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BEHAVIORAL CONTROL OVER SHOCK BLOCKS BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF LATER SOCIAL DEFEAT

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Abstract—Experience with behavioral control over tailshock (escapable shock, ES) has been shown to block the behavioral and neurochemical changes produced by later uncontrollable tail shock (inescapable shock, IS). The present experiments tested, in rats, whether the protective effect of control over tailshock extends beyond reducing the behavioral and neurochemical impact of a subsequent tailshock experience to stressors that are quite different. Social defeat (SD) was chosen as the second stress experience because it has few if any cues in common with tailshock. SD produced shuttlebox escape learning deficits ("learned helplessness") and reduced juvenile social investigation 24 h later, as does IS. IS is notable for inducing a large increase in dorsal raphe nucleus (DRN) serotonergic (5-HT) activity as measured by extracellular levels of 5-HT within the DRN, and SD did so as well. ES occurring 7 days before SD blocked this SD-induced DRN activation, as well as the SD-induced interference with shuttlebox escape and reduction in social investigation. Prior exposure to yoked IS did not reduce the DRN 5-HT activation or later behavioral effects produced by SD, and thus the proactive stress-blunting effects of ES can be attributed to it's controllability. Thus, ES confers a very general protection to the impact of a subsequent stress experience.

Key words: stress resilience, serotonin, stressor controllability.

There is considerable interest in psychological/behavioral variables that determine the behavioral and physiological impact of stressors, as well as experiential factors that alter the organism's vulnerability to subsequent aversive experiences (Southwick et al., 2005). Interestingly, the opportunity to exert behavioral control over an aversive event not only blunts the behavioral and neurochemical consequences of that event (Maier and Watkins, 1998; Ravindran et al., 2002; Weiss, 1971), but also reduces the behavioral and neurochemical consequences of exposure to subsequent stressors over which the organism does not have behavioral control (Amat et al., 2006; Williams and Maier, 1977). The latter "immunizing" effect of behavioral control has received only recent attention. In the reported experiments rats exposed to a series of tailshocks, each of which terminated whenever the rat turned a small wheel in the front of the chamber (escapable shock, ES), later failed to show the typical behavioral and neurochemical consequences of exposure to inescapable (uncontrollable) shocks (IS) occurring in a different, but similar apparatus. More specifically, the initial ES treatment was shown to completely block (a) the interference with shuttlebox escape learning (Williams and Maier, 1977) and reduction in social investigation of a juvenile (Christianson et al., 2008b) that is normally produced by IS, and (b) the intense activation of dorsal raphe nucleus (DRN) serotonergic (5-HT) neurons that is typically produced by IS (Amat et al, 2006). DRN 5-HT activity was explored because prior work had found IS-induced activation of these cells to be critical to the mediation of both the escape interference (Maier et al., 1995) and reduced social investigation (Christianson et al., 2008a) produced by IS. An initial experience with exactly yoked inescapable tailshocks (IS) in the wheel-turn boxes did not blunt these behavioral and neurochemical sequelae of later IS, demonstrating that the stress-resistance produced by the initial experience was caused by the controllability of the tailshocks.

The seeming "resilience" in the face of later uncontrollable stress produced by experiencing a controllable aversive event is striking as the controllable events (ES) are nevertheless quite "stressful." For example, ES produces adreno-cortico-trophin-hormone and corticosterone increases that are as large as those produced by yoked IS (Maier et al., 1986), as well as comparable increases in cortico-trophin-releasing-hormone and arginine vasopressin mRNA in the hypothalamic paraventricular nucleus (Helmreich et al., 1999). However, very little is known concerning this immunization phenomenon. Perhaps the most obvious question is whether the blunting effects of a control episode are restricted to subsequent events that are the same as, or similar to, the events over which the organism is given control, or whether instead exerting control over an aversive stimulus such as tailshock confers a more general resistance to the effects of stressors. In selecting a stressor subsequent to the initial ES it seemed desirable to choose an aversive situation that has as few stimuli in common with ES and the ES environment as possible, and one known to activate DRN 5-HT neurons. Social defeat (SD) meets both requirements. Pilot work indicated that SD produces both shuttlebox escape deficits and reduced juvenile social investigation 24 h later, as does IS, and Gardner et al. (2005) in rats, and Cooper et al. (2009) in Syrian hamsters, reported that SD induces

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Abbreviations: ADI, attack/defeat index; ANOVA, analysis of variance; DRN, dorsal raphe nucleus; ES, escapable shock; FR-, fixed ratio; HC, home cage controls; IS, inescapable shock; LH, learned helplessness; PLSD, protected least significant difference test; SD, social defeat; vmPFC, ventral medial prefrontal cortex.

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Fos in DRN 5-HT neurons. Thus here we sought to determine whether prior ES would block the escape deficits, reduced social investigation and DRN 5-HT activation produced by SD.

EXPERIMENTAL PROCEDURES

Subjects

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) weighing 275–350 g, were housed four per cage on a 12 h light/dark cycle (on at 07:00 h and off at 19:00 h). Long Evans retired breeders weighing 600–800 g housed individually, under a similar lighting schedule, were selected as alpha males for SD encounters (see below) if they attacked a Sprague–Dawley rat within the first 5 min of several practice encounters. Experiments were conducted between 9:00 h and 1600 h. All procedures conformed to international guidelines on the ethical use of animals and were approved by the Institutional Animal Care and Use Committee of the University of Colorado at Boulder. The number of animals used and their suffering were kept to the minimum.

