Behavioral Neuroscience
1999, Vol. 113, No. 4, 804–815

0735-7044/99/$3.00

Serotonin Regulation of Aggressive Behavior in Male Golden Hamsters (*Mesocricetus auratus*)

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These studies examined the neurochemistry and neuroanatomy of the serotonin (5-HT) system innervating the anterior hypothalamus (AH) and the interaction of 5-HT receptor agonists with arginine vasopressin (AVP) in the regulation of offensive aggression in golden hamsters. Because specific 5-HT_{1A}, 5-HT_{1B}, and AVP V_{1A} binding sites were observed within the AH by in vitro autoradiography, the hamsters were tested for offensive aggression after microinjections of AVP in combination with either the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino) tetraline (DPAT) or the 5-HT_{1B} agonist CGS-12066A (CGS) directly within the AH. Though treatment with DPAT resulted in a dose-dependent inhibition of AVP-facilitated offensive aggression, CGS was ineffective. In addition, a retrograde tracer was injected within the AH to localize the distribution of 5-HT neurons projecting to the area. Retrogradely labeled 5-HT neurons were found within the dorsal, median, and caudal linear raphe nuclei and are suspected to inhibit AVP-facilitated offensive aggression by an activation of 5-HT_{1A} receptors in the AH.

Many preclinical and clinical studies have reported a role for serotonin (5-HT) in the control of aggressive behavior (for reviews, see Eichelman, 1990; Miczek & Donat, 1989; Olivier & Mos, 1990). Animals treated with fluoxetine and other selective 5-HT reuptake inhibitors show elevations in extracellular levels of 5-HT in the brain (Auerbach, Minzenberg, & Wilkinson, 1989; Ferris, 1996; Guan & McBride, 1988; Perry & Fuller, 1992). This increase in 5-HT levels after peripheral injection of 5-HT reuptake inhibitors suppresses multiple measures of aggressive behavior in a wide range of animals, for example, lizards (Deckel, 1996), rats (Dalta, Mitra, & Bhattacharya, 1991; Molina, Ciesielski, Gobailles, Insel, & Mandel, 1987; Olivier, Mos, van Oorschot, & Hen, 1995), mice (Ogren, Holm, Renyi, & Ross, 1980; Olivier, Mos, Van der Heyden, & Hartog, 1989; Sanchez & Hyttel, 1994), golden hamsters (Delville, Mansour, & Ferris, 1995; Ferris et al., 1997), voles (Villalba, Boyle, Caliguri, & De Vries, 1997), and dogs (Dodman et al., 1996), as well as humans (Coccaro & Kavoussi, 1997).

With over 14 different 5-HT receptor subtypes (Hoyer & Martin, 1997) to choose from, 5-HT appears to have its antiaggressive effect by interacting with 5-HT_{1A} and 5-HT_{1B} receptors. Several nonselective 5-$HT_{1A/1B}$ receptor agonists, for example, N-(3-Trifluoromethylphenyl)piperazine hydrochloride, 1-(3-Chlorophenyl)piperazine hydrochloride, and eltoprazine, effectively suppress aggression (Mos, Olivier, Poth, & van Aken, 1992; Sanchez, Arnt, Hyttel, & Moltzen, 1993; Sijbesma, Schipper, & De Kloet, 1990). Eltoprazine has a serenic-like profile, blocking offensive aggression (i.e., initiation of attacks and bites) with no demonstrable effect on other social behaviors or exploratory activity. There is evidence that the primary inhibitory effect of eltoprazine is mediated through the postsynaptic 5-HT_{1B} receptor subtype (Olivier et al., 1995; Sijbesma et al., 1991). Most recently, it was shown that provoked and alcohol-enhanced aggression in mice could be specifically suppressed by the 5-HT_{1B} agonist CP94253 without affecting other behaviors (Fish, Faccidomo, & Miczek, 1998). Furthermore, homozygous mutant mice lacking the 5-HT_{1B} receptor show enhanced aggression toward intruders (Saudou et al., 1994).

Still other reports have shown a critical role for 5-HT_{1A} receptors in the suppression of aggression (for review, see Bell & Hobson, 1994). Giving the 5-HT_{1A} receptor antagonist (+)-WAY-100135 to male mice results in a dose-dependent increase in offensive behavior (Bell, Mitchell, & Hobson, 1996). 5-HT receptor agonists with a high affinity for the 5-HT_{1A} receptor, such as 8-hydroxy-2-(di-n-propylamino) tetraline (DPAT), inhibit aggression (McMillen, Scott, Williams, & Sanghera, 1987; Miczek, Hussain, & Faccidomo, 1998; Olivier et al., 1989; Sanchez et al., 1993; White, Kucharik, & Moyer, 1991). The antiaggressive effects of low doses of DPAT have little or no obvious effect on motor activity (White et al., 1991).

A majority of the studies reporting that 5-HT diminishes aggression are based on the peripheral administration of 5-HT reuptake inhibitors and 5-HT_{1A} and 5-HT_{1B} ligands. This systemic approach does not address where these drugs act in the brain or their interaction with other neurotransmit-

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This work was supported by National Institute of Mental Health (NIMH) Grant MH-52280. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIMH. We are indebted to Maurice Manning for his generous gift of HO-LVA. We are also grateful to Garth Brown for his generous gift of [^{125}I]-HO-LVA.

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ter systems involved in the regulation of aggression. One neurotransmitter, arginine vasopressin (AVP), enhances aggressive behavior in several species of rodents and acts at different neural sites (Delville, Mansour, & Ferris, 1996; Ferris et al., 1997; Koolhaas, Van den Brink, Roozendal, & Boorsma, 1990; Koolhaas, Moor, Hiemstra, & Bohus, 1991). Microinjection of AVP into the anterior hypothalamus (AH) of male golden hamsters increases offensive aggression toward smaller male intruders (Ferris et al., 1997). Conversely, blockade of AVP V_{1A} receptors in the AH with receptor antagonists inhibits aggression in hamsters (Ferris & Potegal, 1988; Potegal & Ferris, 1990). The aggressive activity resulting from AVP microinjected into the AH appears to be inhibited by 5-HT, as peripheral treatments with fluoxetine block AVP-facilitated aggression of resident hamsters toward smaller intruders (Ferris et al., 1997).

The present studies were undertaken to examine (a) the role of 5-HT_{1A} and 5-HT_{1B} receptor subtypes in mediating AVP-facilitated aggression in the AH of male golden hamsters and (b) the source of 5-HT innervation to the AH that is involved in the control of aggression in this species. To accomplish these goals, AVP-facilitated offensive aggression was monitored after the microinjection of several doses of 5-HT_{1A} and 5-HT_{1B} receptor agonists into the AH. To identify afferent connections from the raphe complex to the AH, a retrograde tracer, Fluoro-Gold (FG) was injected into AH, and retrogradely labeled neurons were double-stained for FG and tryptophan hydroxylase (TH).

Method

Animals

Male golden hamsters (*Mesocricetus auratus,* 110–120 g) were obtained from Harlan Sprague–Dawley Laboratories (Indianapolis, IN), housed individually in Plexiglas cages (24 cm × 24 cm × 20 cm), maintained on a reverse 14:10-hr light–dark cycle (14 hr light, 10 hr dark; lights on at 19 00), and provided with food and water ad libitum. Hamsters were acclimated to the reverse light–dark cycle for at least 2 weeks before testing. All behavioral tests were conducted during the dark phase of the circadian cycle.

