

Effects of 5,7-Dihydroxytryptamine Depletion of Tissue Serotonin Levels on Extracellular Serotonin in the Striatum Assessed with In Vivo Microdialysis: Relationship to Behavior[†]

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ABSTRACT Effects of i.c.v. administration of 5,7-dihydroxytryptamine (5,7-DHT) on biochemistry and behavior were studied in awake Sprague-Dawley rats. It was found that 5,7-DHT depletion of striatal tissue levels of serotonin (5-HT) does not diminish extracellular levels until substantial depletions occur. This finding is similar to those observed after 6-hydroxydopamine lesions of the brain dopamine systems. Although varying amounts of 5,7-DHT produced serotonin depletions in striatal tissue, decreases in extracellular levels were only observed at tissue depletions greater than 60% compared to saline-injected control subjects. Thus, the effects of serotonin lesions which produce only moderate depletions may not be the result of decreased extracellular serotonin, but instead may be the result of compensatory changes in remaining neurons which maintain normal extracellular serotonin concentrations. Different degrees of striatal serotonin depletion were associated with opposite behavioral effects. Moderate levels of serotonin depletion (50–75%) produced evidence of increased anxiety, while these effects were no longer seen in rats with more severe 5-HT depletions (>75%). **Synapse 33:16–25, 1999.** © 1999 Wiley-Liss, Inc.

INTRODUCTION

Substantial compensation in intact neurons is known to occur after damage to the central monoaminergic neuronal systems. Microdialysis studies have shown that 6-hydroxydopamine (6-OHDA) lesions of the dopamine (Robinson and Wishaw, 1988; Abercrombie et al., 1990; Castaneda et al., 1990) or norepinephrine (Abercrombie and Zigmond, 1989) systems do not decrease basal extracellular levels until quite large depletions of tissue content have occurred. Recently, it has been shown that similar compensatory changes take place in central serotonin (5-HT) systems after depletion with 5,7-dihydroxytryptamine (5,7-DHT) (Kirby et al., 1995). However, in that study, despite the production of substantial 5-HT depletions in tissue samples no decreases in basal extracellular 5-HT levels were observed. Since anesthetized rats were used and the basal levels of 5-HT were quantitated only 3 h after implantation of the microdialysis probe, vascular damage-related 5-HT may have contributed to estimations of basal extracellular levels. A previous study which found decreased extracellular 5-HT in the hippocampus of anesthetized

rats after 5,7-DHT treatment (Sharp et al., 1989) used citalopram in the perfusate. Thus, it is uncertain whether basal levels of 5-HT were altered by the lesion or the response to citalopram.

Therefore, we conducted an in vivo microdialysis experiment on freely moving Sprague-Dawley rats to assess basal 5-HT levels in the striatum after 5,7-DHT induced 5-HT lesions. The microdialysis probes were implanted 16 h prior to the collection of basal dialysis samples. A second experiment examined the behavioral sequelae of differing levels of 5-HT depletion in the

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open-field and the elevated-plus maze. In this experiment, the content of 5-HT in the striatum was determined as the measure of tissue depletion for comparison to Experiment 1.

MATERIALS AND METHODS

The subjects were male Sprague-Dawley rats (Charles River, Frederick, MD). They were individually housed in the same colony room with a 12:12 light:dark cycle, lights on at 0700 and food and water ad libitum. The microdialysis and behavioral studies were conducted during the lights-on phase (0900–1700).

Experiment 1: Relationship between extracellular and tissue concentrations of 5-HT in the striatum following i.c.v. injections of 5,7-DHT

After a 1-week acclimation period, the rats were anesthetized with chloral hydrate (400 mg/kg, IP) for surgery. The subjects received an injection of desmethylimipramine (Sigma, St. Louis, MO; 25 mg/kg IP) 30 min prior to surgery. Each subject then received an injection of saline or 5,7-DHT creatine sulfate (Sigma; $n = 4$ 100 μg , $n = 7$ 200 μg , $n = 5$ 300 μg , $n = 6$ 400 μg , and $n = 6$ saline-treated subjects) in a volume of 10 μL into the right lateral ventricle (AP +7.9, ML +1.7, DV +6.9, relative to interaural zero). A microdialysis guide cannula (CMA 12) was also stereotaxically placed into the left striatum (AP +9.9, ML +3.0, DV +6.0, relative to interaural zero).

In vivo microdialysis was performed under freely moving conditions 2 weeks postlesion. Microdialysis probes (CMA 12/3; Carnegie Medicin, Sweden) were of concentric design with a 3 mm dialyzing region. In vitro studies demonstrated that recovery of the probe for 5-HT was approximately 25% at a 1.0 $\mu\text{L}/\text{min}$ flow rate. Microdialysis probes were implanted in the striatum via the indwelling guide 16 h prior to testing. After this time the subjects were placed in a 20" cylindrical Plexiglas chamber. Inlet and outlet tubing (FEP tubing; Carnegie Medicin) were attached to the probe and support tubing was attached to the cap. Artificial CSF (4.0 mM KCl, 147.0 mM NaCl, 2.2 mM CaCl₂, 1.0 mM MgCl₂, pH 6.5) was perfused at 1.0 $\mu\text{L}/\text{min}$. Four microdialysis samples were collected at 60-min intervals beginning 3 h after flow onset. At the end of the experiment the subjects were sacrificed by decapitation, the brains removed, and tissue dissected by hand for HPLC analysis of regional brain monoamine levels.

