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Intraventricular 6-Hydroxydopamine Lowers Isolation-Induced Fighting Behavior in Male Mice

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CRAWLEY, J. N. AND J. F. CONTRERA. *Intraventricular 6-hydroxydopamine lowers isolation-induced fighting behavior in male mice.* PHARMAC. BIOCHEM. BEHAV. 4(4) 381–384, 1976. – Male mice with high isolation-induced fighting tendencies were administered 200 μg 6-OHDA or vehicle intraventricularly and tested for fighting tendency for up to 10 weeks until sacrifice, and assayed for brain NE levels. A strong correlation was found between NE depletion and reduced fighting tendency after 6-OHDA treatment. The depressed fighting by mice with less than 200 ng NE/g persisted throughout a series of test fights, indicating no recovery in fighting behavior throughout the survival time.

Isolation-induced mouse fighting Neurotransmitters and behavior 6-Hydroxydopamine
Norepinephrine depletion

BEHAVIORAL neuroanatomists since the turn of the century have been using lesion techniques to relate brain structures to behavior, while the neurochemical coding of these brain regions has remained obscure. Methods for assaying endogenous levels of neurotransmitters [7] and for visualizing neurochemically specific fiber tracts [9,16] have been developed in the past decade. These methods open possibilities for studying the central nervous system neurotransmitters involved in behavioral patterns in much the same way as anatomical structures are investigated. Notable successes relating neurochemistry to behavior include Jouvet's [15] elucidation of 5-hydroxytryptamine-norepinephrine ratios during the sleep cycle; and clinical findings that dopamine in the corpus striatum is involved in motor dysfunctions such as Parkinson's disease [12]. Interesting and often controversial studies are in progress by many investigators to determine the neurochemical basis of eating, drinking, locomotion, activity, exploration, motor coordination, grooming, and self-stimulation, to name a few [1, 10, 13, 21, 22, 27, 28, 29]. This paper reports some preliminary results relating norepinephrine levels to spontaneous male mouse fighting behavior.

The few experiments to date on the neurochemistry of rodent fighting behavior emphasize the uselessness of terms like aggression for neuropharmacologists as well as for ethologists. Thoa *et al.* [31] found that 6-hydroxydopa (6-HDP) depletion of norepinephrine (NE) in whole brains of rats increased shock-induced fighting behavior. A decrease in attack on frogs [14], but increased attack on mice [2, 14, 18], by 6-hydroxydopamine (6-OHDA) treated rats with whole brain NE depletion has been found in other studies. Our results indicate that 6-OHDA depletion of NE in isolated male mice will decrease the tendency for these depleted mice to attack and fight with strange male opponent mice. The decreased fighting appears to be directly and perhaps specifically correlated with decreased NE levels.

METHOD

Animals

The 33 mice were the offspring of breeding pairs of CF-1 albino laboratory mice (Carworth Farms, New City, N.Y.). Mice were kept in a soundproof room on a light schedule of 12 hr (on 6:00–18:00) at a temperature of about 22°C to facilitate survival of isolated males. Mice were housed in clear plastic cages, 29 × 18 × 13 cm (Scientific Products, Washington, DC) covered with 1/2 cm^2 wire mesh lids, visually separated by 3 × 14 × 0.1 cm cardboard panels between cages. Animals were weaned at age 1 month and placed either 1 male per cage (isolated male) or 1 male housed with a female littermate per cage (male + female). Our previous paper [6] showed that both of these housing conditions tend to equally increase fighting tendencies of the adult male mice. Water and laboratory rodent pellet food were always available; litter was changed and cages washed once a week. Except for the test fights, drug administration, and weekly cleaning, mice were undisturbed in their home cages.

Test fights

The test for fighting tendency consisted of a subject male being placed in an empty clean clear plastic arena cage (29 × 18 × 13 cm) for 10 min with a designated Standard Opponent Winner or Loser mouse. (Standard Opponents, as described by Brain and Poole [4], are mice external to the experimental group, who had been previously trained to

consistently attack (= Opponent Winner) or to consistently lose and never attack (= Opponent Loser) a strange male mouse.) The experimenter observed the 10 min test and scored it as one of 4 easily distinguishable outcomes: 1) the test mouse did not fight (No Fight) or 2) attacked and fought (Won) in the presence of a Loser Opponent; and 3) the test mouse either fought back (Draw) or 4) did not fight back (Lost) in the presence of an attacking Winner Opponent. The experimenter also noted time (sec) spent fighting, latency to attack the Loser, presence of tail rattling, body postures, squeaking, body wounds, etc., but the most reliable criteria of fighting, used in the data analysis below consists of Wins against the Loser and Draws against the Winner. For further discussion of behavioral methods, see Crawley *et al.* [6].