All hamsters were acquired and cared for in accordance with the guidelines published in the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, 1985).

AVP V_{1A}, 5-HT_{1A} and 5-HT_{1B} Receptor Binding

AVP receptor binding sites were labeled by in vitro autoradiography (a modification of a previously published protocol, Ferris, Delville, Gronka, Luber-Narod, & Insel, 1993) and were adapted to a new linear ligand, HO-Phenylacetyl 1-D-Tyr(Me)2-Phe3-Gln4-Asn5-Arg6-Pro7-Arg8-NH_2 (HO-LVA; Barberis et al., 1995). In brief, hamsters ($n = 6$) were decapitated, and their brains were removed, frozen on dry ice, and kept at −80° C until sectioning. Coronal sections (20 μm) were cut in a −10° C cryostat, thaw-mounted on gelatin-coated slides, air dried, and stored at −80° C. Later, the sections were brought to room temperature and dipped for 5 min in a solution of 0.2% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4) supplemented with 0.1% bovine serum albumin (BSA; Fraction V, Sigma Chemical, St. Louis, MO). The slides were then incubated for 60 min at room temperature in a 0.05 M Tris–HCl buffer (pH 7.3) containing 10 mM $MgCl_2$, 0.1% BSA, 0.05% Bacitracin, and 50 pM ^{125}I-HO-LVA. The sections were then washed three times in ice-cold incubation medium for 5 min each, followed by a final wash of ice-cold distilled water. Nonspecific binding was achieved by incubations containing 1 μM unlabeled HO-LVA. After drying at room temperature, the sections were apposed to Hyperfilm ^{3}H (Amersham, Arlington Heights, IL) in X-ray cassettes for 2–3 days at room temperature. After development of the film, the sections were counterstained with 0.5% thionin to identify the neuroanatomical location of the labeled sites. Sections contiguous to those used for V_{1A} receptor binding were examined for 5-HT_{1A} and 5-HT_{1B} receptor binding. Sections for 5-HT_{1A} receptor binding were preincubated in a 0.05 M Tris–HCl buffer containing 2 mM $MgCl_2$, followed by a 120-min incubation in the same buffer in the presence of the iodinated ligand, 8-hydroxy-2-(N-n-propyl-N-3′-iodo-2′-propenyl)aminotetralin ([^{125}I]-8-OH-PIPAT; 70 pM), a 5-HT_{1A} receptor ligand (NEN Research Products, Billerica, MA; Mei-Ping, Frederick, Zhi-ping, & Kung, 1995). These incubations were followed by several washes in ice-cold 0.05 M Tris-HCl buffer with a final wash of ice-cold distilled water. Once dried, the sections were apposed to Hyperfilm ^{3}H overnight in X-ray cassettes. Nonspecific binding was determined in the presence of 10 μM DPAT (Research Biochemicals International, Natick, MA).

Sections for 5-HT_{1B} receptor binding were processed by the same procedure as those for 5-HT_{1A} but with [^{125}I]-iodocyanopindolol (NEN Research Products) as ligand (Hoyer, Engel, & Kalkman, 1985). Incubation was performed in the presence of 30 μM isoproterenol to suppress binding to the β-adrenoreceptors. Nonspecific binding was determined in the presence of 1 μM unlabeled 5-HT.

Offensive Aggression After Injections of AVP and 5-HT Receptor Agonists

A resident–intruder paradigm was used to evaluate the role of 5-HT in the control of AVP-facilitated offensive aggression in male golden hamsters. At least 2 days before testing, hamsters were anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg, Abbott Laboratories, North Chicago, IL) and stereotaxically implanted with 26-gauge, unilateral microinjection guide cannulas aimed at the AH, as previously described (Ferris, Meenan, Axelson, & Albers, 1986). The stereotaxic coordinates were 1.1 mm anterior to bregma, 1.8 mm lateral (left) to the midsagittal suture, 7.5 mm ventral from dura, angled at 8°. The incisor bar was held at the level of the interaural line.

On the day of testing, hamsters were microinjected with AVP (0.09 μM in 0.9% NaCl) alone or in combination with various concentrations of the 5-HT_{1A} receptor agonist DPAT (Sigma Chemicals) or the 5-HT_{1B} receptor agonist CGS-12066A (CGS, Research Biochemicals International) in a volume of 100 nl. The injections were given through a 33-gauge needle attached to a 1-μl Hamilton syringe by PE-20 tubing. All microinjections were performed on unanesthetized hamsters and took no longer than 15 s. After microinjection, the needle was left in place for about 30 s before being removed to minimize the spread of the injected fluid along the track of the needle. Afterward, the hamsters were returned to their home cage and immediately tested for aggression in the presence of a younger, smaller male intruder (40–50 days old, 90–100 g). The intruders used for the experiment had experienced defeat before the tests and were submissive. Intruders were used once or twice on each testing day, with an interval of at least 1 hr between tests. The resident was scored for latency to bite the intruder, total number of bites, contact time, and flank marks over a 10-min test period, as previously described (Ferris et al.,

1997). *Contact time* was defined as the period of time during which the resident deliberately initiated contact with the intruder either through olfactory investigation or aggression. All behavioral tests were videotaped under dim red illumination and scored by two independent observers who were unaware of the hamsters' treatment. No resident met the same intruder more than once.

These studies were designed to examine the effect of 5-HT_{1A} and 5-HT_{1B} receptor agonists on offensive aggression facilitated by the microinjection of AVP into the AH. Fifteen hamsters were tested under four different types of treatment: AVP alone, or AVP in combination with 100 μM, 10 μM, or 1 μM DPAT. Another group of fifteen hamsters was tested with AVP alone or AVP in combination with 1 mM, 330 μM, or 100 μM CGS. In each study, treatments were counterbalanced and presented in random order. The studies produced tables of repeated measures. The latency to bite and duration of contact time were compared between groups with repeated measures analyses of variance (ANOVAs) followed by Newman–Keuls post hoc tests. Total number of bites and flank marks were analyzed with nonparametric tests (Friedman followed by Wilcoxon, two-tailed).

FG, 5-HT, and TH Immunocytochemistry

FG was delivered to the AH by iontophoresis according to the stereotaxic coordinates noted above. Hamsters ($n = 12$) were anesthetized with Nembutal, and a glass micropipette (tip: 40–50 μm diameter) loaded with FG (2% in 0.9% NaCl; Fluorochrome, Englewood, CO) was lowered into the AH. The injections were performed by running a current (5 μA; 7 s on, 7 s off) through the micropipette for 10 min. Two weeks later, the hamsters were anesthetized and perfused transcardially after an intracardiac injection of heparin (5,000 units in 1 ml saline) with saline containing 0.2% sodium nitrite (to dilate blood vessels) followed by a solution containing 4% paraformaldehyde and 2.5% acrolein in 0.1 M potassium phosphate buffered saline (KPBS, pH 7.4) for 15–20 min, and followed with saline again for 3 min. Later, the brains were removed from the skull and saved in 20% sucrose–KPBS at 4° C until they were sliced into 40 μm-thick coronal sections with a freezing microtome. The sections were saved in a cryoprotectant (Watson, Weigand, Clough, & Hoffman, 1986) and labeled by immunocytochemistry (Chang, Kuo, Whittaker, & Cooper, 1990).