In some of the lesioned ($n = 4$ 200 μg , $n = 3$ 300 μg , $n = 6$ 400 μg) and unlesioned ($n = 7$) subjects the effects of tetrodotoxin (TTX; Sigma) on extracellular 5-HT levels were also assessed. After the establishment of a stable baseline, the perfusate was changed to artificial CSF containing TTX (100 nM) using a Carnegie Medicin fluid switch. Sampling continued at 20-min intervals for 2 h.

Microdialysis samples were analyzed by HPLC-EC for 5-HT and 5-hydroxyindolacetic acid (5-HIAA) levels using a microbore column (Spherisorb ODS5; BAS, Lafayette, IN). The mobile phase consisted of 152 mM citric acid, 15 mM sodium acetate, 1.98 mM octane sulphonic acid, 0.8 mM EDTA, and 8% methanol (pH 3.6). Following chromatographic separation the compounds of interest were quantified by amperometric electrochemical detection (+0.7 V; Intro detector; Rainin, Woburn, MA) and the chromatograms collected on a Power Macintosh 7200 using Method Manager (Rainin). The lowest detectable 5-HT concentration was 10^{-10} M in a 50 μL sample.

The subjects were decapitated and the striatum on the side of the probe was dissected freehand on an ice-cold plate. Subsequently, it was assayed for content of 5-HT and 5-HIAA by HPLC-EC. The tissue was immediately placed in 200 μL 0.1 M perchloric acid, homogenized, and centrifuged. Monoamines in the effluent were assessed using a refrigerated autosampler (CMA 200, Carnegie Medicin) coupled to an HPLC-EC (Coulchem 5100A and a 5014A Detector, ESA, Bedford, MA), using ESA MD-TM mobile phase and an ESA MD-150 column.

Experiment 2: Relationship between 5-HT depletions in the striatum produced by i.c.v. injections of 5,7-DHT and anxiety-related behavior

After a 1-week acclimation period the rats were anesthetized with chloral hydrate for surgery as described for Experiment 1. The subjects received an injection of desmethylimipramine (Sigma; 25 mg/kg IP) 30 min prior to surgery. Different groups were injected i.c.v. with varying amounts of 5,7-DHT creatine sulfate or vehicle ($n = 13$ 100 μg , $n = 8$ 200 μg , $n = 6$ 300 μg , $n = 4$ 400 μg , and $n = 7$ unlesioned subjects). Behavioral testing was conducted 2 weeks postlesion. One day later, the subjects were sacrificed for determination of tissue 5-HT concentrations as described for Experiment 1.

Behavioral testing consisted of a 5-min exposure to an open-field followed by a 5-min exposure to the elevated-plus maze. In both cases testing was conducted under white light. The behavioral data were collected using the Timer 1.4 program (NIH, 1987) on a Macintosh computer. The observer was sitting 1 meter from the apparatus at a height sufficient to allow a clear view of the experimental subject at all times. The observer was blind to the group assignment of the subjects. The open-field apparatus was a 1 m² white Plexiglas box enclosed by 40 cm high walls. The floor of the open-field was divided into a 25-square grid by black tape. Prior to their exposure to the open-field, subjects were placed in a holding cage for 15 min to allow them to acclimate to the testing room. Each subject was individually tested by placing them in a corner square of the open-field at the start of each 5-min

test session. The behavioral measures scored were locomotion in the center and periphery of the open-field, rearing, and grooming. The number of line crosses was used as the index of locomotion. The number of bouts were noted for grooming and rearing. Between each test session, the open-field apparatus was thoroughly cleaned using 40% ethanol.

The elevated-plus maze was of black Plexiglas construction with two open arms in which the subjects can see over the sides, to the ground 1 meter below, and two closed arms with 40 cm high walls. The duration of the test was 5 min, during which the number of open and closed arm entries, the duration of time spent in the open and closed arms, the number of fecal boli, and the latency to enter the open arm were monitored by an observer using the Timer 1.4 program described above. Between each test session, the apparatus was thoroughly cleaned using 40% ethanol.

Data analysis

Neurochemical data from Experiments 1 and 2 were analyzed by ANOVA with the between-subjects factor of treatment group followed by post hoc analysis using Duncan's Multiple Range Test. In addition, the relationship between extracellular striatal 5-HT and percent tissue depletion in lesioned subjects was examined by regression analysis (Statview 4.02, Abacus).

Behavioral subjects from Experiment 2 were divided into groups based on the actual tissue depletions in the striatum relative to saline controls: saline, low (0–50% depletion), moderate (51–75% depletion), and high (76–100% depletion). The data from the open-field and elevated-plus maze were then analyzed by ANOVA with the between-subjects factor of lesion group followed by post hoc analysis using Duncan's Multiple Range Test.

RESULTS

Experiment 1: Relationship between extracellular and tissue concentrations of 5-HT in the striatum following i.c.v. injections of 5,7-DHT

Tissue levels of 5-HT ($F[4,23] = 5.61$, $P < 0.003$; Fig. 1A) and 5-HIAA ($F[4,23] = 4.52$, $P < 0.008$; Fig. 1B) were decreased in 5,7-DHT-treated subjects relative to controls. Significant depletions of 5-HT were produced by administration of more than 200 μg of 5,7-DHT. Maximal depletions were achieved by 300 or 400 μg of 5,7-DHT. The ratio of 5-HIAA/5-HT was unchanged after the 5,7-DHT treatment ($F[4,23] = 0.78$, n.s.; Fig. 1C).

