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Research report

Effect of number of tailshocks on learned helplessness and activation of serotonergic and noradrenergic neurons in the rat

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Abstract

Adult male albino rats were exposed to varying numbers of tailshocks (0, 10, 50 or 100). The following day, their escape latencies in a shuttlebox were measured in order to estimate the degree of learned helplessness (LH) produced by the varying number of shocks. Only the groups exposed to 50 or 100 shocks displayed evidence of LH. In a parallel experiment, *c-fos* activation was used to determine the degree of activation of raphe serotonergic neurons (FosIR + 5-HT) and locus coeruleus (LC) noradrenergic neurons (FosIR + TH) produced by the same shock conditions. Compared to unhandled cage controls, all shock groups (0 shocks was a restrained group) significantly activated both raphe and LC neurons. The 50 and 100 shock groups had significantly higher degrees of activation of serotonergic neurons in the rostral raphe groups and the LC than the 0 and 10 shock groups. These data are consistent with the hypothesis that activation of rostral raphe serotonergic neurons and LC noradrenergic neurons beyond a certain threshold may be critical for the development of LH. The relevance of these results for elucidating the neural bases of psychopathology is discussed.

Keywords: Raphe; Serotonin; Locus coeruleus; Norepinephrine; Inescapable shock; Learned helplessness; Depression; *c-fos*

1. Introduction

A major question regarding the etiology of mental illness is at what point the precipitating conditions cross the threshold from being merely bothersome and annoying to producing frank pathology. This raises the issue of what are the attendant CNS and peripheral physiological changes associated with crossing this boundary, and therefore pinpointing those that are potentially causal in the illness.

It has long been known that exposing organisms to a series of uncontrollable stressors produces behavioral, neurochemical, and endocrine sequelae that resemble those characteristic of a number of disorders [14,23]. This has typically been studied by exposing subjects to a series of inescapable tailshocks or footshocks, a procedure which results in the laboratory phenomenon called "learned helplessness" (LH) [13]. However, the exact stimulus parameters for producing this phenomenon have not been systematically explored. In the first of the two experiments presented here, we examined the efficacy of the number of tailshocks (0–100) in producing LH in adult rats. The second experiment followed up on these findings and, using *c-fos* activation in conjunction with double-labeling, examined the effects of varying the numbers of shocks on the activation of both serotonin (5-HT) containing neurons, throughout the brain stem raphe complex, and of norepinephrine (NE) containing neurons of the locus coeruleus (LC). Serotonergic neurons were chosen because of their well-known involvement in human psychopathology [15,17] and because previous work in one of our laboratories had shown that serotonergic neurons in the dorsal raphe nucleus (DRN) play a particularly important role

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in the development of LH in rats [5,11]. We have also previously reported that serotonergic neurons throughout the raphe complex are significantly activated by 100 tailshocks [19] and that DRN 5-HT neurons are responsive to the dimension of stressor controllability [4]. Noradrenergic neurons of the LC were also chosen for study because of their involvement in human psychopathology [2], LH [22], and stress and arousal [3,6].

The critical issue examined here is whether crossing the threshold of the number of shocks needed to produce LH also appears to cross a boundary in producing a significantly larger activation of brain raphe-serotonergic and/or LC-noradrenergic neurons.

2. Materials and methods

2.1. Animals

Adult male Harlan Sprague–Dawley rats weighing 300–350 g were housed in pairs in standard Plexiglas cages, with food and water ad libitum, in a room on a 12 h light:12 h dark cycle (light on at 07:00 h). All procedures were in accordance with NIH animal care guidelines and were conducted with approval of the University of Colorado Institutional Animal Care and Use Committee.

2.2. Inescapable stress

Rats were randomly assigned in pairs to the following groups: restraint (0), 10, 50, or 100 shocks. The experimental groups ($n = 8$ per group for experiment 1 and 6 per group for experiment 2) were exposed to either restraint or inescapable shock (IS) in a single session of approximately 100 min, which took place between 09:00 and 11:00 h. Rats were restrained in Plexiglas tubes (23.4 cm long and 7.0 cm in diameter). A bar protruded from the back of each tube to which the rat's tail was taped. Copper electrodes were attached to each tail. Rats were given shocks (5 s, 1.0 mA) in yoked pairs on variable-interval schedules corresponding to the number of shocks received such that all sessions were approximately the same length. Thus, the average intertrial interval times were 645, 125 and 60 s for the 10, 50 and 100 shock groups, respectively. In the second experiment a group of home cage controls (CC; $n = 4$) were also studied. They remained undisturbed in the animal colony. Cagemates each received the same experimental treatment. Shocks were created by shock sources modeled after the Grason-Stadler model 700 shock source.