Immunocytochemistry to FG was performed with a rabbit polyclonal antibody to FG (1/1,000; AB 153; Chemicon International, Temecula, CA). Briefly, the sections were successively pretreated in 1% sodium borohydrite (to eliminate residual aldehydes) followed by 20% normal goat serum containing 1% hydrogen peroxide and 0.3% Triton X-100 (to block nonspecific labeling, eliminate endogenous peroxidase activity, and permeabilize the sections). Afterward, the sections were incubated for 1 hr in the primary antibody (rabbit anti-FG, 1/1,000) containing 2% normal goat serum and 0.3% Triton X-100 at 37° C. After washing, the sections were incubated in a secondary antibody (7.5 μg/ml, biotinylated goat anti-rabbit immunoglobulin G [IgG]; Vector Laboratories, Burlingame, CA) followed by a tertiary incubation (Vectastain ABC Elite Kit, Vector Laboratories). Between incubations, the sections were washed with 0.05 M Tris buffered saline (pH 7.6). Finally, the sections were labeled with diaminobenzidine (DAB). This procedure led to an intense and optimal labeling of FG within cell bodies and some fibers. This labeling only occurred within areas observed to have fluorescent staining by observation of the FG under ultraviolet (UV) excitation light in unstained sections. Furthermore, omission of the primary antibody prevented all labeling. The distribution of FG-immunoreactive neurons was mapped on a Leitz microscope equipped with a camera lucida, as noted above.

Immunocytochemistry to 5-HT was performed with a rabbit polyclonal antibody to 5-HT (1/4–5,000; NT102; Eugene Tech International, Ramsey, NJ) in sections originating from hamsters perfused with 4% paraformaldehyde. In these fixations, the hamsters were perfused with 4% paraformaldehyde in 0.1 M PBS (pH 7.2) instead of the paraformaldehyde–acrolein mixture. The brains were taken out and postfixed in 4% paraformaldehyde for an additional 30–60 min before being placed in 20% sucrose–PBS at 4°C. Later, the brains were cut into 40-μm thick coronal slices with a freezing microtome. The sections were saved at −20° C in a cryoprotectant until they were labeled by immunocytochemistry, as previously described (Delville, Melloni, & Ferris, 1998). The distribution of 5-HT-immunoreactive cells within the midbrain was mapped in brain sections labeled with DAB and a primary antibody concentration of 1/4,000. This procedure led to a brown labeling of neurons within the midbrain.

Combined immunocytochemistry to FG and TH (a key enzyme in the synthesis of 5-HT) was performed through similar methods on alternate sections with the same rabbit polyclonal antibody to FG (1/500; AB 153; Chemicon) and a sheep polyclonal antibody to TH (4 μg/ml; AB1541; Chemicon). The sections were incubated in these antibodies for a period of 48–72 hr at 4°C after a preincubation in 20% normal donkey serum. The sections were labeled by incubation with the secondary antibodies (Texas Red conjugated donkey anti-sheep IgG and fluorescein isothiocyanate [FITC]-conjugated donkey anti-rabbit, 5 μg/ml). The resulting label was intensified by using the primary antibody solutions as bridging IgGs and by re-incubation into the secondary antibodies. Immunoreactivity was observed on a Zeiss microscope equipped with fluorescent illumination. Texas Red labeling was observed under green excitation light, and FITC labeling was observed under blue excitation light.

FITC immunoreactive labeling only appeared within cells containing FG labeling as observed under UV excitation light. In the midbrain, Texas Red immunoreactive labeling only appeared within known serotoninergic cell groups of the raphe nuclei. The distribution of FITC-labeled FG-immunoreactive cells matched the distribution of DAB-labeled FG-immunoreactive cells. The distribution of Texas Red-labeled TH-immunoreactive neurons matched the distribution of DAB-labeled 5-HT-immunoreactive cells mapped within the raphe nuclei.

Results

Vasopressin and 5-HT Binding Sites in the AH

The receptor autoradiograms from contiguous coronal sections (see Figure 1) reveal specific AVP V_{1A} receptor binding in the hypothalamus. As shown in the figure, certain sites such as the suprachiasmatic nucleus and the area of the AH (arrow) contain a high density of V_{1A} receptor binding. A different pattern was observed for specific 5-HT_{1A} and 5-HT_{1B} receptor binding in the AH. Indeed, 5-HT_{1A} and 5-HT_{1B} receptor binding sites appeared more homogenous throughout the area.

Inhibiting AVP-Facilitated Aggression With 5-HT Receptor Agonists

Treatment with the 5-HT_{1A} agonist DPAT affected aggressive behavior in a dose-dependent manner (see Figure 2). The latency to bite after treatment with AVP alone was just

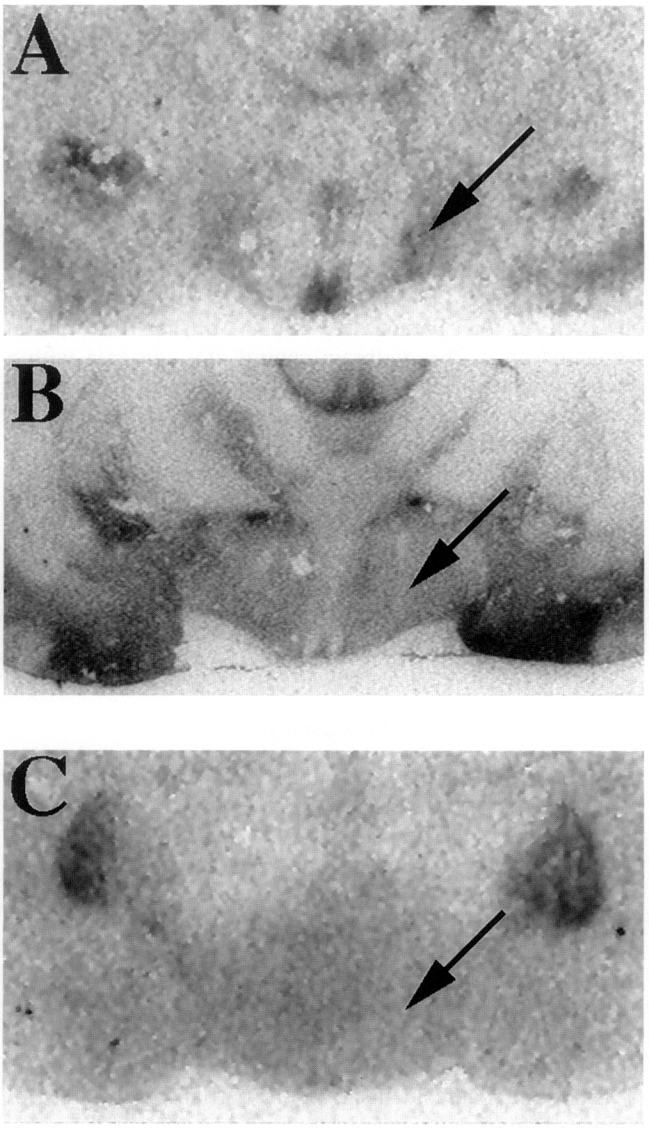

Figure 1. Arginine vasopressin (AVP; Panel A) and serotonin (5-HT; Panels B and C) receptor binding in the anterior hypothalamus (arrows) of golden hamsters. Shown are autoradiograms of specific binding for [^{125}I]-hydroxy-phenylacetyl-1-D-Tyr(Me)2-Phe3-Gln4-Asn5-Arg6-Pro7-Arg8-NH_2 ([^{125}I]-HO-LVA), a selective AVP V_{1A} receptor ligand (Panel A); 8-hydroxy-2-(N-n-propyl-N-3′-iodo-2′-propenyl)aminotetraline, a selective 5-HT_{1A} receptor ligand (Panel B); and (−)[^{125}I]iodocyanopindolol, a selective 5-HT_{1B} receptor ligand (Panel C). The binding shown was performed on contiguous, 20-μm coronal sections.