Potential subject mice were screened for fighting tendencies in 5 test fights. At age 2 months, each male was tested against an Opponent Loser, and 2 days later against an Opponent Winner. At age 3 months, each male was again tested against a different Opponent Loser and 2 days later against a different Opponent Winner. Within one week before drug administration, each male (adult, 3–5 months old) was tested against a different Loser Opponent. Only males who had fought in at least 3 out of 5 of these tests, and Won in the last test, were used in this experiment.

Four days after drug administration, subject mice were tested against a Loser, and on Day 6 against a Winner Opponent. On Day 11, each was again tested with a new Loser, and on Day 13 with a new Winner. These 2 tests per week usually continued for 4 weeks, until sacrifice, but in some cases survival was extended to 10 weeks, with 2 fights each week. Before the first test for each week, the animal was given a 5 min open field test to measure locomotion, exploration, grooming, etc. This data, to be reported in a future communication, generally showed no significant motor differences between 6-OHDA treated mice and vehicle controls.

Intraventricular Drug Administration

Subject mice under Nembutal anesthesia were stereotaxically injected with 200 μg of 6-OHDA-HBr (Sigma Chemical Co., St. Louis, Mo.) in 0.1% ascorbic acid-0.9% NaCl vehicle solution, into either the right or the left lateral ventricle. Injection volume was 5 μl administered over a 3 min period using a Hamilton syringe connected to a 27 ga needle. A single 200 μg dose was used in this experiment to give a wider range of partially depleted brain amines. Vehicle controls were given 5 μl of ascorbic-saline intraventricularly.

A separate group of mice was identically treated, except that 50 mg/kg desmethyl imipramine (DMI, kindly contributed by Lakeside Laboratories, Milwaukee, Wisc.) was administered intraperitoneally 30 min before injection of 6-OHDA. This procedure depletes dopamine (DA) without destroying NE terminals [5,29]. DMI-pretreated vehicle controls were also performed.

Assay of Brain NE and DA

Mice were sacrificed by cervical dislocation, at least 3 days after the last test fight. Brains were quickly removed, frozen on dry ice, weighed, and individually stored in 5 ml of 10% trichloroacetic acid at −5°C. Each brain was homogenized in the trichloroacetic acid and centrifuged at 5,000 × g at 5°C for 10 min. The supernatant was separated on alumina [7], the eluted catecholamines were assayed spectrofluorimetrically using the trihydroxyindolamine reaction for norepinephrine [7] and for dopamine [35].

Statistical Analysis

To compensate for the inherent variability of behavioral test results, each mouse was tested against a Loser and a Winner Opponent once a week for 4 or more weeks until sacrifice, to get a broader picture of the fighting tendencies of the individual mice. A rough estimate of fighting tendency was calculated by adding total number of Draws against Winner Opponents and dividing by total number of fights (Wins + No Fights against Losers plus Draws + Losses against Winners). The Spearman Rank Correlation Coefficient, with correction for tied behavioral scores [26], was calculated to test the degree of correlation between whole brain content of NE and fighting tendency for 6-OHDA treated mice.

Mice were also grouped for statistical analysis according to brain levels of NE: Vehicle controls 350–500 ng NE/g brain tissue, and 6-OHDA treated 0–100 ng NE/g (most depleted), 101–200 ng NE/g, 201–300 ng NE/g, and 301–500 ng NE/g (relatively undepleted). For each of these NE ranges, the number of mice fighting against the Loser on Day 4 after drug administration was divided by the total number of mice in that range. Similar calculations were performed to obtain % mice fighting in each NE range for each of the test days up to 10 weeks of testing. A Chi Squared 2 × 2 Contingency Test (Hewlett-Packard Stat Pack V-9) was used to compare % mice fighting between NE ranges to determine significant differences in amount of fighting between mice with various degrees of brain NE depletion on each testing day. The Chi Squared test was also used to examine the changes over time after drug administration within a given NE range, e.g., comparing Wins against Loser at week 1 with Wins against Loser at week 4 for the 0–100 ng NE range, to detect possible recovery of fighting with time after 6-OHDA administration.

