Pharmacology Biochemistry and Behavior, Vol. 42, pp. 25–28, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Domestication Alters 5-HT_{1A} Receptor Binding in Rat Brain

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Received 12 August 1991

HAMMER, R. P., JR., K. M. HORI, R. J. BLANCHARD AND D. C. BLANCHARD. *Domestication alters 5-HT_{1A} receptor binding in rat brain.* PHARMACOL BIOCHEM BEHAV **42**(1) 25–28, 1992.—Serotonin-1A receptor binding density was compared in the brains of wild and domesticated adult male *Rattus norvegicus* using in vitro receptor autoradiography of [^{3}H]8-hydroxy-2-[*n*-dipropylamino]tetraline (DPAT). While both groups exhibited similar patterns of labeling, [^{3}H]DPAT binding density was significantly ($p \leq 0.05$) lower in the median raphe nucleus and greater in superficial entorhinal cortex and rostral dentate gyrus of domesticated compared to wild rats. The results suggest that specific serotonergic circuits from the median raphe nucleus to the entorhinal and hippocampal regions might be involved in regulation of the defensive behaviors that differ profoundly between wild and domesticated rats. The relationship of these putative differences to behavioral disorders such as anxiety and depression in humans is discussed.

Domestication Serotonin 5-HT_{1A} Wild rat Median raphe nucleus Hippocampus Defensive behavior

BEHAVIORAL analyses of wild and domesticated rats have revealed distinct differences in defensive reactivity and response. Defensive threat vocalization and biting in response to human approach and handling have been virtually eliminated during domestication of the laboratory rat, and flight and avoidance behaviors have also been dramatically reduced (6, 24). In contrast, conspecific attack or offensive behaviors of wild rats are similar to those exhibited by laboratory rats (24).

Both offensive and defensive attack behavior of wild rats could be classified as "aggressive" behavior by the inexperienced observer, but differences including those of the underlying structural and neurochemical substrates are readily apparent on closer examination. For example, gamma-amino butyric acid antagonist (10) or excitatory amino acid (3) microinjection into the periaqueductal gray matter elicits defensive, but not offensive, behavior, while lesion of lateral forebrain serotonin (5-HT) tracts increases offensive, but not defensive, behavior (26) in the rat. Interestingly, a strong component of defensive behavior is also associated with 5-HT innervation as serotonergic lesions of the raphe nuclei increase defensive behavior in cats (21). These seemingly contradictory lesioning results might be due to the different 5-HT terminal regions affected by lesions located in different brain regions. Different 5-HT receptor subtypes located in these different regions could also be involved. For example, selective serotonin-1A (5-HT_{1A}) agonist treatment reduces defensive threat and attack behavior in wild rats (7). We utilized autoradiographic analyses to compare 5-HT_{1A} receptor density in discrete brain regions of wild and domesticated rats of the same species. The results of these experiments suggest that 5-HT_{1A} receptors located in median raphe and hippocampal circuits might be associated with differences in defensive reactivity between wild and domesticated rats.

METHOD

A group of wild-trapped adult male *Rattus norvegicus* were housed in facilities maintained for this purpose by the University of Hawaii Laboratory Animal Services for at least 60 days with ad lib access to food and water. Another group of adult male, domesticated *R. norvegicus* (Long-Evans strain) were

These results were presented, in part, at the 1988 International School of Ethology, held at the Ettore Majorana Centre for Scientific Culture in Erice, Italy.

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selected from laboratory-bred and -reared animals. All animals were healthy with nutritionally adequate diets at the time of the study.

Animals were briefly anesthetized with CO_2 and decapitated and brains were removed and frozen in 2-methylbutane at −35°C. Tissues were then sectioned in a cryostat at −15°C, mounted onto cold gelatin-coated glass slides, dried in an evacuated desiccator jar at 0°C, and stored in a freezer at −70°C until all tissues were collected.

A complete set of sections from each brain was incubated in [^{3}H]8-hydroxy-2-[*n*-dipropylamino]tetraline (DPAT), which selectively labels 5-HT_{1A} receptors (16). Briefly, tissues were preincubated in 50 mM Hepes buffer, pH 7.4, for 20 min at 23°C, incubated in the same buffer containing 4 mM CaCl, 0.1% ascorbate, 10 μM pargyline, and 0.8 nM [^{3}H]DPAT (110 Ci/mMol, Research Products International) for 30 min at 22°C, rinsed 3 × 40 s at 0°C in buffer, and dried under a cooled airstream. The preincubation procedure utilized is assumed adequate to eliminate the influence of endogenous 5-HT, alteration of which might affect autoradiographic results. Nonspecific binding was determined in the presence of 10 μM 5-HT to be less than 18% of total binding. Sections were exposed to ^{3}H-sensitive X-ray film (Ultrofilm, LKB) in cassettes for 12 weeks.