over 2 min. However, it was more than twice as long after treatments with 10 μM and 100 μM DPAT. These differences were statistically significant, $F(3, 42) = 9.6$, $p < .001$. Compared with treatment with AVP alone, treatment with 10 μM and 100 μM DPAT led to a statistically significant elongation of the latency to bite ($p < .01$ and $p < .001$, respectively). Furthermore, the different treatments also had a significant effect on the number of bites, $\chi^2(3, N = 60) = 13.36$, $p < .01$, recorded during testing (see Figure 2). Although treatment with AVP alone stimulated all hamsters to bite, treatment with 100 μm DPAT treatment blocked 6 of 15 hamsters from biting. As a result, treatment with 10 μM and 100 μM DPAT led to a significant reduction in the number of bites ($p < .01$) compared with treatment with AVP alone. Contact time was also significantly reduced by treatment with DPAT, $F(3, 27) = 4.34$, $p < .05$. Treatment with 10 μM but not 1 μM or 100 μM DPAT significantly reduced the amount of time the resident spent in contact with the intruder. In contrast, the number of flank marks recorded during the 10-min observation periods was not significantly affected by treatment with DPAT, $\chi^2(3, N = 60) = 2.38$, $p > .1$.

Treatment with the 5-HT_{1B} receptor agonist CGS did not have any statistically significant effect on aggressive behavior (see Figure 3). There were no between-group differences in the latency to bite or the number of bites recorded during the tests, $F(3, 42) = 1.36, p > .1$; $\chi^2(3, N = 60) = 1.94, p > .1$, respectively. There was no significant effect on contact time, $F(3, 42) = 0.46$, $p > .1$. However, there was a significant increase in flank marking, $\chi^2(3, N = 60) = 8.44$,

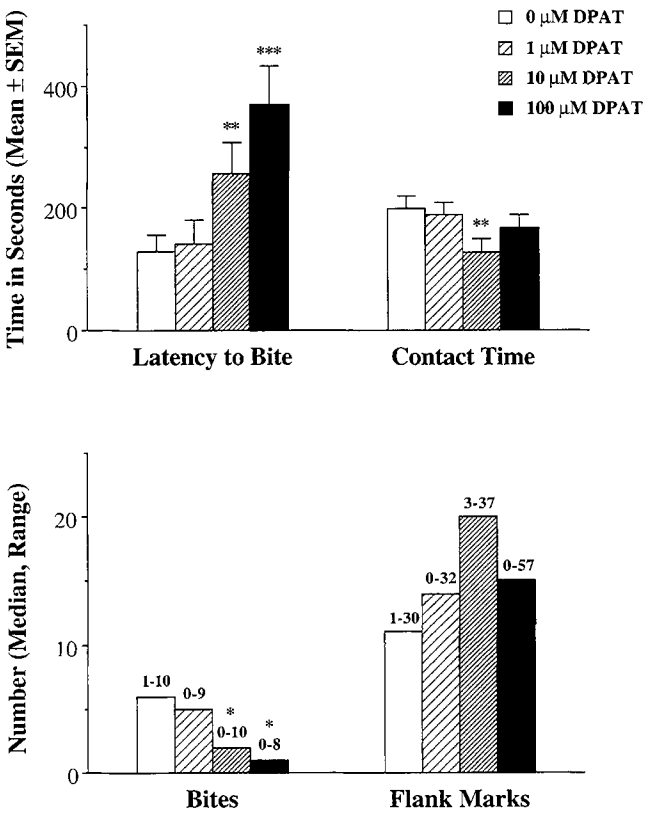

Figure 2. Offensive aggression tested in a resident–intruder paradigm. The hamsters were tested immediately after hypothalamic microinjections of 0.09 μM arginine vasopressin, alone or in combination with various concentrations of 8-hydroxy-2-(di-n-propylamino) tetraline (DPAT), a serotonin 1A receptor agonist. The latency to bite, contact time, number of bites, and number of flank marks were compared between treatments. *$p < .05$, **$p < .01$, ***$p < .001$, compared with hamsters injected with 0 μM DPAT.

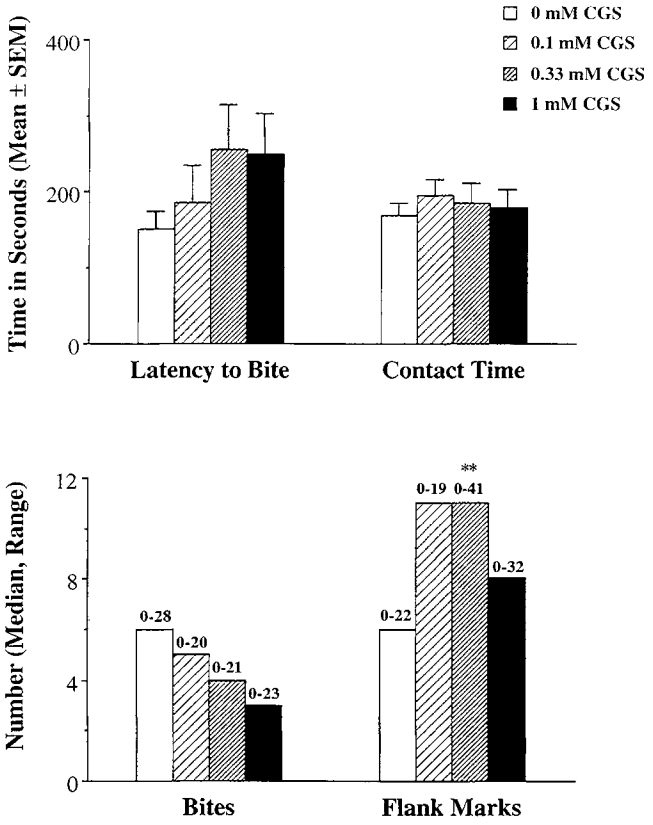

Figure 3. Offensive aggression tested in a resident–intruder paradigm. The hamsters were tested immediately after hypothalamic microinjections of 0.09 μM arginine vasopressin, alone or in combination with various concentrations of CGS, a serotonin 1B receptor agonist. The latency to bite, contact time, number of bites, and number of flank marks were compared between treatments. $^{**}p < .01$, compared with hamsters injected with 0 μM 8-hydroxy-2-(di-n-propylamino) tetraline.

$p < .05$. Treatment with 0.33 mM CGS led to an increase in flank marking activity compared with treatment with AVP alone ($p < .05$).

