Fig. 4).

Besides these various behavioural manifestations of fear/anxiety, the effects of putative anxiolytic/anxiogenic manipulations may also be assessed on the autonomic/endocrine responses associated with shock-prod burying as described in the preceding section. To date, however, only a few studies have employed such an approach. De Boer et al.

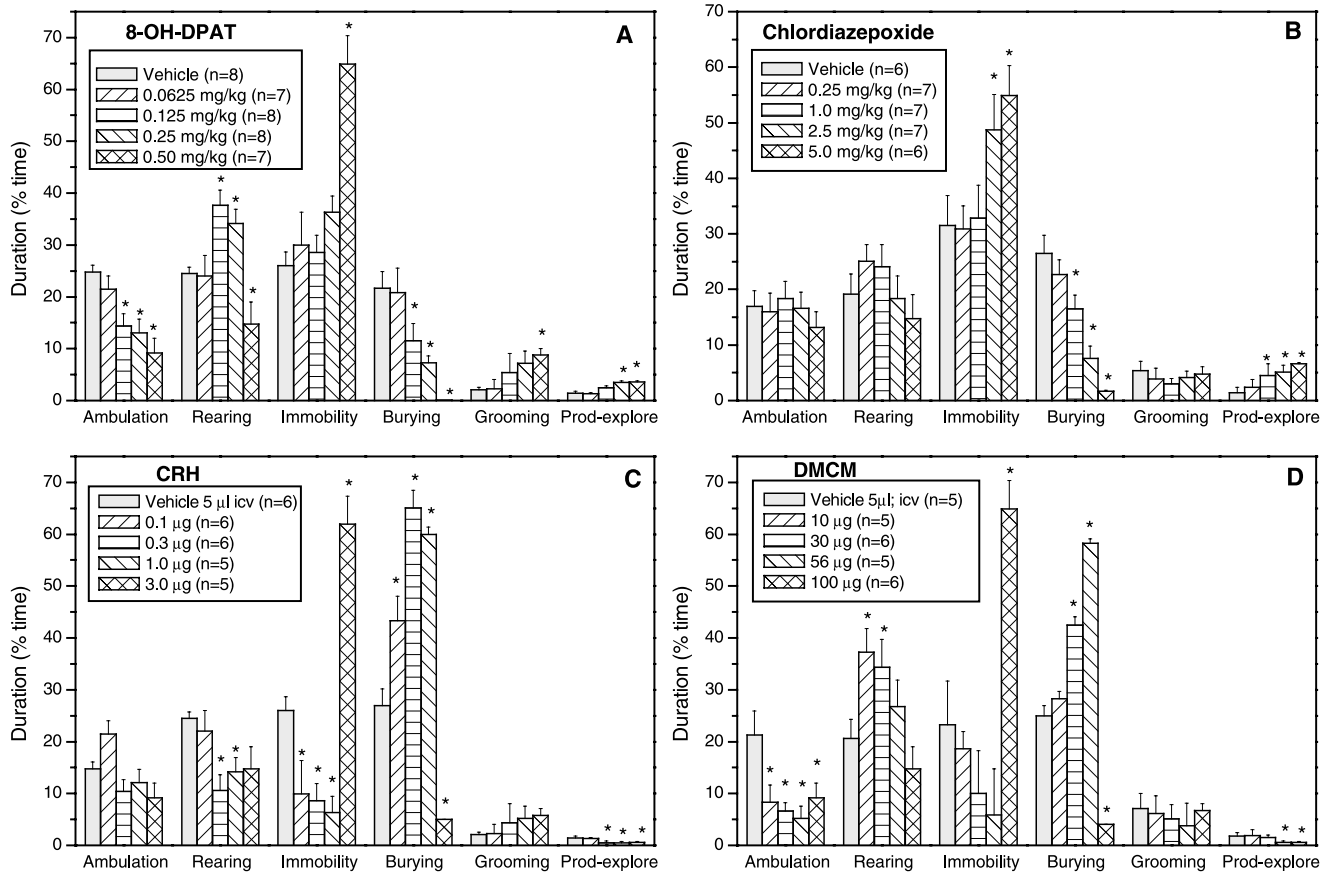

Fig. 4. Effects of 8-OH-DPAT (panel A; anxiolytic prototypical 5-HT_{1A}-receptor agonist), chlordiazepoxide (panel B; classical anxiolytic BDZ-receptor agonist), corticotropin-releasing hormone (panel C; anxiogenic stress-peptide CRH-receptor agonist) and methyl-6,7-dimethyl-4-ethyl-B-carboline-3-carboxylate (DMCM, panel D; anxiogenic BDZ receptor inverse-agonist) on six behavioral categories of rats in the unconditioned shock-prod burying test. *Values are significantly (at least $P<0.05$) different from the vehicle.