RESULTS

As seen in Fig. 1, there is a significant correlation between NE depletion and lowered fighting tendency (Spearman rank correlation coefficient $r_s = 0.73$, $t = 0.47$, $p<0.025$). As seen in Fig. 2, on a given test day, 6-OHDA treated mice with brain NE levels of 0–100 and 101–200 ng NE/g brain tissue fought much less than 6-OHDA treated mice with 201–300 ng NE/g, less than mice with more than 300 ng NE/g, and less than vehicle controls with 350–500 ng NE/g. No significant difference was found between the 201–300, the greater than 300, and the control group at any testing date. However, 0–100 ng NE/g depleted mice fought significantly less than the 201–300 group ($\chi^2 = 6.00$, $p<0.02$), less than the over 300 ng NE/g treated group ($\chi^2 = 6.00$, $p<0.02$), and less than the vehicle control group ($\chi^2 = 7.54$, $p<0.01$) during the first test against the Loser, and this significance persisted through all tests until sacrifice. The 101–200 ng NE/g group fought significantly less than vehicle controls ($\chi^2 = 3.85$, $p<0.05$) at the first day of testing, and varied between non-significance and $p<0.02$ when compared to the fighting scores of higher NE level mice in subsequent tests. This 101–200 ng NE/g group also showed some significant variability in % mice

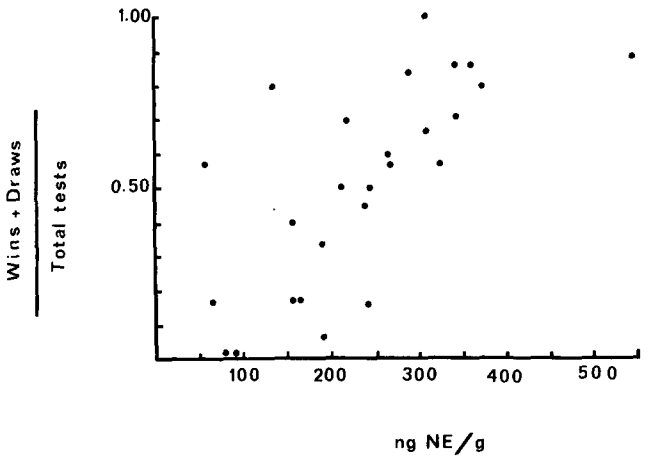

FIG. 1. Correlation between 6-OHDA-induced depletion of norepinephrine (NE) and male mouse fighting behavior. Mice are tested once a week against a Standard Opponent Loser and 2 days later against an Opponent Winner for at least 4 weeks after 6-OHDA administration. An estimate of fighting tendency is calculated by adding total Wins against Losers plus total Draws against Winners and dividing by total number of test fights (Wins + No Fights against Loser plus Draws + Losses against Winner). Whole brain norepinephrine is assayed by the alumina-trihydroxyindoleamine method and expressed as nanograms of norepinephrine per gram of brain tissue weight. (Spearman rank correlation coefficient r_s = 0.73, t = 0.47, $p<0.025$.)

fighting between the testing dates. None of the other groups had any significant differences in % mice fighting among the tests against Winners and Losers throughout the survival weeks. This lack of change over time gives reliability to the estimated fighting tendency scores of Fig. 1, and suggests no recovery of fighting behavior for up to 10 weeks after 6-OHDA destruction of brain norepinephrine terminals.

Since 6-OHDA depletion of NE is generally accompanied by some smaller depletion of DA (usually DA about 60–90% of controls in NE-depleted mice), it was possible that actually this small reduction of DA was the factor interfering with fighting. Therefore, DMI pretreated 6-OHDA treated mice were tested for fighting tendencies. High mortality occurred in the DMI pretreated group. However, 4 DMI-6-OHDA treated mice were obtained with normal NE levels but with 80%, 35%, 30% and 20% of control levels of DA, and each of these mice fought as much as controls.