Extracellular levels of 5-HT assessed by in vivo microdialysis were decreased only in the groups with the highest level lesion (L400; Fig. 2A). This was demonstrated by an ANOVA which contrasted the 400 μg lesion group against all the other groups ($df = 1$, $F =$

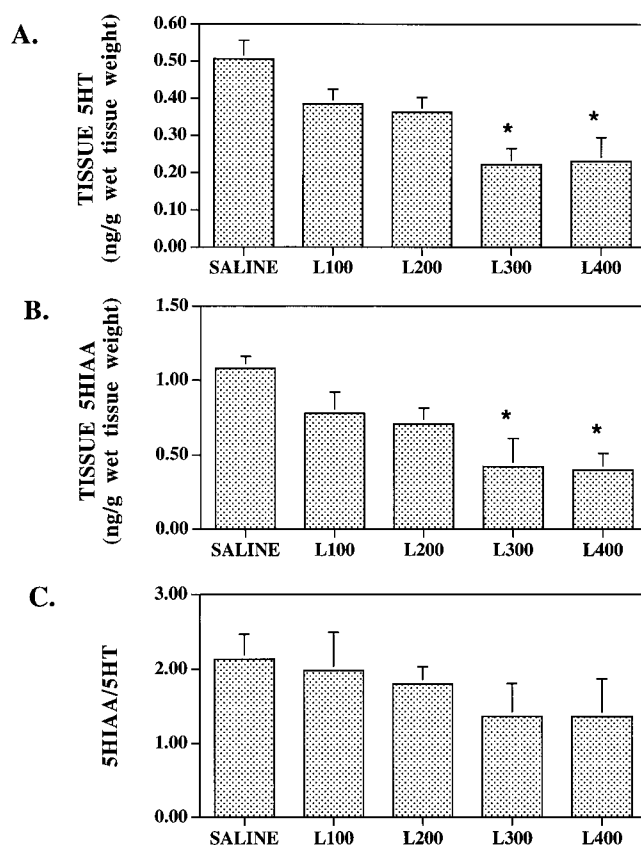


Fig. 1. The effect of 5,7-DHT on striatal tissue levels of (A) 5-HT, (B) 5-HIAA in ng/g wet tissue weight, and (C) the ratio of the tissue concentration of 5-HIAA to the tissue concentration of 5-HT. Data expressed as mean \pm SEM. (*) Significant difference from saline condition by post hoc *t*-test, Duncan Multiple Range Test ($P < 0.05$).

7.42, $P < 0.02$). In all other groups the extracellular levels of 5-HT were not different from saline treated control subjects. An ANCOVA, using the tissue level of 5-HT as a covariate, demonstrated the same result, a significant group difference ($F[4,18] = 4.21$, $P < 0.02$). The relationship is even more readily apparent when the percent depletion of 5-HT is plotted against extracellular levels (Fig. 2B). As can be seen, only when the tissue levels of 5-HT were depleted by more than 60% did the extracellular levels of 5-HT decrease substantially. This relationship was best fit by a second order polynomial ($y = 3.3 + 0.025x - 0.001x^2$), although the fit was not particularly robust ($r = 0.36$, $P < 0.10$). However, using this formula a 68% tissue depletion resulted in a 90% decrease in extracellular levels of 5-HT. Although the fit was not robust, the dialysate concentration of 5-HT at 0% depletion was estimated to be 3.3×10^{-9} M, very similar to the actual value of 3.7×10^{-9} M.

Administration of tetrodotoxin produced profound decreases in extracellular concentrations of 5-HT to an equal extent in all lesioned and unlesioned groups, such that there was no significant effect of group in the