Following film development, regional labeling was analyzed using a quantitative densitometry system (Microcomp DS, Southern Micro Instruments). The system was first calibrated in terms of fmol/mg using radiostandards (American Radiolabeled Chemicals) coexposed with tissue on film (11). Brain regions identified on autoradiographic images were then outlined bilaterally in several adjacent sections and the mean of these data was recorded to yield a regional labeling value for that brain. These regional values were subsequently compared across groups using analysis of variance (ANOVA).

RESULTS

Autoradiographs of [^{3}H]DPAT labeling revealed the typical pattern of 5-HT_{1A} binding in the CNS (16) of both wild and domesticated animals. Labeling was very dense in the hippocampus (CA1), dentate gyrus, lateral septum, and entorhinal cortex, moderate in raphe, interpeduncular, and hypothalamic nuclei, and low in neocortex. Other brain regions showed little or no labeling. Data in Table 1 represent total binding of [^{3}H]DPAT, some portion of which is nonspecific; therefore, binding density was not assessed in brain regions that contain little or no labeling.

[^{3}H]DPAT binding was significantly altered in relatively few of the brain regions examined, and a distinct pattern emerged from these results. The significant ($p \leq 0.05$) effects were largely limited to limbic regions receiving serotonergic projections, including the superficial entorhinal cortex and dentate gyrus, in which labeling was increased in domesticated rats, and the median raphe nucleus, in which labeling was decreased (Fig. 1).

TABLE 1

EFFECT OF DOMESTICATION ON REGIONAL [^{3}H]8-OH-DPAT BINDING (fmol/mg ± SEM)

Brain Region (*n*)	Wild (6)	% Change	Domestic (6)
Limbic system			
Entorhinal cortex			
Superficial	68.02 ± 2.93	+37.0*	93.17 ± 10.16
Deep	58.52 ± 3.51	+12.3	65.73 ± 3.73
Hippocampus			
CA1	65.84 ± 5.72	+10.8	72.96 ± 6.38
CA3	26.70 ± 1.98	−0.1	26.71 ± 2.56
Dentate gyrus			
Rostral	32.86 ± 3.45	+52.9†	50.27 ± 2.81
Caudal	54.41 ± 4.05	+11.6	60.74 ± 3.02
Interpeduncular nucleus	49.86 ± 3.14	−13.7	43.06 ± 1.66
Septum, lateral	60.23 ± 5.68	−2.0	59.04 ± 2.63
Neocortex			
Somatosensory cortex			
Superficial	11.29 ± 2.73	−5.5	10.67 ± 1.02
Deep	15.15 ± 3.93	+17.4	17.78 ± 0.81
Visual cortex			
Superficial	11.54 ± 1.63	+22.4	14.13 ± 3.23
Deep	14.25 ± 2.17	+20.1	17.12 ± 3.56
Hypothalamus			
Medial preoptic area	13.95 ± 2.93	+14.1	15.92 ± 0.76
Ventromedial nucleus	18.50 ± 3.11	−37.3	11.59 ± 1.48
Brain stem			
Dorsal raphe nucleus	40.90 ± 3.57	−13.6	35.33 ± 3.44
Median raphe nucleus	14.08 ± 2.48	−47.4*	7.41 ± 0.88

*Significant ($p \leq 0.05$) difference using one-way ANOVA; †Significant ($p \leq 0.01$) difference using one-way ANOVA.

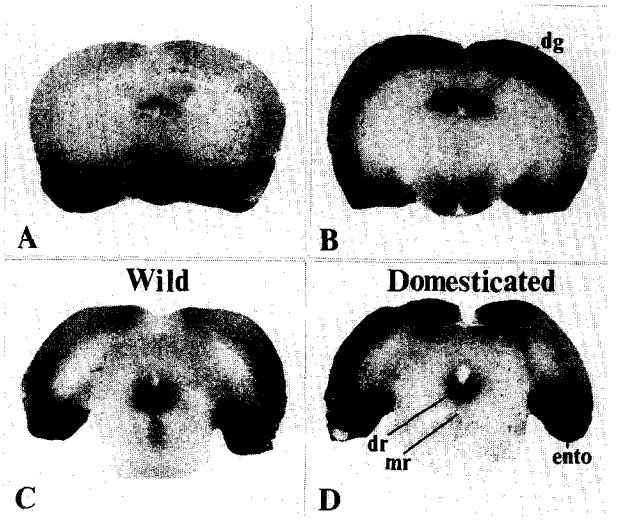

FIG. 1. Autoradiographs of [^{3}H]DPAT binding in brain sections from (A,C) wild-trapped and (B,D) domesticated animals. Binding density was significantly greater in the rostral dentate gyrus (dg) and superficial entorhinal cortex (ento), but significantly less in the median raphe nucleus (mr) of domesticated animals. In contrast, binding density did not differ significantly in the dorsal raphe nucleus (dr).

DISCUSSION

Activity of endogenous 5-HT systems has been implicated in various behavioral conditions, such as dominance (18,22) and depression (1,12,27). In fact, selective 5-HT$_{1A}$ agonists appear to demonstrate clinical anxiolytic (17) and antidepressant (20) efficacy.

