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ANTIDEPRESSANTS REDUCE EXTINCTION-INDUCED WITHDRAWAL AND BITING BEHAVIORS: A MODEL FOR DEPRESSIVE-LIKE BEHAVIOR

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Abstract—The withholding of expected rewards results in extinction of behavior and, hypothetically, to depression-like symptoms. In a test of this hypothesis, we examined the effects of extinction of food-reinforced lever-pressing on collateral behaviors that might be indices of depression. Operant extinction is known to be aversive to the organism and results in avoidance behavior. We hypothesized that avoidance of, or withdrawal from, the former source of reward may serve as a marker for "despair." Adult male Wistar rats (*n*=6–7 animals per group) were exposed to a Skinner box attached to a second compartment of the same size, providing opportunity for the animals to leave the operant chamber and to enter the "withdrawal" compartment. The animals spent a portion of the time during the extinction trials in this second chamber. To assess the predictive validity of this behavior as a potential marker of "despair," we tested the effects of chronic administration of two common antidepressant drugs on this measure. The tricyclic antidepressant imipramine (20 mg/kg) as well as the selective serotonin reuptake inhibitor citalopram (20 mg/kg) reduced the number of entries and time spent in the withdrawal compartment. We propose that entries into and time spent in the withdrawal compartment may operationalize "avoidance," a core symptom of major depression. Rearing as well as biting behaviors during the extinction trials were also attenuated by the antidepressant treatment. These results lend support to the hypothesis that extinction of positively reinforced operants evokes behaviors that reflect elements of "despair/depression" because these behaviors are modulated by antidepressant treatment. The avoidance of the operant chamber as a consequence of extinction, together with rearing and biting behaviors, may serve as useful measures for the testing of antidepressant treatments.

Key words: depression, extinction, operant behavior, despair, imipramine, citalopram.

Chronic stressors, as well as the absence or withdrawal of positive reinforcers (rewards) (Lewinsohn, 1974), such as the loss of employment, partner, friends, and health, are associated with the risk for the onset of major depressive disorder (MD) (for review see Hammen, 2005; Kessler, 1997). The main symptoms are a depressed mood, low drive, loss of interest and pleasure, hopelessness, feelings of guilt or worthlessness, suicidal intentions, avoidance, and agitated behavior.

Given the importance of loss, or lack of reinforcers in the etiology of human clinical depression, we have begun to investigate the possibility of developing an animal model for extinction-induced despair/depression-like behavior. Initially we employed extinction of negatively reinforced behavior in the search for behavioral and physiological markers of extinction-induced "despair" (see Huston et al., 2009, for review). Specifically, we found that extinction of successful escape in the Morris water maze was accompanied by behaviors akin to depression, such as an increase in immobility (Schulz et al., 2004, 2007a,b) that correlated with measures of anxiety (Schulz et al., 2007b) and was attenuated by chronic antidepressant treatment (Schulz et al., 2007a). The despair-like behavior also correlated with changes in physiological parameters that are well-known biomarkers for depression (Schmidt et al., 2011), including changes in monoamine neurotransmitters (Schulz et al., 2004; Topic et al., 2008b), neurotrophins (Topic et al., 2008a), and hypothalamus-pituitary-adrenal axis (HPA)-related stress markers (Topic et al., 2008b). Although negative reinforcements are an important component of human life, the loss of positive reinfocers may be equally aversive. Thus, we set out to search for behaviors that were emitted during the course of operant extinction as a result of the withdrawal of expected appetitive reinforcement and might serve as markers for "despair." Although it is well established that the extinction of instrumental behavior or the reduction in the magnitude of the reinforcer is aversive to the organism and is accompanied by behaviors indicative of "aggression" (Azrin et al., 1966), "frustration" (Papini and Dudley, 1997), and "stress" (Coover et al., 1971; Kawasaki and Iwasaki, 1997), there has been no serious attempt to relate the effects of operant extinction to "despair" or "depression," and consequently, there is no available animal model for extinction-induced depression-like behaviors.

Given that extinction of appetitive behavior is aversive to the organism, we hypothesized that the withholding of reward should lead to avoidance of, or, escape from the site or source of the reinforcer. To assess avoidance behavior, we attached a second compartment devoid of lever and feeder to the operant chamber, so that during the extinction trials the animals had the "choice" to either remain in the compartment where the food pellets were

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Abbreviations: ANOVA, analysis of variance; HPA, hypothalamus-pituitary-adrenal; MD, major depressive disorder.

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delivered or traverse into the empty compartment that was not associated with food reward. In addition to the time and number of entries into the "withdrawal" chamber, we assessed rearing and biting behaviors. Because any animal model must have predictive validity—in the present case—validity as a model of depression, we examined the influence of two common antidepressant drugs (citalopram and imipramine) administered daily over the course of the extinction trials. We hypothesized that, to be considered as a potential marker of "despair/depression," the behavior emitted during the extinction trials must be modified by an antidepressant treatment. We expected that the antidepressant treatments would decrease avoidance of the former source of reward and, thus, decrease the number of entries and duration of time spent in the "withdrawal" compartment and, potentially, influence other behaviors emitted during the extinction trials.