(1990) showed that the benzodiazepine anxiolytic chlordiazepoxide, despite the paradoxical increase in immobility behavior, reduced the elevations in plasma catecholamine and corticosterone associated with both active and passive coping with the shock-prod. This strongly indicates that CDP treatment indeed does reliably reduce the heightened fear/anxiety state induced by the presence of the electrified prod. In contrast, however, the behavioral anxiolytic effects of the 5-HT_{1A} receptor agonists flesinoxan and ipsapirone are not reflected in these hormonal measurements. Flesinoxan did not suppress the shock-prod stress-induced rises in plasma corticosterone and glucose levels despite clear anxiolytic-like reductions in burying and freezing behavior (Groenink et al., 1995). A similar dissociation of shock-prod behavior and neuroendocrine parameters has been reported by Korte et al. (1992) for the partial 5-HT_{1A} receptor agonist ipsapirone, although this compound diminished the tachycardia observed during presentation of the shock-prod (Korte and Bohus, 1990). The inability of 5-HT_{1A} receptor agonists to reduce stress-induced rises in neuroendocrine parameters in this test is possibly due to their potent stimulating effects on these parameters under basal conditions and, therefore, mask possible stress hormone-reducing effects.

Overall, defensive burying as an active coping response in the shock-prod paradigm fulfils the major pharmacological criteria of therapeutic class specificity, dose-dependent sensitivity, correlation with clinical potency, behavioral selectivity and absence of tolerance upon chronic treatment. Together with the good interlaboratory reliability and the virtual absence of false positives or false negatives, defensive burying serves well for screening potential anxiolytic properties of drugs. In addition, it should be apparent that by scoring the complete defensive behavioral repertoire of animals in the shock-prod test, the effects of drugs on both active (i.e., burying) and passive (freezing) defensive behaviors, as well as on some general motor behaviors, can be assessed simultaneously.

To further facilitate the interpretation of drug actions in this paradigm, the associated physiological/hormonal indices of these behaviours can be assessed concomitantly. The picture emerging from several studies that have employed such an approach is that anxiolytic/anxiogenic agents from different pharmacological classes provoke reliable changes in active burying and prod exploration but not in the competitive passive immobility/freezing responses. Especially, the nonuniform attenuation of different behavioral manifestations of fear/anxiety by prototypical anxiolytics seems to make the interpretation of drug effects in this paradigm at first sight somewhat problematic. Although, if one accepts the view that different neuronal networks subserve active and passive coping behaviors (Henry and Stephens, 1977; Keay and Bandler, 2001; Bandler et al., 2000; Davidson et al., 2000), then it should not at all be surprising that agents from different pharmacological classes may differently affect neuronal activity in these circuitries and, hence, affect passive and active coping responses differently. Therefore, the distinct behavioural profiles of the various pharmacological agents may not only be relevant from a psychopharmacological point of view but also from a neurobiological one.

Furthermore, a complex relationship appears to exist between levels of fear/anxiety, activation of the associated underlying neuronal systems and the generation of burying, flighting and freezing responses. It has been found that increasing a rat's putative fear/anxiety level, either behaviourally (pretest manipulations and/or test condition, see Table 2) or pharmacologically (medium to high doses of anxiogenic drugs, e.g., Fig. 4C and D), potentiate freezing (passive coping) behaviour and disrupts burying (active coping).