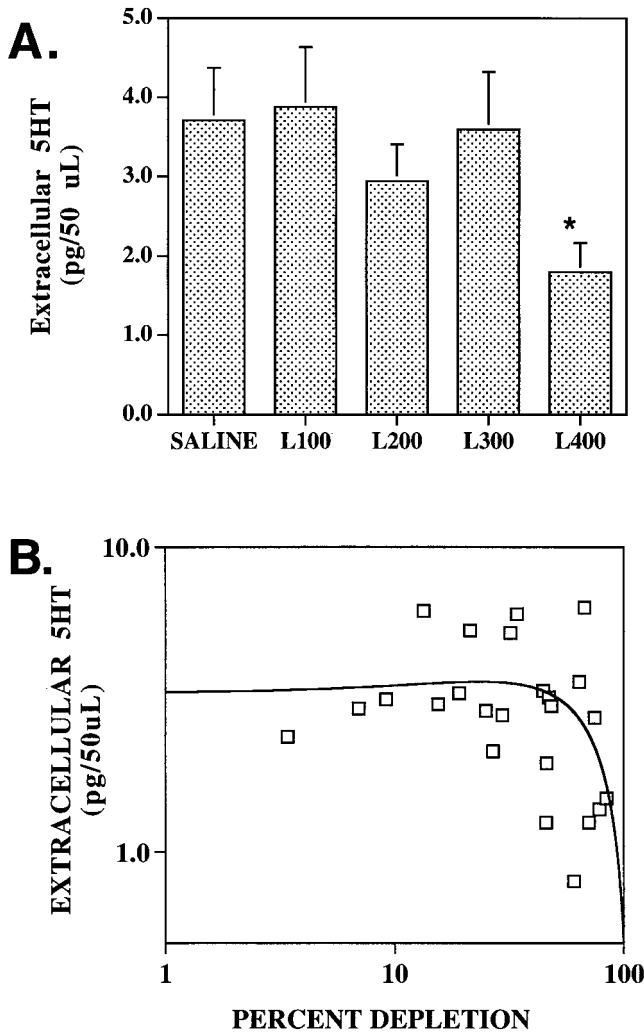


Fig. 2. (A) The effect of administration of 5,7-DHT on extracellular levels of 5-HT in the striatum assessed with in vivo microdialysis expressed as pg 5-HT per 50 μ L sample, (*) significant difference from saline condition by post hoc *t*-test, Duncan Multiple Range Test ($P < 0.05$). (B) The relationship of the log percent depletion of striatal tissue levels of 5-HT to extracellular levels of 5-HT expressed as pg 5-HT per 50 μ L sample. These data were best fit by a second order polynomial equation ($r = 0.36$, $P < 0.01$).

TABLE I. The effect of TTX on extracellular 5-HT*

Group	Maximal decrease in extracellular 5-HT by TTX (percent)
Saline	71.9 \pm 4.5
200 μ g	74.0 \pm 8.1
300 μ g	60.3 \pm 14.1
400 μ g	79.7 \pm 6.6

*The maximal depletion of extracellular 5-HT in the striatum produced by TTX as assessed by in vivo microdialysis in subjects previously treated with saline or 5,7-DHT (200, 300, or 400 μ g i.c.v.) expressed as percent decrease from basal values.

ANOVA ($F[3,16] = 0.97$, n.s.; Table I). These data indicate that the sampled 5-HT originated in the area of the probe and did not perfuse there from more distant sites.

TABLE II. The effects of 5,7-DHT on striatal 5-HT in Experiment 2*

Group	Tissue concentration of 5-HT (ng/g wet tissue)
Saline	0.666 \pm 0.127
100 μ g	0.413 \pm 0.067
200 μ g	0.454 \pm 0.157
300 μ g	0.174 \pm 0.034*
400 μ g	0.051 \pm 0.024*

The effect of 5,7-DHT on striatal tissue levels of 5-HT from the subjects in Experiment 2. Data expressed as mean \pm standard error of the mean. Significant difference from saline condition () by post hoc *t*-test, Duncan Multiple Range Test ($p < 0.05$).

Experiment 2: Relationship between 5-HT depletions in the striatum produced by i.c.v. injections of 5,7-DHT and anxiety-related behavior

Analysis of tissue 5-HT levels in the striatum revealed that administration of 5,7-DHT produced substantial depletion of 5-HT, with maximal depletions observed after 300 or 400 μ g of 5,7-DHT ($F[4,33] = 3.63$, $P < 0.02$; Table II). These data were used to calculate the percent depletion for each animal relative to saline controls and the subjects were placed into 5-HT depletion groups accordingly: low (0–50% depletion relative to saline controls, $n = 9$), moderate (51–75% depletion relative to saline controls, $n = 9$), high (76–100% depletion relative to saline controls, $n = 11$). The behavioral results were analyzed in this manner so that the behavioral effects of the actual, not the expected, 5-HT depletions could be evaluated.

Depletion of central 5-HT produced substantial changes in behavior assessed in the open-field, and these changes were highly dependent on the severity of the depletion (Fig. 3). Peripheral locomotion decreased significantly only in the high depletion group ($F[3,32] = 3.52$, $P < 0.03$; Fig. 3A). However, low or moderate levels of 5-HT depletion reduced locomotion in the center of the open-field, but not higher depletion ($F[3,32] = 3.16$, $P < 0.04$; Fig. 3B). The interaction of these two factors meant that the distribution of activity, center vs. periphery of the open-field, was differentially affected by low/moderate and high levels of 5-HT depletion. That is, low/moderate levels of 5-HT depletion increased the percentage of locomotion in the periphery ($F[3,32] = 4.08$, $P < 0.02$; Fig. 3C), and decreased the percentage of locomotion in the center of the open-field ($F[3,32] = 4.08$, $P < 0.02$; Fig. 3D). However, subjects with high levels of 5-HT depletion were not different from saline controls. The effects of 5-HT depletion were limited to peripheral and center locomotor activity. No significant differences were observed in either grooming ($F[3,32] = 0.86$, n.s.; Fig. 3E), or rearing behavior ($F[3,32] = 0.27$, n.s.; Fig. 3F).