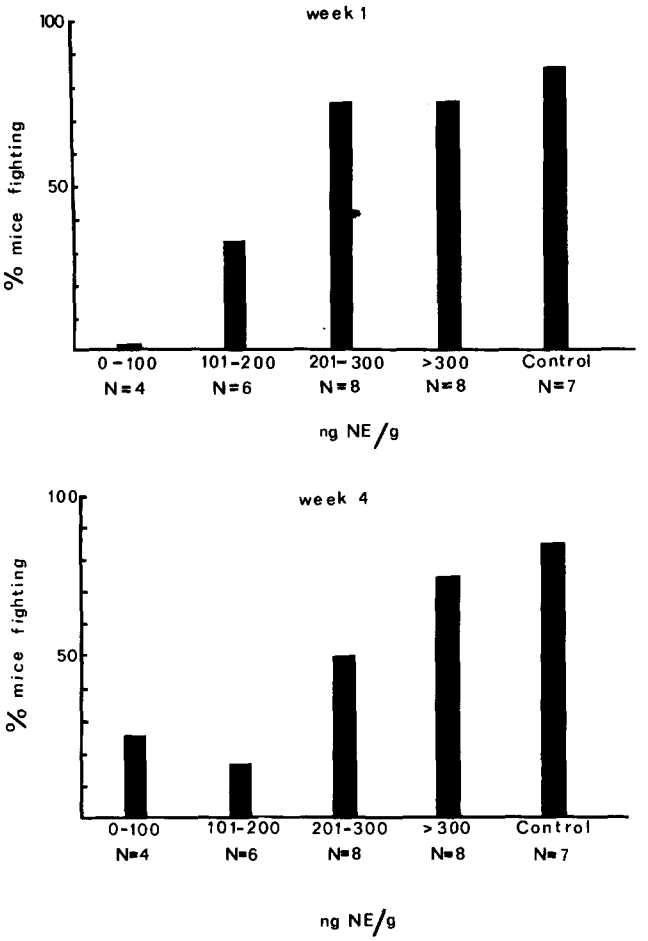

FIG. 2. Relation between brain NE levels and number of mice fighting on a given test day. Fighting is significantly less for mice severely depleted of brain NE by 6-OHDA. When compared to partially depleted mice with more than 200 ng NE/g and to vehicle treated controls with 350–500 ng NE/g, a much smaller percentage of severely depleted mice with less than 200 ng NE/g fight against Loser Opponents one week (upper graph) and 4 weeks (lower graph) after intraventricular drug administration. This significance persists in fighting tests for up to 10 weeks after treatment, indicating no recovery in fighting tendencies after 6-OHDA destruction of brain norepinephrine terminals.

DISCUSSION

Our results indicate a 6-OHDA depletion-dependent correlation between whole brain levels of norepinephrine and isolation-induced male mouse fighting behavior. Previous studies by Welch [34], Valzelli [11], and others have shown that monoamines change during isolation and during the actual fighting session. The depletion data above may be suggesting that some critical amount and/or location of norepinephrine is necessary for the initiation of spontaneous attack of a strange male by a male laboratory mouse. This study also supports previous work using generalized catecholamine depletors, such as alpha-methyl tyrosine, which also reduce mouse attack and fighting behavior [33]. Since intraventricular 6-OHDA does not cross the blood-brain barrier and does not directly affect the peripheral sympathetic nervous system [24], this experiment specifies the central nervous system involvement in attack behavior. Open field behavioral data, to be reported at a later date, indicates no locomotory impairment in the centrally depleted subject males who are not fighting.

From the data herein, one cannot yet comment on the neurochemical or anatomical specificity of this mechanism. Biochemically, other transmitters may be involved in mouse fighting, as some other pathway(s) interacting with the norepinephrine pathway(s), for example. The few cases of dopamine-depleted mice indicate that dopamine depletion without norepinephrine depletion is insufficient to significantly change fighting behavior in this testing situation. Another prominent transmitter, 5-hydroxytryptamine, is generally unaffected by this type of 6-OHDA treatment

[32]. However, the seeming specificity of NE in this behavioral paradigm requires further testing.

Anatomically, the whole brain depletion approach sheds no light on which NE-containing brain structures and pathways are most depleted, or whether these regions correspond to the neuroanatomical areas classically considered as important to social behavior, for example hypothalamus [19,30], limbic connections [3, 17, 25], olfactory tracts [8, 20, 23]. Experiments are in progress and planned to elucidate with more specificity the neurochemical-anatomical brain substrate for isolation-induced male mouse fighting. This biochemically applied classical approach of gross depletion of neurotransmitters, analysis of behavioral deficits, and further indepth analysis of within-brain dynamics can prove valuable as a first estimation of neurochemical coding of many important behaviors.

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