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Original article

A panic experimental model: Validation of a complex operant behavioral method in undernourished rats, with desipramine to provide a template effect profile

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Abstract

Introduction: Clinical studies have shown that some antidepressants may be more efficient than benzodiazepines to alleviate anxiety associated with panic disorders; however, operant conflict procedures in rats developed so far seem not particularly able to model human anxiety sensitive to antidepressant treatments. Previous panic models with learned responses did not statistically subtract the effect of confounding factors from the variable of interest. **Methods:** Undernourished rats were selected due to their behavioral and neurobiological resemblance to human patients suffering from panic disorder. The Geller–Seifter paradigm represented the stressful environmental condition in adult life. Desipramine (10 mg/kg/day) or saline were administered IP during 7 days under a cross over design ($N=10$). Five daily 15 min-operant sessions were carried out on each experiment. Unpunished, unrewarded and punished operant behavioral periods were identical both in their duration and in their reward system (the FR1 schedule) in order to measure response suppression, which has not been considered in previous studies with the Geller–Seifter paradigm. The dependent variable was the difference between comparable unpunished and punished periods. **Results:** A significant Diet × Drug interaction was observed in the dependent variable, which represented the level of "suppression/suppression release" induced by treatments. **Discussion:** Compared to control rats, deprived rats showed a significant and selective anticonflict effect of desipramine on the stressful and complex operant performance. The animal model of perinatally protein-deprived rats along with the Geller–Seifter's operant behavioral paradigm may represent a more sensitive approach to model human anxiety sensitive to antidepressant treatments by considering the combined impact of both early biological trauma and adult learned experiences under the same design.

Keywords: Antidepressants; Conditioned anxiety; Desmethylimipramine; Geller–Seifter paradigm; Operant behavioral methods; Panic disorder; Perinatal protein malnutrition; Rat

1. Introduction

1.1. Geller–Seifter paradigm

There is a wide range of animal models and measures designed to assess anxiety or fearfulness. Many of these rely on the so-called approach–avoidance conflict paradigms. These tests have been extremely useful as initial screens for drugs affecting anxiety, but the components of anxiety assessed by these models remain poorly defined (Shekhar et al., 2001).

One of the most widespread animal models to assess anxiety–anti-anxiety effects is the Geller–Seifter paradigm (Beaufour, Ballon, Le Bihan, Hamon, & Thiébot, 1999; Geller & Seifter, 1960). It consists of a conflict operant procedure in which the feeding behavior (lever pressing) is suppressed by conditioned anxiety (an aversive stimulus associated with reinforcement). Researchers usually infer an anticonflict effect of drugs by measuring the difference between non-drug and drug responses during the punished period alone. They generally refer to this change as "release of the conditioned response suppression". But the response suppression itself is not actually measured because

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the reference criterion is generally the response rate under another schedule of reinforcement (assessed under another unit of measurement and under periods of different duration). In other words, the response suppression is generally assumed (but not statistically measured) by visually comparing the response rate under the punished period vs. the response rate under the unpunished one and by inferring that any difference is sufficient to be regarded as "suppression".

We consider that it could be more useful to incorporate also the information provided by the unpunished periods into the model of analysis particularly since an anticonflict effect of drugs should not affect normal activity in these periods. If a significant behavioral change in the unpunished period is accompanied by a simultaneous increase in the number of shocks received in the punished one, this could be interpreted as a cognitive deleterious effect of the drug as opposed to an anxiolytic or antidepressive one. In addition, the lack of a control group to test confounding appetitive, motor or analgesic effects, may also mask the final psychopharmacological effect. The use of animals predisposed to develop anxiety symptoms may help to better elucidate these questions. The analysis of the interaction between biological and environmental anxiogenic factors can provide a more realistic approach to the understanding of these psychopathological processes.