Although a similar pattern was observed in the elevated-plus maze, few of the differences were statistically significant. There was no substantial difference in closed arm entries ($F[3,32] = 0.77$, n.s.; Fig. 4A). Open

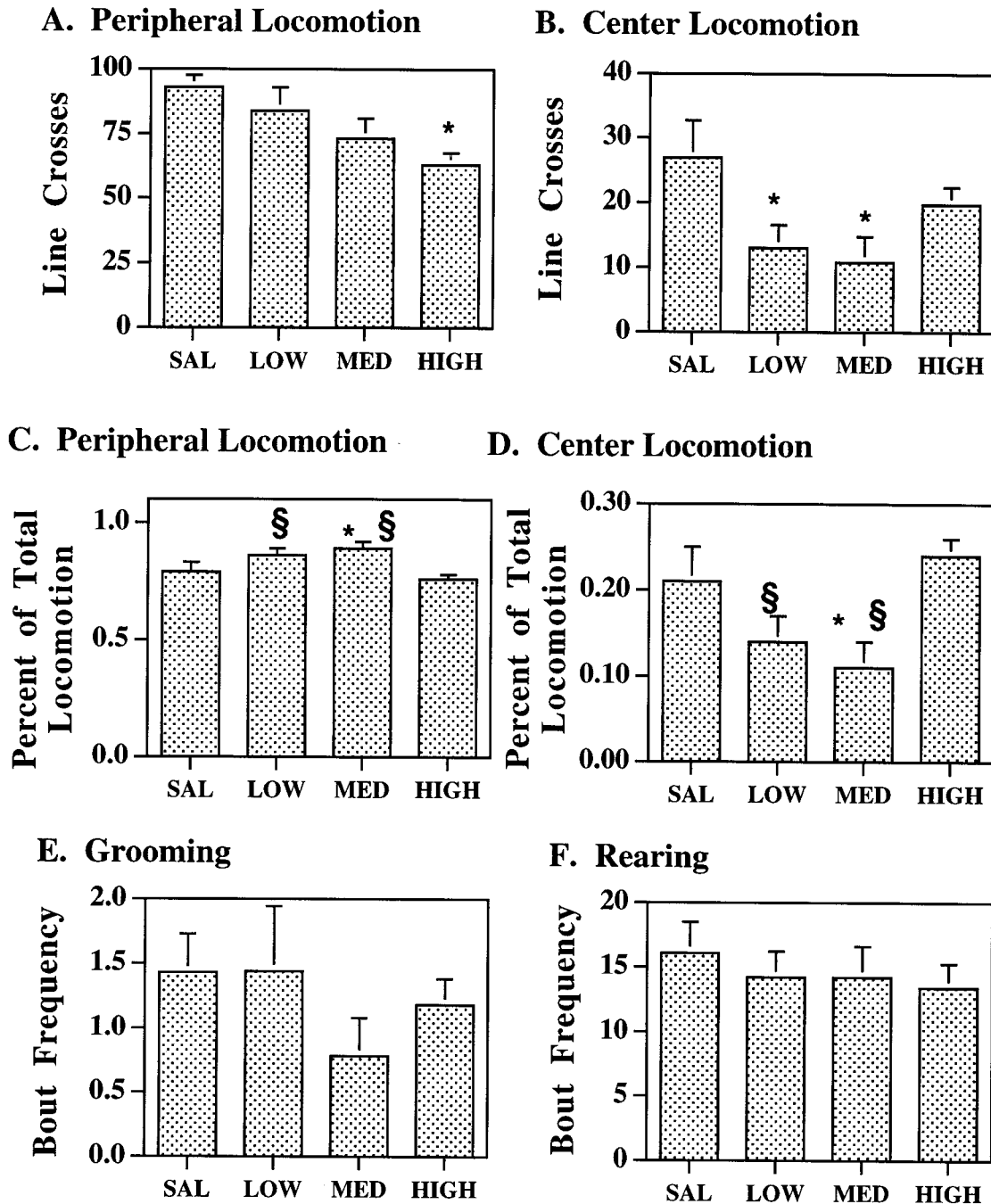


Fig. 3. The effect of 5,7-DHT lesions (low: 0–50%, moderate: 51–75%, high: 76–100% of saline controls) on behavior in the open-field: (A) peripheral locomotion frequency, (B) center locomotion frequency, (C) peripheral locomotion as percent of total locomotion, (D)

center locomotion as percent of total locomotion, (E) frequency of grooming, (F) frequency of rearing. Significant difference from saline condition (*), or high level lesion (§) by post hoc *t*-test, Duncan Multiple Range Test ($P < 0.05$).

arm entries decreased slightly at low levels of depletion and then increased at higher levels of depletion, but this effect was not statistically significant ($F[3,32] = 2.24$, n.s.; Fig. 4B). Moderate and high levels of 5-HT depletion produced nonsignificant decreases in closed arm duration ($F[3,32] = 1.75$, n.s.; Fig. 4C) and increases in open arm duration ($F[3,32] = 0.69$, n.s.; Fig.

4D). The subtle pattern observable for open entries was more obviously apparent in defecation ($F[3,32] = 3.09$, $P < 0.05$; Fig. 4E) and latency to enter the open arm (Fig. 4F), although this latter effect was not significant. Low levels of tissue 5-HT depletion were associated with increased defecation and this difference was reversed by greater levels of 5-HT depletion.