5-HT-Immunoreactive Perikarya in the Pons

Immunocytochemistry for 5-HT was performed in untreated adult male hamsters ($n = 12$). The distribution of 5-HT-immunoreactive perikarya throughout the raphe complex at the level of the pons was mapped with a camera lucida in a representative hamster (see Figures 4 and 5). A dense population of 5-HT-immunoreactive neurons was observed within the dorsal raphe nucleus, spreading laterally into the posterior dorsal raphe nucleus. As seen on the camera lucida drawings (see Figure 5), the median raphe nucleus also contained a high density of 5-HT-immunoreactive neurons, with a diffuse population of neurons spreading laterally into the paramedian raphe nucleus. Diffuse populations of 5-HT-immunoreactive neurons were also seen in the caudal linear nucleus, the serotonergic B9 group, and extending laterally into the ventral tegmental area.

FG Injections in the AH

Immunocytochemistry for FG iontophoresed into the AH was performed in 6 hamsters. The location of each FG injection site at the level of the AH is shown in Figure 6. The resulting distribution of FG-immunoreactive neurons was mapped with a camera lucida within the raphe complex at the level of the pons in a representative hamster (see Figures 4 and 5). Populations of FG-immunoreactive neurons were observed within the dorsal, median, and caudal linear raphe nuclei. However, FG-immunoreactive cells were never observed in the paramedian raphe, B9, or the ventral tegmental area. The midbrain central gray, an area with a scarce number of 5-HT neurons, also showed a moderate number of FG-immunoreactive neurons.

Separate sections from the raphe complex at the level of the pons were processed for combined immunocytochemistry to FG and TH. These sections were used to confirm the presence of 5-HT neurons located within the dorsal, median, and caudal linear raphe nuclei and projecting to the AH. In all hamsters, double-labeled neurons were easily apparent within these nuclei (see Figure 7), as well as within the pontine raphe nucleus.

Discussion

The present results show that AVP V_{1A}, 5-HT_{1A}, and 5-HT_{1B} receptors are present in the AH of male golden hamsters where a plexus of AVP and 5-HT fibers was previously reported (Ferris et al., 1997). Furthermore, this study suggests that the release of 5-HT in the AH acts through 5-HT_{1A} receptors to inhibit AVP-facilitated offensive aggression (i.e., longer latencies and fewer bites). The antiaggressive effect of 5-HT_{1A} receptor activation is not surprising. Isolated male resident mice given peripheral treatments of compounds with 5-HT_{1A} activity show diminished offensive aggression toward intruders. McMillen et al. (1987) found that residents given the partial 5-HT_{1A} receptor agonists buspirone and gepirone showed diminished aggression toward intruders without the side effects of sedation or ataxia. White and coworkers (1991) screened numerous compounds with 5-HT_{1A} activity including buspirone, gepirone, and DPAT, all of which reduced aggression at doses below those that produce loss in motor coordination. In a parallel study, Sanchez et al. (1993) also screened multiple 5-HT_{1A} compounds, using increase in attack latency as a simple measure of antiaggressive activity. The ability of 5-HT_{1A} compounds to increase attack latency is positively correlated with their 5-HT_{1A} receptor affinity as measured in an in vitro binding assay using rat brain membranes. In addition, there is a highly significant, positive correlation between inhibition of aggression by these compounds and their generalization to DPAT-induced discriminative stimulus in rats. In a recent study by Miczek et al. (1998), quantitative behavioral analysis showed a systematic reduction in all major measures of offensive aggression by resident mice after DPAT treatment. The dose-dependent diminution in aggression is accompanied by a reduction in motor activities, principally the duration of walking. How-

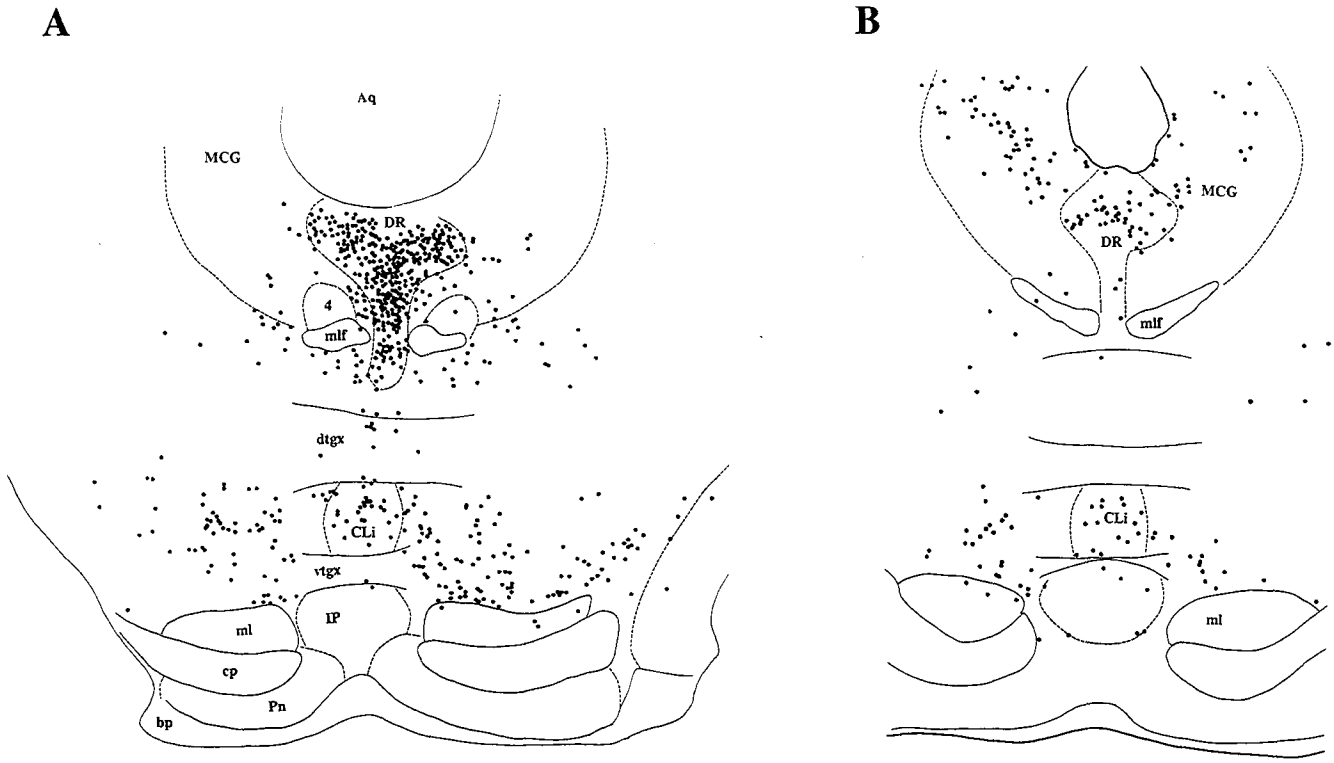

Figure 4. Camera lucida drawings showing the distribution of serotonin (A) and Fluoro-Gold (B) immunoreactivity in the midbrain of male golden hamsters. Sections used for Fluoro-Gold drawings originate from Hamster 10 (see Figure 6), which was injected in the anterior hypothalamus. Aq = aqueduct; MCG = midbrain central gray; DR = dorsal raphe nucleus; 4 = trochlear nucleus; mlf = medial longitudinal fasciculus; dtgx = dorsal tegmental decussation; CLi = caudal linear nucleus of the raphe; vtgx = ventral tegmental decussation; ml = medial lemniscus; IP = interpeduncular nucleus; cp = basal cerebral peduncle; Pn = pontine nuclei; bp = brachium pontis (Paxinos & Watson, 1986).

ever, at any dose, the antiaggressive effects of DPAT far exceed the reduction in motor activity. In the present study, there was no specific measure of motor activity during behavioral testing. Nonetheless, most hamsters showed robust flank marking behavior across all treatments (see Figure 2). Flank marking is a stereotyped scent-marking behavior that requires concerted motor activity (Johnston, 1975).