Ethopharmacological analyses have distinguished certain behaviors peculiar to feral rats, which exhibit enhanced emotional response to a variety of threat stimuli (4). In particular, increased defensive threat and attack behaviors are observed in wild as compared to domesticated rats (6). It is significant that the most consistent effect of selective 5-HT$_{1A}$ agonist treatment of wild rats is a reduction in defensive threat and attack behavior in response to proximal stimuli (7). Hence, 5-HT$_{1A}$ receptor activity could be linked to the particular behavioral traits that best distinguish wild from domesticated rats. This does not, of course, preclude the possible influence of other 5-HT receptor subtypes (14).

5-HT$_{1A}$ sites are present in high density in limbic and brain stem regions (16). The 5-HT$_{1A}$ receptors located to the raphe nuclei on cells that product 5-HT are assumed to be autoreceptors, which regulate firing of these neurons. Thus, acute administration of selective 5-HT$_{1A}$ agonists inhibits dorsal raphe neuronal activity (23,25), which is restored following chronic administration due to desensitization of these somatodendritic 5-HT autoreceptors (8). Although functionally different, these autoreceptors may be structurally similar to all other 5-HT$_{1A}$ receptors as the mRNA that regulates the expression of the autoreceptor is not differentiated from that of other 5-HT$_{1A}$ receptors by various 5-HT$_{1A}$ receptor-selective oligonucleotide probes (15). Differential activity could be produced, however, by differential regional or synaptic localization of the receptor. For example, projections from dorsal and median raphe nuclei differentially supply serotonergic terminal regions; fibers from median raphe neurons project to the hippocampus, dentate gyrus, and medial forebrain structures, whereas most other regions are supplied by dorsal raphe neurons (2).

Reduced 5-HT$_{1A}$ binding density in the median raphe nucleus of domesticated rats might reflect a higher level of neuronal firing and 5-HT release in this region, which could produce compensatory downregulation of receptor density. Neurochemical studies have shown that 5-HT content is greater in the midbrain of wild rats selectively bred for reduced attack in response to human handling compared to high attacking lines, a procedure that might effectively mimic one of the processes involved in domestication (13). Thus, domestication might be associated with elevated 5-HT activity. The results of the present study suggest that this change might involve median raphe neurons and the brain regions to which they project. Reduced [^{3}H]DPAT binding density in the median raphe nucleus could result from lower affinity or reduced number of 5-HT autoreceptors on median raphe neurons. Moreover, these neurons project to the hippocampus and dentate gyrus, wherein 5-HT$_{1A}$ receptor density is *increased* with domestication, suggesting that serotonergic activity in this circuit is selectively increased with domestication. 5-HT$_{1A}$ receptor labeling is also increased in the entorhinal cortex, whose cells project to the dentate gyrus. Thus, 5-HT activity in brain circuits projecting to the hippocampus seems particularly affected by domestication. Even if the reduction of median raphe 5-HT$_{1A}$ receptor density with domestication reflects decreased number of 5-HT neurons in this region, the relative efficacy of hippocampal 5-HT circuits would be enhanced due to increased 5-HT$_{1A}$ binding in this region. These data suggest that an enhanced action of 5-HT in hippocampal circuits might be related to reduced defensive behavior occurring with domestication.

The severity of panic attack in humans, which involves abnormal parahippocampal blood flow (19), is reduced by 5-HT uptake inhibition (9). Thus, panic behavior could be related to reduced 5-HT activity in hippocampal regions producing a human manifestation of increased defensiveness. [^{3}H]DPAT binding is also increased in the dentate gyrus following chronic antidepressant treatment (27), although chronic 5-HT$_{1A}$ agonist treatment affects binding only in raphe nuclei (27). Thus, some antidepressant effects could be similar to those observed with domestication, suggesting a link between the neurobiology of depression and that underlying defensive behavior. Such a link is further supported by the similar pattern of behavioral isomorphisms observed in the symptomatology of depression and in defensive behaviors following chronic social stress (5).

Domestication also affects 5-HT$_{1A}$ binding in the ventromedial hypothalamus (Table 1), albeit without significance in this size sample. The trend toward decreased labeling in this region might reflect an increased 5-HT content and metabolism observed in the hypothalamus of domesticated animals (13).

The selective, regional alteration of 5-HT$_{1A}$ receptor labeling observed herein suggests that 5-HT activity is associated with decreased defensive behavior in domesticated animals. The localization of these effects suggests that enhanced 5-HT$_{1A}$ receptor activity in hippocampal circuits could be selectively involved and provides a likely substrate for 5-HT$_{1A}$ agonist-induced alterations of defensive behavior (7).

ACKNOWLEDGEMENTS

This research was supported by USPHS Awards DA04081, MH42803, RR08125, and RR03061 and NSF Award BNS 9111524.

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