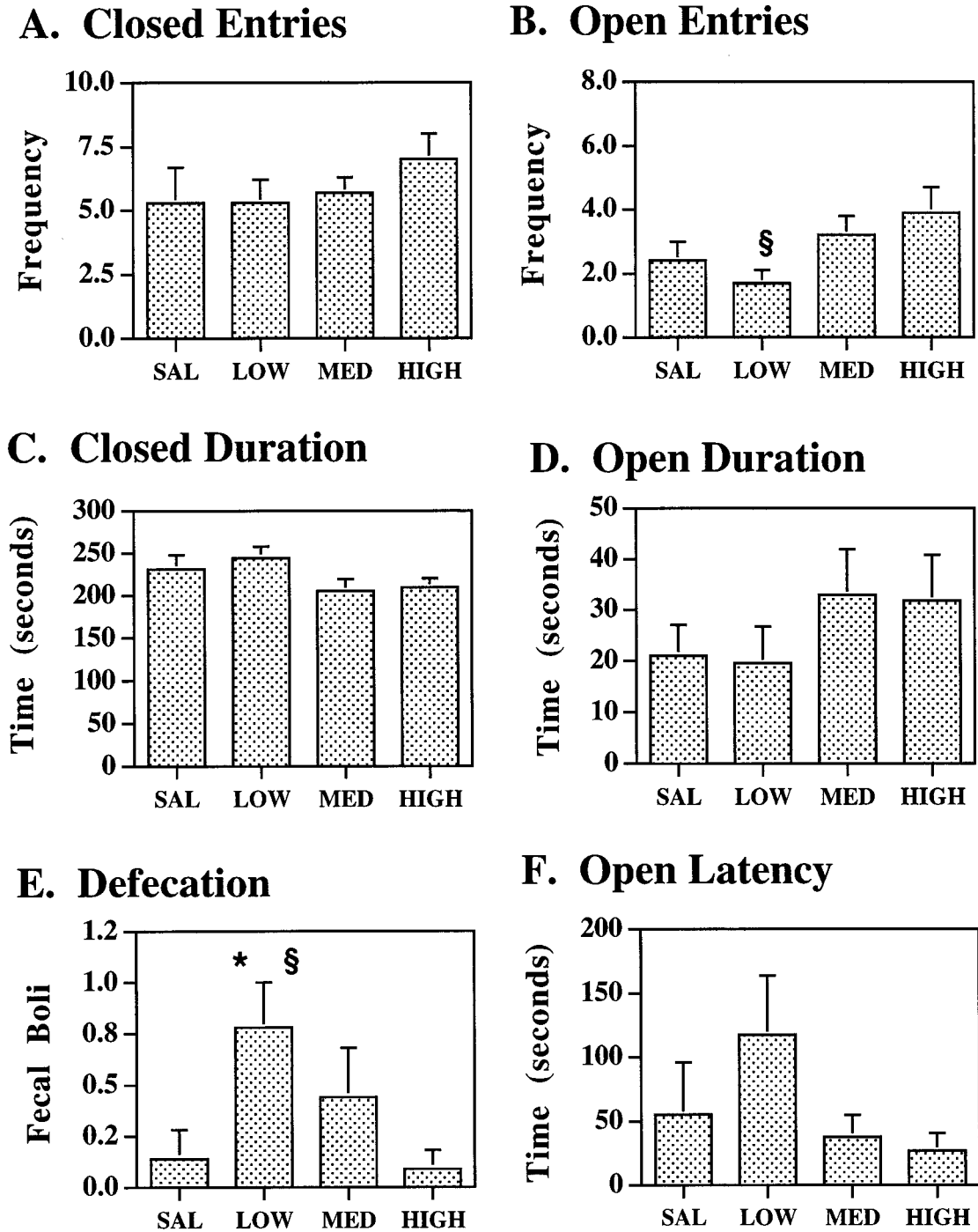


Fig. 4. The effect of 5,7-DHT lesions (low: 0–50%, moderate: 51–75%, high: 76–100% of saline controls) on behavior in the elevated-plus maze: (A) closed entries, (B) open entries, (C) closed duration, (D)

open duration, (E) defecation, (F) latency to enter open arms of plus maze. Significant difference from saline condition (*), or high level lesion (§) by post hoc *t*-test, Duncan Multiple Range Test ($P < 0.05$).

DISCUSSION

Despite substantial depletions of tissue 5-HT and 5-HIAA levels, extracellular levels of 5-HT in the striatum were maintained near control levels until substantial depletions (>60%) were observed. Although extracellular 5-HT levels did not decrease in the L300

group, despite apparently equivalent tissue 5-HT depletions compared to the L400 group, a rather specific relationship between extracellular and tissue 5-HT levels was apparent in a scatterplot of the data (Fig. 2B). This pattern is qualitatively similar to that seen in the catecholamine systems following 6-OHDA lesions

(Robinson and Wishaw, 1988; Abercrombie and Zigmond, 1989; Abercrombie et al., 1990; Castaneda et al., 1990). Furthermore, the pattern is more quantitatively similar to NE lesions, since although extracellular levels are maintained after mild to moderate lesions, decreases are observed after 65% depletion, rather than the 90% necessary for DA systems. Demonstration of compensatory maintenance of extracellular 5-HT levels despite substantial tissue 5-HT depletions supports a previous study that measured basal levels of extracellular 5-HT in anesthetized rats (Kirby et al., 1995). In that study, microdialysis was conducted 4 weeks after the 5,7-DHT lesions, allowing more time for compensatory mechanisms to develop. More importantly, the failure to demonstrate decreased extracellular 5-HT levels after substantial lesions in that study may have been due to residual 5-HT from vascular damage due to the recency of the probe implantation. In the present study, the TTX data indicated that most of the 5-HT measured was of neuronal origin, even after substantial depletion of tissue 5-HT. Furthermore, since the TTX was perfused through the probe, 5-HT originated from the area directly around the probe and was not diffusing from other areas. Thus, the present results agree with previous findings that 5-HT depletions do not necessarily reduce basal extracellular levels (Kalen et al., 1988; Kirby et al., 1995), particularly when only partial lesions are produced (Romero et al., 1998), although stimulated 5-HT release is attenuated (Kirby et al., 1995; Romero et al., 1998). Since it was possible to observe decreases in basal extracellular levels of 5-HT, this finding emphasizes the advantages of using freely moving and awake animals when examining small changes, or decreases, in extracellular neurotransmitter concentrations, and the importance of allowing sufficient time for damage from probe implantation to subside when examining 5-HT with microdialysis.