The involvement of 5-HT_{1A} receptors in the regulation of aggression also extends to clinical studies. In prospective, placebo-controlled studies, mentally retarded patients with aggressive and self-injurious behavior showed some beneficial effects with buspirone (Ratey, Sovner, Parks, & Rogentine, 1991; Ricketts, Goza, & Ellis, 1994; Verhoeven & Tuinier, 1996). Daily drug treatments in mentally retarded patients can lessen outbursts, self-injury, and impulsivity as well as improve sociability without sedation and depression of intellectual capacities. Buspirone was also reported to improve behavior in a small percentage of children hospitalized with symptoms of moderately severe aggression (Pfeffer, Jiang, & Domeshek, 1997), whereas in a retrospective study, the antiaggressive effects of buspirone were noted in patients with psychiatric illness associated with traumatic brain injury (Stanislav, Fabre, Crimson, & Childs, 1994).

5-HT_{1A} receptors are found on postsynaptic membranes at distant sites of 5-HT neurotransmission and on the soma and dendrites of 5-HT neurons in the anterior raphe nuclei (Sotelo, Cholley, Mestikawy, Gozlan, & Hamon, 1990; Verge et al., 1986). Activation of 5-HT_{1A} receptors causes a hyperpolarizing increase in potassium conductance and an inhibition in neuronal activity (Andrade & Nicoll, 1987). Mos et al. (1992) reported that intraventricular injection of DPAT had no effect on offensive aggression in rats. However, in a later study they reported that direct injection of DPAT into the raphe complex of male rats decreases offensive aggression (Mos, Olivier, Poth, van Oorschot, & van Aken, 1993), a finding corroborated by others in females (de Almeida & Lucion, 1997). From these data, the authors concluded that postsynaptic 5-HT_{1A} receptors are not critical in the control of aggression (Mos et al., 1993). Instead, de Almeida and Lucion argued that DPAT suppresses aggression by activating somatodendritic autoreceptors and inhibiting 5-HT neuronal activity. This notion seems implausible because aggression is reduced by the site-specific injection of DPAT into the medial preoptic area (Cologer-Clifford, Simon, Lu, & Smoluk, 1997), the corticomedial amygdala (de Almeida & Lucion, 1997) and the AH, as found in the present study. Additional data suggest that postsynaptic

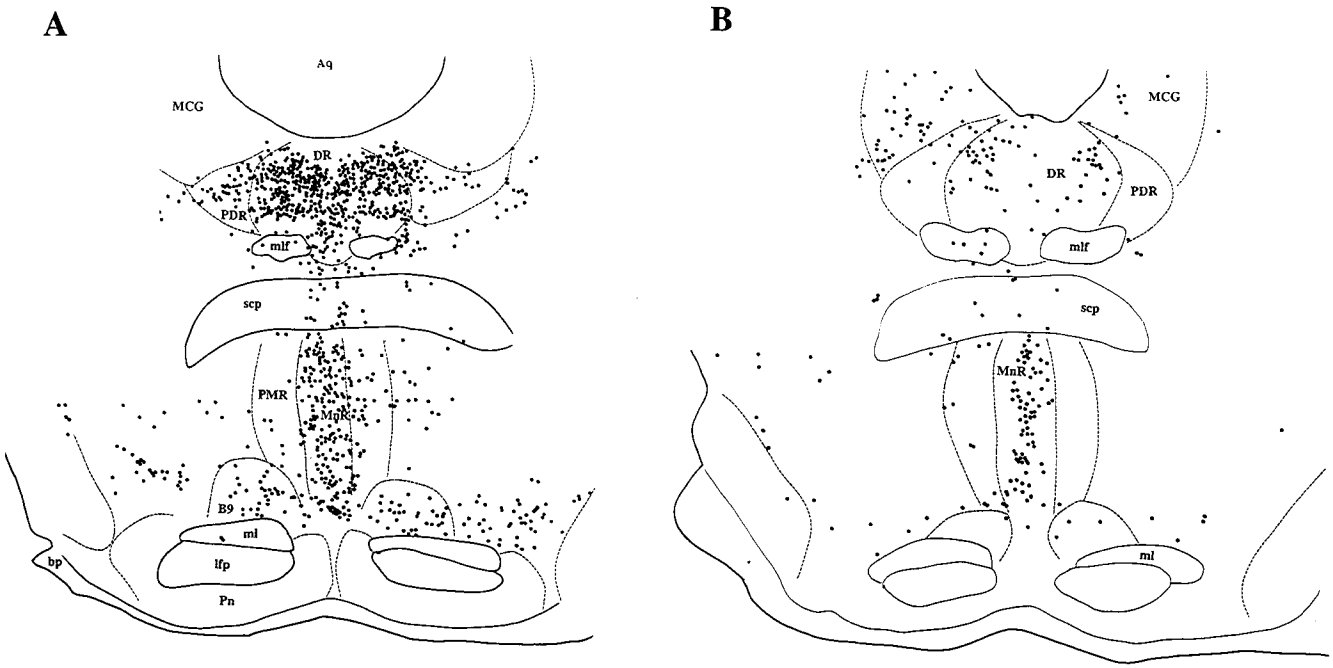

Figure 5. Camera lucida drawings showing the distribution of serotonin (A) and Fluoro-Gold (B) immunoreactivity in the midbrain of male golden hamsters. Sections used for Fluoro-Gold drawings originate from Hamster 10 (see Figure 6), which was injected in the anterior hypothalamus. Aq = aqueduct; MCG = midbrain central gray; DR = dorsal raphe nucleus; PDR = paradorsal raphe nucleus; mlf = medial longitudinal fasciculus; scp = superior cerebellar peduncle; PMR = paramedial raphe nucleus; MnR = median raphe nucleus; B9 = B9 serotonin cells; ml = medial lemniscus; lfp = longitudinal fasciculus of the pons; bp = brachium pontis; Pn = pontine nuclei (Paxinos & Watson, 1986).

receptors within the forebrain are more important than autoreceptors within the raphe nuclei in the control of aggression. Although 5,7-dihydroxytryptamine lesions of 5-HT neurons in the anterior raphe nuclei reduce 5-HT_{1A} binding in this area, they do not eliminate the antiaggressive effect of eltoprazine, a 5-$HT_{1A/1B}$ agonist (Sijbesma et al., 1991). Presumably, eltoprazine is activating postsynaptic 5-HT_{1A} and/or 5-HT_{1B} receptors outside the raphe complex to reduce aggression.