2.3. Shuttle-box apparatus

Behavioral testing occurred in shuttle boxes measuring 46 cm long × 20.7 cm wide × 20 cm high. Scrambled 0.6 mA foot shocks were administered through a grid floor made of stainless steel. The shuttle box was divided into two equal halves with an aluminum partition that contained an archway that allowed passage from one side to the other.

2.4. Shuttle-box procedure

Twenty four hours after exposure to inescapable shocks, rats were placed in the shuttle boxes and were administered five 0.6 mA foot shocks that were delivered on a 1 min variable-interval schedule and could be terminated by crossing to the other side one time [fixed ratio-1 (FR-1) trials]. This procedure was followed by 25 FR-2 trials during which rats were required to cross the shuttle box twice to terminate the shock. It is during the FR-2 trials that the escape deficits produced by IS are normally revealed. All shocks were terminated after 30 s if the proper escape response did not occur.

2.5. Immunohistochemistry

The rats were deeply anesthetized with sodium pentobarbital (Nembutal) 2 h after the last shock (or control period). They were then perfused through the left ventricle with heparinized 0.9% saline followed by 250 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PBS; pH 7.4) at 4 °C for 15 min. Extracted brains were postfixed in the same 4% paraformaldehyde for 4 h and then transferred to 30% sucrose in PBS until sectioning. The brains were then shipped cold from the University of Colorado at Boulder to Princeton University by overnight courier. Brains were cut into 40-μm thick coronal sections. Every fifth section was collected in a separate series of wells containing antifreeze solution (37.5% ethylene glycol, 20% sucrose in 0.03 M PBS) and then stored at −20 °C. Tissue from each animal was processed for double labeling of both Fos and 5-HT or Fos and TH (tyrosine hydroxylase) using immunohistochemistry techniques.

Briefly, free-floating sections were incubated for 10 min in 0.1% hydrogen peroxide to remove endogenous peroxidase activity. After rinses with cold PBS the sections were incubated for 1 h in 2% normal goat serum and 0.3% triton X-100 in PBS to block nonspecific binding. They were then incubated for 36–48 h with primary polyclonal rabbit anti-Fos antiserum (Santa Cruz Biotechnology, Santa Cruz, CA) in block solution (1:2000) at 4 °C. After this period, the slices were rinsed with PBS and incubated for 90 min with biotinylated goat anti-rabbit secondary antiserum (Vector Laboratories, Burlingame, CA, USA) in block solution (1:200). Sections were then incubated with avidin–biotin–horseradish peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories) in cold PBS (1:100) for 90 min. After rinses with cold PBS and sodium acetate buffer (0.1 M, pH 6.0), the tissue was reacted with 3,3′-diaminobenzidine tetrahydrochloride (DAB) as a chromogen. Nickel sulfate was used to intensify the reaction product. When sufficient Fos staining was obtained, as determined by microscopic examination, the tissue was then stained for either 5-HT or TH. One series of sections was incubated with primary polyclonal rabbit anti-5-HT antiserum (1:2000; Protos Biotech, New York, NY, USA) and another series with primary polyclonal rabbit anti-TH antiserum (1:2000; Calbiochem, San Diego, CA, USA), at 4 °C for 40–48 h, in block solution. 5-HT and TH were both visualized after incubation with the fluorescent secondary antibody Cy3 (Jackson Immuno Research Laboratories, West Grove, PA, USA) for 2 h at room temperature, in block solution (1:100). After additional rinses with PBS, the sections were mounted on slides (Fisherbrand Superfrost/Plus, Fisher Scientific, Pittsburgh, PA, USA), dried overnight in a dark environment, coverslipped with DPX mounting media (Fluka BioChemika, Steinheim, Switzerland) and stored at 4 °C.

2.6. Data analysis

The slides were examined with an Olympus BX-60 microscope (Olympus America Inc., Melville, NY, USA) using the brightfield