Experiment 1 demonstrated that depletions of less than 65% of tissue serotonin produce no appreciable effects on basal extracellular 5-HT levels (Fig. 2). The mechanisms of this compensation probably include a variety of processes such as enhanced synthesis of 5-HT (Stachowiak et al., 1986; Tsuiki et al., 1995), accompanied by increased tryptophan hydroxylase levels in surviving 5-HT neurons (Bendotti et al., 1990), and decreased autoreceptor feedback and 5-HT reuptake (Gobbi et al., 1994). In addition, the pattern of release or cell firing may change as well, although the proportion of burst firing 5-HT neurons does not appear to increase after 5,7-DHT lesions (Hajos and Sharp, 1996). The mechanisms of compensation are apparently somewhat different from those for other monoamines, since the ratio of tissue 5-HIAA to tissue 5-HT was unchanged following lesions. After 6-OHDA lesions, the tissue concentrations of HVA and DOPAC are somewhat less depleted than DA, which results in increased

metabolite:neurotransmitter ratios (Robinson and Wishaw, 1988).

In addition to presynaptic compensation, the maintenance of basal levels of extracellular 5-HT must be considered in the light of postsynaptic receptor supersensitivity (Nelson et al., 1978; Wang et al., 1979; Ashby et al., 1994; Sawynok and Reid, 1994), which may very well produce enhanced 5-HT function overall. Furthermore, although increased inhibitory actions of iontophoretically applied 5-HT have been described after 5,7-DHT lesions (Wang et al., 1979), serotonin receptor subtype composition appears to be altered as well. For instance, after intrathecal 5,7-DHT supersensitivity of 5-HT₁, but not 5-HT₂ or 5-HT₃, receptors was observed in the spinal cord (Sawynok and Reid, 1994). Receptor changes after 5,7-DHT treatment may be highly regionally specific. Although no changes in 5-HT₃ sensitivity have been observed in spinal chord (Sawynok and Reid, 1994), supersensitivity has been observed in frontal cortex (Ashby et al., 1994).

The failure of 5,7-DHT to reduce basal extracellular 5-HT concentrations except at high levels of tissue depletion has important implications for the use of 5,7-DHT as a tool to study the role of 5-HT systems in behavior, and may explain many contradictory or negative findings, particularly when other compensatory changes are also taken into account. Behavioral studies of the effects of 5-HT lesions have often sought to determine the role of 5-HT systems by methods that do not produce complete lesions. In part, this is because diverse methods are used to produce these lesions, including administration of 5,7-DHT into the lateral ventricles, the 5-HT cell body regions (dorsal or median raphe), and into specific terminal regions.

With regard to anxiety, it has most often been shown that 5,7-DHT lesions have an anxiolytic effect. For instance, in conflict tests (Tye et al., 1977; Iversen, 1983; Thiebot et al., 1983; Soderpalm and Engel, 1991), the social interaction test (File et al., 1979), and the elevated-plus maze (Briley et al., 1990). Some of these effects have been shown to be dependent on intact adrenocortical function (Soderpalm and Engel, 1992), and 5,7-DHT lesions impair adrenocortical responses to stressful stimuli (Feldman et al., 1991). However, anxiolytic effects of 5,7-DHT have not always been observed, for instance, in the defensive burying model (Salivar et al., 1991), and the Vogel conflict test (Shimizu et al., 1992; Takao et al., 1992). It is interesting to note that in the Shimizu et al. (1992) study, although there was a >90% depletion of hippocampal 5-HT, the depletions in other areas were far less complete. This illustrates one weakness of the present study. In general, it is assumed that i.c.v. administration of 5,7-DHT should produce global 5-HT depletion and, therefore, the depletions in particular areas should be highly correlated. This might not be the case, particularly for partial lesions. Different brain structures might have quite different sensitivi-

ties to the toxin, resulting in more severe depletions in some regions. Other brain areas (e.g., dorsal raphe, hippocampus, hypothalamus) are thought to have a more prominent role in the effects of 5,7-DHT lesions on anxiety and stress (Jacobs et al., 1974; Feldman et al., 1991; Feldman and Weidenfeld, 1991, 1995; Critchley et al., 1992; Takao et al., 1992). However, dorsal raphe administration of 5,7-DHT produced minimal hippocampal 5-HT depletion, but substantial striatal 5-HT depletion and produced alterations in responses to anxiolytic compounds (Critchley et al., 1992). Future studies should examine the relationship of 5-HT depletion severity in multiple brain regions to alterations in anxiety mechanisms.