In these studies, the activation of the 5-HT_{1B} receptor by CGS has no significant effect on aggressive behavior, although there was a trend toward inhibition. Interestingly, treatment with this drug enhanced flank marking, a stereotyped motor behavior activated by AVP (Ferris, Albers, Wesolowski, Goldman, & Leeman, 1984) and inhibited by fluoxetine (Ferris et al., 1997). The present data suggest that fluoxetine-inhibited flank marking is not affected by 5-HT_{1B} receptors. Indeed, neither DPAT nor CGS blocked flank marking under the present study's experimental conditions. It was anticipated that the 5-HT receptor subtype regulating aggression would also be involved in the regulation of flank marking. Both offensive aggression and flank marking contribute to the ethogram of agonistic activity in golden hamsters (Johnston, 1975), and both behaviors are activated by AVP and inhibited by 5-HT. However, it seems the regulation of agonistic behavior in male golden hamsters may involve several 5-HT receptor subtypes.

The present data are consistent with findings reported after intracerebroventricular injections of the same 5-HT receptor agonists in male hamsters (Joppa, Rowe, & Meisel, 1997). However, the present data do not preclude the possibility that activation of 5-HT_{1B} receptors in other brain areas and under other neuroendocrine conditions could affect aggressive behavior. Recent work shows that the antiaggressive effects of 5-HT_{1A} and 5-HT_{1B} agonists are site-specific, synergistic, and influenced by the hormonal environment (Cologer-Clifford et al., 1997; Cologer-Clifford, Simon, Richter, Smoluk, & Lu, in press). Treatment of mice with estrogens or androgens alters sensitivity to DPAT and CGS. Essentially, estrogens produce a more restrictive environment for serotinergic inhibition than do androgens. Mice treated with androgens show suppression in aggression after injections of either CGS or a cocktail of CGS and DPAT into the lateral septum. Treatment with estrogen renders the septum insensitive to both of these 5-HT receptor agonists. Conversely, the medial preoptic area is responsive to both receptor agonists alone or in combination, regardless of the hormonal environment.

Camera lucida drawings of 5-HT immunoreactive perikarya in the brainstem of male golden hamsters show a neuronal distribution, as previously described by Botchkina and Morin (1993). The organization of 5-HT neurons in the golden hamster's anterior raphe nuclei is comparable to that of other mammals (Jacobs & Azmitia, 1992). In golden

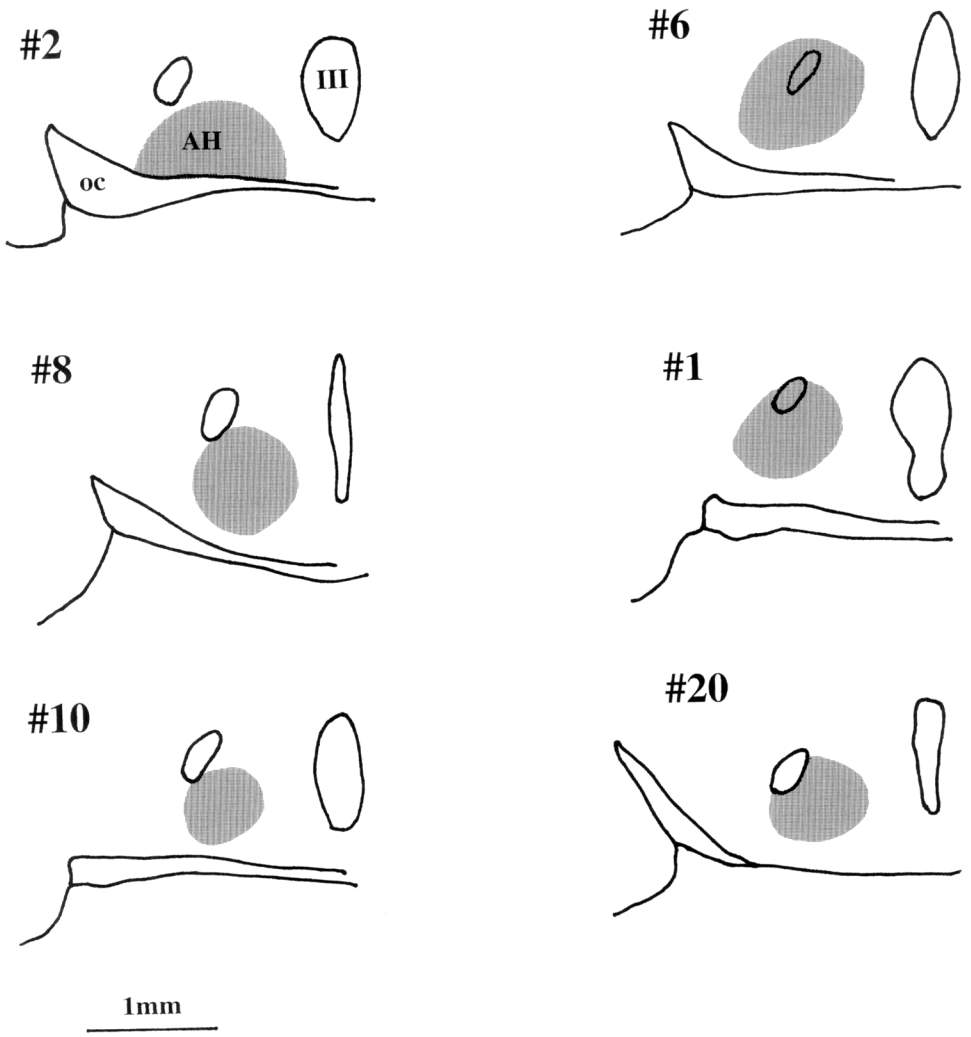

Figure 6. Camera lucida drawings of Fluoro-Gold injection sites (shaded area) located within the anterior hypothalamus (AH) in 6 hamsters. III = third ventricle; oc = optic chiasma.

hamsters, the anterior raphe nuclei include aggregations of neurons in the dorsal and median raphe and caudal linear nucleus. FG injections into the AH retrogradely labelled neurons in the dorsal and median raphe nuclei and the caudal linear nucleus; but there were no double-labeled cells in the lateral populations of serotoninergic neurons. The distribution of retrograde labeling was bilateral and showed a crossover of afferent connections from the raphe to the AH. Many of the neurons labeled with FG double stain for TH immunoreactivity. In a developmental study, Botchkina and Morin (1993) reported a dense 5-HT innervation from the medial forebrain bundle to the lateral portion of the AH in golden hamsters. Hence, the primary source of this 5-HT innervation to the AH appears to be neurons from the dorsal and medial raphe nuclei and caudal linear nucleus ascending through the medial forebrain bundle.

AVP is an important neurotransmitter affecting agonistic behavior associated with the establishment and maintenance of dominant–subordinate relationships between hamsters (for review, see Ferris, 1992). Microinjection of AVP into the AH facilitates offensive aggression toward intruders. AVP receptor antagonists microinjected into the AH produced a dose-dependent inhibition of offensive aggression by resident males toward intruders (Ferris & Potegal, 1988) and a decrease in aggression between pairs of conspecifics in a neutral arena (Potegal & Ferris, 1990). The ability of AVP to modulate offensive aggression is not limited to the AH. In hamsters, microinjection of AVP into the ventrolateral hypothalamus facilitates offensive aggression (Delville et al., 1995). In castrated rats, infusion of AVP into the amygdala or lateral septum facilitates offensive aggression (Koolhaas et al., 1990; 1991).