Overall organization

This report contains two types of experiments: (a) some examined the effects of SD on DRN 5-HT efflux during the SD encounters, and on behavior 24 h later (learned helplessness (LH)), and (b) some examined the effects of an initial experience with controllable tail shock (ES) in modulating the effects of SD occurring 7 days later, both on DRN 5-HT efflux during the SD encounter and on changes in behavior 24 h after SD. The behaviors measured in both types of experiments were: attack/defeat Index (ADI, see below) during the SD encounters, and shuttlebox escape learning and social exploration 24 h after SD.

Surgery and cannulation

Rats to be used for microdialysis underwent surgery using anesthesia with a mixture of 100 mg kg^{-1} ketamine (Fort Dodge Animal Health, Fort Dodge, IA, USA) and 6.4 mg kg^{-1} xylazine and 1.6 mg kg^{-1} acepromazine (Vedco Inc., St. Joseph, MO, USA). A cannula guide for microdialysis probes (CMA 12), was stereotaxically implanted with the tip terminating just above the caudal DRN: 8.3 mm caudal to bregma and 5 mm ventral from the dura matter at the midline (Amat et al., 2005). A screw cap of a 15 ml conical centrifuge tube, whose central portion was removed, was also affixed to the skull so that its threads were exposed and it encircled the cannulae guide. This was done so that the skull assembly could be protected during microdialysis. Rats were allowed to recover for 1–2 wk before experimentation. All surgical procedures to any given animal were performed the same day.

Wheel-turn escape learning

Each rat was placed in a Plexiglas box ($14\times11\times17$ cm^3) with a wheel mounted in the front and a Plexiglas rod extending from the back. The rat's tail was taped to the Plexiglas rod and affixed with copper electrodes. Rats received shocks in yoked pairs (ES and IS). The treatment consisted of 100 trials with an average intertrial interval of 60 s. Shocks began simultaneously for both rats in a pair and terminated for both whenever the ES rat met a response criterion. Initially, the shock was terminated by a quarter turn of the wheel. The response requirement was increased by one-quarter turn when each of three consecutive trials was completed in less than 5 s. Subsequent latencies under 5 s increased the requirement by 50% up to a maximum of four full turns. If the requirement was not reached in less than 30 s, the shock was terminated and the requirement reduced to a single quarter turn. This procedure was used to insure that the ES rats learned an operant response. Shock intensity was 1.0 mA for the first 30 trials, 1.3 mA for the second 30 trials and 1.6 mA for the last 40 trials, to maintain good escape responding. Non-shocked home cage control (HC) rats remained undisturbed in the colony, except during the microdialysis experiments, where they remained undisturbed in the dialysis room.

In vivo microdialysis

The afternoon before the experiment, a CMA 12 microdialysis probe (0.5 mm in diameter, 1 mm membrane with a 20-kDa molecular weight cut-off; CMA/Microdialysis Inc., North Chelmsford, MA, USA) was introduced through the cannula guide so that the membranous tip of the probe was within the DRN. A portion of a 15 ml Eppendorf tube (Eppendorf of North America Inc., Westbury, NY, USA) was screwed onto the skull-mounted screw cap, through which the dialysis tubing, protected within a metal spring, entered and attached to the probe. Each animal was placed individually in a Plexiglas bowl (Bioanalytical Systems, West Lafayette, IN, USA) and infused with isotonic Ringer's solution (Baxter, Portage, MI, USA) at a rate of 0.2 μl min^{-1} overnight. At 9:00 h the next day, the flow rate was increased to 1.5 l min^{-1} and a 90-min stabilization period was allowed. The infusion flow remained constant throughout the experiment and samples were collected every 15 min. After taking four baseline samples, 45 min social encounters took place, with either an alpha male, or with a Sprague–Dawley rat similar in size to the target subject. The same size Sprague–Dawley never fought with the dialyzed target rat. During collection of the last sample, brisk movements of the skull-mounted screw cap were performed to test for possible 5-HT increases due to rat head movement during the dialysis. The data from the rat were discarded if that procedure caused 5-HT increases.

5-HT analysis

5-HT concentration was measured in dialysates by high performance liquid chromatography with electrochemical detection. The system consisted of an ESA 5600A Coularray detector with an ESA 5014B analytical cell and an ESA 5020 guard cell. The column was an ESA MD-150 (C-18, 3 μm, 150×3.2 mm^2) maintained at 37 °C, and the mobile phase was the ESA buffer MD-TM. The analytical cell potentials were kept at μ75 mV and +250 mV and the guard cell at +300 mV. Dialysate (25 μl) was injected with an Environmental Sciences Associates Bioscience Inc (ESA) 542 autosampler that kept the dialysates at 6 °C. External standards (Sigma) were run each day to quantify 5-HT by means of peak height, using ESA software, the experimenter being blind to experimental condition.

Dialysis probe verification

At the end of the experiment an overdose of pentobarbital was administered and brains were removed and frozen. A cryostat was used to take 40 μm sections, which were then stained with Cresyl Violet for cannula placement verification. Only rats with the dialysis probe at least 70% within the intermediate and caudal DRN (−7.8 to −8.8 from bregma) were included (see Fig. 1).

Social defeat

SD encounters were carried out both with freely moving rats as well as during microdialysis. When freely moving, the encounters took place in $45\times60\times40$ cm^3 plastic boxes with rat bedding. The Long Evans alpha males were placed in the boxes 1 h in advance and then the target Sprague–Dawley was introduced. The encounters lasted 45 min and were recorded on DVD for later analysis by a researcher unaware of the treatment previously received by the target rat. Two types of behaviors were timed: (a)