Many previous studies of the effects of 5-HT lesions on anxiety sought to examine anxiolytic effects. The ability to observe anxiogenic effects, such as those observed after mild/moderate levels of depletion in Experiment 2, might be impaired by floor effects. Indeed, this may have been the case in the elevated-plus maze in Experiment 2, where there were very few entries into the open arms in saline-treated rats and then a trend towards more entries (an anxiolytic effect) in more depleted subjects. In a similar fashion, anxiolytic effects of dorsal raphe 5,7-DHT lesions were not observed in the elevated-plus maze in another study (Critchley et al., 1992). Interestingly, although the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) was anxiogenic in nonlesioned rats in that study, it was anxiolytic in lesioned subjects. Despite the apparent anxiolytic effects of 5,7-DHT lesions in many instances, such lesions have been shown to block the anxiolytic actions of some compounds, including ipsapirone (Critchley et al., 1992) and buspirone (Takao et al., 1992), and also the anxiolytic action of sexual intercourse (Salivar et al., 1991) in the absence of any basal effects on anxiety of the lesions alone. It has been suggested that differences in general locomotion may be a confounding factor in the elevated-plus maze (Dawson and Tricklebank, 1995). However, in Experiment 2 increased defecation was found in the plus maze after mild/moderate 5-HT depletions, which cannot be accounted for by changes in general locomotor activity.

One explanation for these varying results is that serotonin lesions may have biphasic effects on anxiety, depending on the severity of depletion, as was observed in Experiment 2. In this instance, some behavioral effects of 5-HT lesions were observed only after small to moderate tissue depletions of 5-HT, notably, increased anxiety measured in the open-field and increased defecation in the plus maze. It appears that processes related to more severe lesions reverse these effects, since no behavioral differences were observed between control subjects and those with maximal (>75%) lesions. Indeed, this may have been why so few effects were observed in the elevated-plus maze. That is, in this

case, the anxiety increasing effects of mild lesions were not pronounced, perhaps due to floor effects, and the subsequent reversal obscured this effect, which was thus only significant in the defecation measurements. This reversal could be alternatively interpreted as an anxiolytic effect of the lesion (relative to moderate depletions), a description which is consistent with many studies that have produced nearly complete 5-HT lesions. Alternatively, this overall pattern could suggest that increases in anxiety after partial 5-HT lesions are associated with compensatory changes rather than 5-HT loss. Thus, the effect is eliminated with complete lesions in which compensation, in terms of basal extracellular levels of 5-HT, is no longer achieved.

In nonlesioned rats, biphasic effects of serotonergic manipulations on anxiety have also been observed. For instance, after L-5-HTP administration in the Vogel conflict test (Hjorth et al., 1987), anxiolytic effects were observed at low doses and anxiogenic effects at high doses. The opposite findings in that study and the present study might lie in different 5-HT systems underlying fear and anxiety (see review by Graeff et al., 1996) which may be differentially affected by lesions, and different paradigms may reflect one or the other functions to a greater or lesser extent. Indeed, some of the effects of lesions, particularly partial lesions, may be more the result of altering the balance of the serotonergic systems, rather than an even destruction of the serotonin systems. The present experiments were unable to address this issue. However, consistent with such an interpretation, the effect of different stressors on extracellular 5-HT levels is dependent on the region assessed (Kirby et al., 1997).

The effects of 5-HT lesions on open-field behavior in Experiment 2 are most consistent with changes in anxiety rather than changes in general locomotion, since mild/moderate depletions selectively affected center locomotion and center time. Indeed, there was no overall effect on locomotion (center line crosses + peripheral line crosses). Previous research has generally found that 5-HT lesions, produced by various means, increase locomotor activity in familiar or nonaversive environments (Jacobs et al., 1974; Gately et al., 1985; Erinoff and Snodgrass, 1986; Williams et al., 1990) or the perimeter of the open field (Carter and Pycock, 1979). On the other hand, 5-HT lesions decrease locomotion in unfamiliar or aversive environments (Hole et al., 1976; Williams et al., 1990) such as the open field (for see review Gerson and Baldessarini, 1980), particularly when center locomotion is analyzed separately (Lipska et al., 1992) or the distribution of activity (center/total) is analyzed. Although overall measures of locomotor activity are considered to be a poor index of anxiety or emotionality (Lister, 1990), the more specific measures used in the present experiments support the notion that the present differences reflect changes in anxiety mechanisms.

Increased anxiety as a result of partial brain serotonin depletion supports recent clinical work examining the consequences of the serotonin neurotoxins 3,4-methylenedioxymethamphetamine (MDMA), fenfluramine, and dexfenfluramine in humans (McCann and Ricaurte, 1991, 1992; McCann et al., 1994, in press). Although serotonergic damage was associated with a number of outcomes, anxiety and panic attacks were common consequences. Thus, in agreement with clinical findings, the present data suggest that behavioral changes can occur as a consequence of 5-HT depletion even in the absence of complete tissue depletions. These effects may actually be the result of overcompensation rather than elimination of 5-HT function. Although decreased release of serotonin occurs following a pharmacological challenge in 5,7-DHT-lesioned rats under baseline conditions (Kirby et al., 1995), it remains to be seen whether that occurs under physiological conditions in response to stimuli which increase serotonin cell firing and release. In any case, the most important implication of the present work for the use of 5,7-DHT to study 5-HT systems is that substantial tissue depletions may not result in substantial changes in behavior or extracellular 5-HT, except under particular conditions. It must also be noted, particularly with reference to the potential clinical implications of these findings, that further insults or the decline of normal aging might produce deficits at later points in time in lesioned subjects.

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