The facilitation of offensive aggression by AVP at the level of the AH is inhibited by peripheral treatment with fluoxetine (Ferris et al., 1997). Presumably, the elevation of 5-HT in the AH after fluoxetine (Ferris, 1996) can have its effect by inhibiting AVP release or by antagonizing the action of AVP on its postsynaptic site. There is evidence

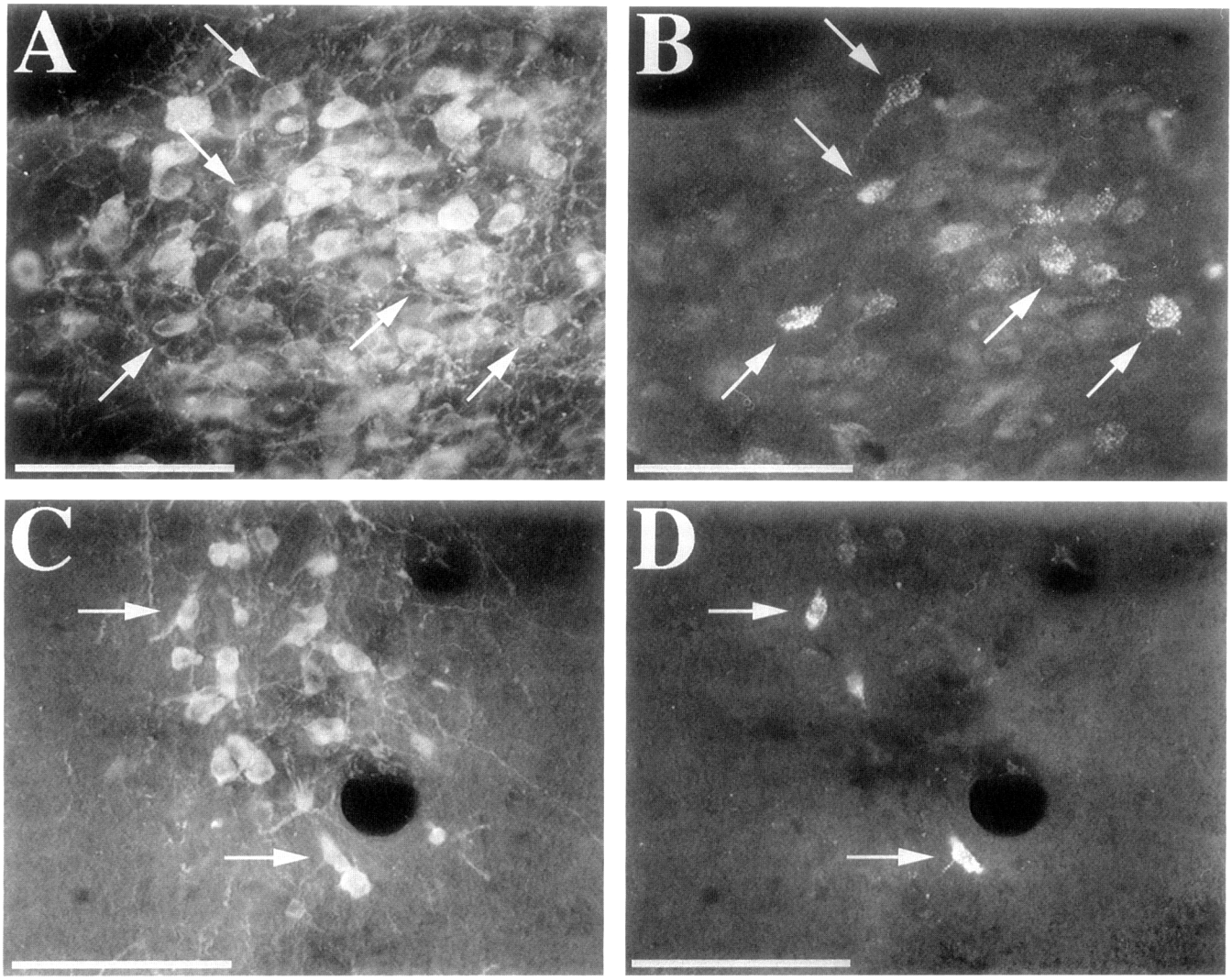

Figure 7. Photomicrograph showing combined labeling of tryptophan hydroxylase-immunoreactive (A and C) and Fluoro-Gold-immunoreactive (B and D) neurons within the dorsal (A and B) and median (C and D) raphe nuclei. Arrows point to cells containing both immunoreactive signals. Scale bars = 100 μm.

from microdialysis of the AH that 5-HT acts directly on AVP neurons to suppress AVP release. Fluoxetine treatment results in a decrease in AVP release within the hypothalamus in hamsters (Ferris, 1996) and rats (Altemus, Cizza, & Gold, 1992). Kia et al. (1996) reported intense immunocytochemical staining for 5-HT_{1A} receptors in the supraoptic nucleus of rats, supporting the notion that activation of 5-HT_{1A} receptors can influence the activity of AVP neurons. However, the present data suggest that 5-HT can also block the activity of AVP after its release in the AH, as evidenced by the dose-dependent diminution of aggression after injections combining AVP and the 5-HT_{1A} receptor agonist DPAT. Enhanced aggression caused by activation of AVP V_{1A} receptors in the AH is suppressed by the simultaneous activation of 5-HT_{1A} receptors in the same site. It is not clear whether a common neuron in the AH shares both receptor subtypes, or whether AVP and 5-HT act on separate neurons in the AH.

Environmental stressors during development can have a pronounced effect on aggressive behavior later in life (Anderson & Mason, 1978; Harlow, Harlow, & Suomi, 1971). It is possible that inappropriate aggressive behavior is caused by stress-induced alterations in the AVP and/or 5-HT systems. Male golden hamsters become very submissive when subjected to the stress of daily threat and attack by dominant conspecifics (Ferris, Axelson, Martin, & Roberge, 1989). This decrease in aggressive behavior is associated with a loss of AVP immunoreactivity in the AH. In a recent study, male hamsters that were stressed during early puberty showed significant changes in AVP and 5-HT levels in the AH as young adults (Delville et al., 1998). These stress-induced neurochemical changes are associated with a context-

dependent change in aggression. Animals with a history of early stress are very aggressive toward smaller conspecifics but are submissive toward animals of the same size. The stress of socially intermixing three strains of male mice over extended periods alters aggressive behavior and 5-HT levels. The most aggressive animals present with the lowest levels of 5-HT in the supraoptic nuclei of the hypothalamus (Serri & Ely, 1984), an area with a high density of AVP neurons. Alterations in AVP and 5-HT may also be associated with inappropriate aggressive behaviors in humans. Impulsive aggressive patients with personality disorder show blunted prolactin release after administration of fenfluoramine (Coccaro, Kavoussi, Hauger, Cooper, & Ferris, 1998; O'Keane et al., 1992). This blunted response to fenfluoramine challenge is suggestive of a hyposensitive 5-HT system. Interestingly, the blunted response to fenfluoramine is also associated with a significant elevation in cerebrospinal fluid levels of AVP (Coccaro et al., 1998). Perhaps the hyposensitive 5-HT system results in enhanced release of AVP, which contributes to impulsive, aggressive behavior.

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Received December 10, 1998
Revision received March 5, 1999
Accepted March 6, 1999 ■