1.2. Deprived rats and panic disorder

Protein deprivation at perinatal age has long-lasting effects on morphological, neurochemical and behavioral parameters that persist in adulthood even after prolonged periods of nutritional recovery (Almeida, Tonkiss, & Galler, 1996; Morgane et al., 1986; Wiggins, Fuller, & Enna, 1984). As regards the catecholaminergic system, perinatally deprived rats showed alterations in noradrenergic neurotransmission (Keller, Munaro, & Orsingher, 1982; Marichich, Molina, & Orsingher, 1979; Nasif, Ramírez, Cuadra, & Orsingher, 2001) that resemble those of patients suffering panic attacks (Goddard & Charney, 1997; Laino, Córdoba, & Orsingher, 1993). Locus coeruleus activity is significantly higher in deprived rats than in controls; likewise, one week of desipramine (DMI) administration reduces the locus coeruleus activity of deprived rats to values comparable to controls, which were not affected after similar treatment (Nasif et al., 2001). Sodero, Valdomero, Cuadra, Ramírez, & Orsingher (2004) hypothesize that neuronal abnormalities observed in deprived rats may represent the neurobiological basis of the pathophysiology of panic disorder.

These abnormalities may account for some of the behavioral consequences observed in deprived rats such as increased avoidance performance, increased immobilization to a loud noise, impaired habituation to an open field after repeated exposures, and an increased number of ineffectual jumps in an active avoidance test (Brioni & Orsingher, 1988).

Considering the association observed between panic attacks and cocaine use in humans (O'Brien, Wu, & Anthony, 2005), it is interesting to note that an increased responsiveness to behavioral effects of cocaine and/or an enhancement of its reinforcement properties have also been observed in rats undernourished at perinatal age (Valdomero, Bussolino, Orsingher, & Cuadra, 2006; Valdomero, Isoardi, Orsingher, & Cuadra, 2005).

In the elevated plus-maze (Laino et al., 1993), drugs with therapeutic efficacy in panic disorders, such as diazepam and alprazolam showed a similar anticonflict effect in control and deprived rats, while buspirone, propranolol, desipramine and phenelzine induced a selective anxiolytic effect on deprived rats. Laino et al. (1993) affirmed that drugs that interact with noradrenergic and/or serotonergic systems exert a selective and anticonflict effect in deprived rats in the plus maze; consequently, deprived rats may represent a useful model for studying antipanic agents.

In previous studies with operant behavior, perinatally protein-deprived rats showed a significantly and gradually better performance than control rats under a variable ratio twenty (VR20) schedule of reinforcement as well as a worse performance under a differential reinforcement of low rate of five seconds (DRLR5) schedule of reinforcement (Brioni & Orsingher, 1988). These effects were attributed to the hyperreactivity of deprived rats to aversive or stressful situations. Under the Geller–Seifter test, the basal performance under FR1 schedule was not significantly different between groups. In the punished period, non-significant differences were observed under the non-drug situation. Nevertheless, 3 mg/kg of diazepam induced a lower anticonflict effect in deprived rats. The effect of this drug on the unpunished period performance was not evaluated (Brioni & Orsingher, 1988).

1.3. Antipanic drugs and desipramine

Models that emulate predisposing environmental events, such as early life stress or adult trauma have been useful for identifying brain circuits that are sensitized by exposure to adverse experiences (Shekhar et al., 2001). Punishment, exposure to novel stimuli and frustrative nonreward are considered as three major classes of anxiogenic environmental stimuli (Gray, 1982). Benzodiazepines, used in the clinic as anxiolytics, have been found in animal models specifically to attenuate behavioural suppression caused by these responses but it is probable that these drugs may alter decision-making by affecting the evaluation of the learned significance of the stimuli in the environment (Ljunberg, Lidfors, Enquist, & Ungerstedt, 1987).

Although in human anxiety disorders habit formation and conditioning of the anxious states will play roles in maintaining pathology, in animal models the cause for anxious behavior is usually acutely presented and, therefore, the attenuation of anxieties by drugs, such as benzodiazepines, may bring about immediate alleviation (Broekkamp, Berendsen, Jenck, & Van Delft, 1989). Interesting exceptions are the studies on the effect of long-term treatments in animal anxiety models. Results showed that drugs such as imipramine and desipramine are inactive with a single treatment but have an anticonflict effect after several weeks of treatments in normal rats (Broekkamp et al., 1989).

Clinical studies have shown that some antidepressants may be more efficient than benzodiazepines to alleviate anxiety associated with panic disorders (Ham, Waters, & Oliver, 2005;