3.1. Marble-burying paradigm

In addition to defensive burying of electrified prods as the dependent variable of putative anxiolytic drug actions, a number of studies have used the suppression of spontaneous burying of harmless objects (usually glass marbles) by mice as the major index of anxiolytic drug action (Broekkamp et al., 1986; Gyertyan, 1995; Abe et al., 1998; Spooren et al., 2000; Millan et al., 2002). In this simple test, mice are individually placed in a novel cage containing a layer of sawdust with a number (approximately 20) of marbles evenly spaced on top of it. Thirty minutes later, the animals are removed and the number of marbles buried in the sawdust recorded. By taking the number of marbles buried as behavioral index, it was initially found that anxiolytic agents reduced this burying index at doses that did not reduce swim-induced grooming, i.e., induce sedation (Broekkamp et al., 1986). Additional behavioral and pharmacological evaluation of marble-burying behavior as a putative test for anxiety/anxiolytic agents revealed that the glass marbles do not provide a fear-provoking stimulus and thereby initiate directed defensive burying, but that they serve as a means of measuring the intensity of digging/burrowing (the bedding material) activity elicited by the presence of a diggable new substrate and which is highly correlated with burying the marbles (Gyertyan, 1995). Inhibition of marble-burying may, therefore, be a correlational test of anxiolytic activity, although selective inhibition of marble-burying was not found to be a property of 5-HT_{1A} receptor-related (e.g., buspirone, gepirone, ipsapirone, ritanserin) and 5-HT_3 receptor antagonist (ondansetron) anxiolytics (Njung'e and Handley, 1991a).

Interestingly, however, it appeared that acute administration of selective serotonin reuptake inhibitors (fluvoxamine, fluoxetine, zimelidine, indalpine, citalopram), the tricyclic agents clomipramine, desipramine and imipramine, the selective noradrenaline reuptake inhibitor roboxetine, the combined noradrenaline/serotonin reuptake inhibitor venlafaxine and the novel selective nonpeptidergic tachykinin NK1 receptor antagonists (GR205,171 and RP67,580)

Table 4
Brain regions involved in defensive shock-prod burying behavior

Brain region	Manipulation	Effect	References
Anterior septum	Electrolytic lesion	No change	Gray et al. (1981)
Posterior septum	Electrolytic lesion	Decrease	Treit et al. (1993a,b,c,d)
Entire septum	Electrolytic lesion	Decrease	Treit and Pesold (1990), Menard and Treit (1996), Treit and Menard (1997)
Posterior septum	Excitotoxic lesion	Decrease	Pesold and Treit (1992)
Entire septum	Midazolam injection	Decrease	Pesold and Treit (1994)
	CNQX injection	Decrease	Menard and Treit (2000)
	AP-5 injection	Decrease	Menard and Treit (2000)
Lateral septum	Midazolam injection	Decrease	Pesold and Treit (1996)
	Muscimol injection	Decrease	Degroot et al. (2001)
	VP-antagonist injection	No change	Everts and Koolhaas (1999)
	Pregnanolone injection	Decrease	Bitran et al. (1999)
Medial septum	Midazolam injection	No change	Pesold and Treit (1996)
	Muscimol injection	Decrease	Degroot et al. (2001)
Central amygdala	Electrolytic lesion	No change, decreased retention	Roozendaal et al. (1991), Treit et al. (1993a,b,c,d), Kopchia et al. (1992)
Entire amygdala	Electrolytic lesion	No change	Treit et al. (1993a,b,c,d), Treit and Menard (1997), Lehmann et al. (2000)
	Midazolam injection	No change	Pesold and Treit (1994, 1996)
Rhinal cortex	Aspiration lesion	No change, decreased retention	Lehmann et al. (2000)
Prefrontal cortex	Electrolytic lesion	No change	Maaswinkel et al. (1996)
Medial PFC	Excitotoxic lesion	Decrease	Shah and Treit (2002)
Dorsal hippocampus	Electrolytic lesion	No change	Treit and Menard (1997)
	MR-antagonist injection	Decrease	Bitran et al. (1998)
	Pregnanolone injection	Decrease	Bitran et al. (1999)
	Physostigmine	Decrease	Degroot et al. (2001)
Nucleus accumbens			
Rostral shell	Muscimol injection	No change	Reynolds and Berridge (2001)
Caudal shell	Muscimol injection	Induction burying	
Bed nucleus stria terminalis	Electrolytic lesion	No change	Treit et al. (1998)
Dorsal raphe	Electrolytic lesion	Decrease	Treit et al. (1993a,b,c,d)

selectively and dose-dependently suppress marble-burying behavior in mice (Broekkamp et al., 1986; Njung'e and Handley, 1991b; Ichimaru et al., 1995; Abe et al., 1998; Millan et al., 2001. Suppression of marble-burying is also observed after chronic treatment with the selective serotonin reuptake inhibitor fluvoxamine (Ichimaru et al., 1995), but not after chronic treatment with zimelidine (Njung'e and Handley, 1991a). Based on this pharmacology, it has been suggested that burying of novel harmless objects by mice may reflect a form of impulsive (obsessive–compulsive) rather than anxiety-like behavior (Millan et al., 2002; Gyertyan, 1995). However, there is currently no compelling evidence to suggest that marble-burying behavior in mice represents a valid model for either OCD or anxiety.