EXPERIMENTAL PROCEDURES

Subjects

Twenty naïve male Wistar rats obtained from the Animal Facility of the University of Düsseldorf were used. They weighed 254 g (±3 g SEM) at the beginning of the experiments. The animals were housed in groups of five in Macrolon cages type IV with a reversed 12-h light: 12-h dark schedule (lights off at 7:00 AM). They were food deprived for 5 days before commencement of experimentation. Ten gram of standard laboratory pellets was given per rat and day no earlier than 1 h after the end of each testing day. The animals were weighed daily for monitoring weight reduction, which ranged between 82 and 94% of their free feeding weight and did not differ between treatment groups over the course of the experiment (analysis of variance [ANOVA]: $F_{[2,\ 16]}=2.485$, $P>0.05$). Water was freely available. The experiments were carried out during the active phase between 8:00 AM and 5:00 PM. Animals were acclimated to the animal holding room for 10 days after arrival before the experiments started. In this time, they were handled on three consecutive days to habituate them to the experimenter. All experiments were carried out in accordance with and approved by the German Animal Protection Law (Bezirksregierung Düsseldorf). The experimental protocol used was chosen to allow minimizing suffering and using a minimal number of animals.

Drugs

According to group assignment, either the tricyclic antidepressant imipramine (10,11-Dihydro-N,N-dimethyl-5H-dibenz[b,f]azepine-5-propanamine hydrochloride, 5-[3-(Dimethylamino)propyl]-10,11-dihydro-5H-dibenz[b,f]azepine hydrochloride; Sigma-Aldrich, Germany) or the selective serotonin reuptake inhibitor citalopram (Cipramil, Lundbeck, Germany) was applied in a dose of 20 mg/kg, which was shown to exert antidepressant–like effects in rodents (e.g., Paul et al., 1990; Cryan et al., 2004; Réus et al., 2010). Both substances were diluted with distilled water (vehicle) and given intraperitoneally in an injection volume of 1 ml/kg.

Experimental schedule

Animals were tested in the Skinner box for 17 days, with one session per day lasting for 15 min. They were exposed to the open field (OF) 24 h later, followed by testing in the forced swim test (FST) the next day. Daily substance administration started on day 13 and was carried out 30 min before testing on every testing day. The behavior of the animals during each task was recorded by video cameras which were connected to a DVD recorder for further post hoc analysis by a trained observer blind to the treatment conditions. The apparatus was cleaned with a 60% ethanol solution after each trial.

Skinner box with withdrawal chamber

Apparatus. The animals were tested for 15 min each day in a dual operant chamber, which is depicted in Fig. 1. Two boxes (29.5×28.5×23.5 cm; modular test cage system, Coulbourn Instruments, USA) with a grid floor were connected via a tunnel (16×12.5×9 cm) made of gray plastic. Transition between the compartments was possible during the whole experiment. Both chambers were equipped with a light mounted near the top, in the center of one of the walls, which was on during the whole testing session (1 lux). In the reinforcement chamber, a lever (2 cm×3.5 cm) was mounted on the wall at 3 cm height. Three cue lights (yellow, green, red) were positioned above the lever. In the middle of the wall, a food magazine for reward pellets (4 cm×3 cm) was placed at 3 cm height. All cue lights were illuminated when the lever was "active," thus, indicating the availability of food reward after lever-pressing. The cue lights ceased glowing when the lever was pressed. The animal had to cross the photo-beams of the food dispenser to retrieve a food pellet before the cue lights could be activated again and another lever-press led to the delivery of the next pellet. In the second box (the withdrawal box) only the

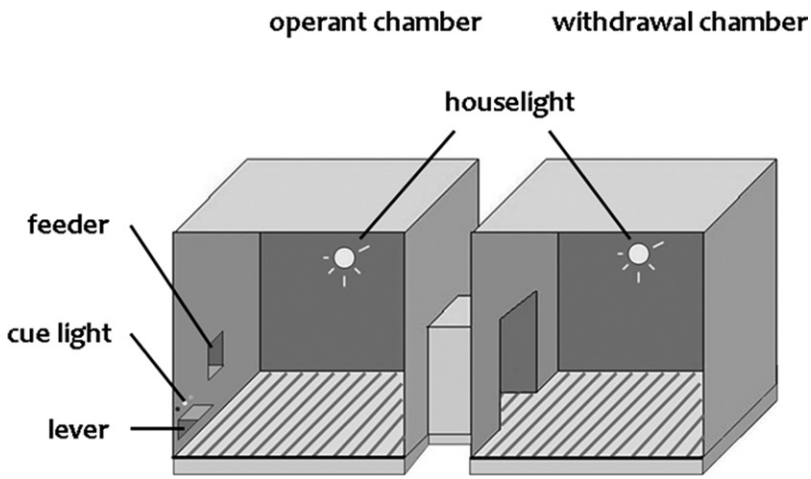

Fig. 1. Scheme of operant chamber with withdrawal compartment, and testing schedule for the treatments with either vehicle, citalopram (20 mg/kg), or imipramine (20 mg/kg).