4. Neurobiology

In order to unravel the neuroanatomical and neurochemical basis of fear/anxiety and/or to examine the brain sites of anxiolytic action of benzodiazepines, several intracerebral electrolytical/neurochemical lesion and stimulation studies have been conducted using the shock-prod defensive burying behavior as the dependent variable. Table 4 presents the results of these investigations. It shows that especially the posterior parts of the septal region, the dorsal hippocampus, the caudal shell of the accumbens and the dorsal raphe nuclei are critical parts of the neural circuitry underlying defensive burying. Within these structures, the inhibitory neurotransmitter GABA via the $GABA_A$ receptor complex seems to play a prominent modulatory role. Interestingly, the amygdala, bed nucleus of the stria terminalis and prefrontal cortex do not seem to play an important role in this form of active defensive behavior. Unfortunately, in all these studies, the reported effects of the brain manipulations were restricted to burying behavior and did not provide information on the more passive forms of avoidance behaviors. It is believed that distinct but parallel neuroanatomical circuits underlie the execution of active and passive emotional coping strategies (Henry and Stephens, 1977; Davidson et al., 2000; Keay and Bandler, 2001). Since both of these coping behaviors can be expressed in this test, the shock-prod burying paradigm is well suited to scrutinize the neural circuitry for active as well as passive coping strat-

egies. Neuroanatomical mapping studies on patterns of neuronal activation using immediate-early gene expression (e.g., c-fos, c-jun, etc.) in high and low burying or freezing animals may be very informative in this respect. A preliminary study by Menard and Meaney (2001) using this approach showed that active and passive forms of shock-prod defense were associated with differential patterns of c-fos immunoreactivity throughout the brain, most notably hippocampal regions (CA1, dentate gyrus) inferior colliculus, septum, periaqueductal gray and nucleus accumbens (shell region). Interestingly, the caudal aspects of this latter brain region (shell n. accumbens) was recently shown to be a critical site to induce burying behavior (Reynolds and Berridge, 2001).

5. Conclusions and remarks

Normal fear and anxiety are emotions that help organisms avoid a wide variety of dangers and threats. These negative emotional states have been conceptualized as a repertoire of species-typical behavioral and physiological defense reactions tailored to meet these different forms of threats. Consequently, disorders of anxiety can, thus, be viewed as the inappropriate activation or exaggeration of these usually adaptive response patterns (Rodgers, 1997; Nesse, 2000; Rosen and Schulkin, 1998). Since the form, function and mechanisms of defense are highly conserved in vertebrate evolution, it has been argued that the behavioral validation of animal tests as models of human anxiety should focus upon the species-typical defensive repertoire (Rodgers, 1997; Blanchard et al., 1993).

From this viewpoint and as discussed in this review, the behavioral and physiological responses displayed in the shock-prod paradigm possess a high degree of face and construct validity of normal human fear and anxious apprehension. As is the case for the human defensive behavioral repertoire, the relative expression of active (i.e., burying) and passive (i.e., immobility) modes of coping with an electrified prod varies considerably between individual animals and seem to be critically dependent on a variety of procedural and organismic variables. When employing a combined ethological and physiological approach, the shock-prod defensive burying/passive avoidance paradigm serves well as an animal test that allows several behavioral and autonomic/endocrine manifestations of fear/anxiety to be measured simultaneously. Such a more refined analysis allows more detailed conclusions on the action of putative anxiolytic compounds. Theoretically, such compounds might reduce either all behavioral and physiological expressions of anxiety, or only one of its expressions or merely cause a shift in the various expression patterns. It is important to realize that the observed behavioral and physiological reactions are expressions of normal and functionally adaptive coping patterns and the extremes of either active or passive responding in this test cannot be regarded as inappropriate, maladaptive or pathological. For this reason, the shock-prod paradigm is not an animal model for anxiety disorder or for any other psychiatric disease. However, the apparent good pharmacological validation (predictive validity) of this test clearly reinforces the view that normal and pathological anxiety involves, at least partly, common neurobiological substrates. Therefore, this paradigm is not only suitable for screening potential anxiolytic properties of drugs but seems to be especially valuable for unraveling the neural circuitry and neurochemical mechanisms underlying the generation of active and passive coping responses.

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