Behavioral Neuroscience
2003, Vol. 117, No. 4, 667–674

Copyright 2003 by the American Psychological Association, Inc.
0735-7044/03/$12.00 DOI: 10.1037/0735-7044.117.4.667

Activation of Serotonergic Neurotransmission During the Performance of Aggressive Behavior in Rats

Bea J. van der Vegt, Natasja Lieuwes, and Esther H. E. M. van de Wall
University of Groningen

Katsunori Kato
University of Tsukuba

Luis Moya-Albiol and Sonia Martínez-Sanchis
Universidad de Valencia

Sietse F. de Boer and Jaap M. Koolhaas
University of Groningen

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

High aggression is often linked to lowered serotonin (5-HT) neurotransmission. Although this may hold for high aggression as a trait characteristic of an individual, serotonergic activity is probably increased during performance of aggressive behavior. To test this hypothesis, first, the 5-HT_{1A} agonist alnespirone and gamma aminobutyric acid-A agonist muscimol were administered into the dorsal raphe nucleus. These treatments, which inhibit 5-HT neuronal activity, were shown to decrease performance of aggressive behavior. Second, after a resident–intruder test, the activation of 5-HT neurons (measured by *c-fos* expression) was increased in high-aggressive rats, compared with low-aggressive rats or control rats that were not subjected to a social confrontation. Results show that performance of aggressive behavior increases 5-HT neuronal activity and that preventing this activation inhibits expression of aggressive behavior.

A close relation between serotonin (5-HT) neurotransmission and aggression has long been recognized. In general, high levels of aggression are associated with low tonic activity of the 5-HT system (Coccaro, 1989; Coccaro & Astill, 1990; Higley et al., 1996; Kavoussi, Armstead, & Coccaro, 1997; Linnoila & Virkkunen, 1992; Mehlman et al., 1994; Popova, Kulikov, Nikulina, Kozlachkova, & Maslova, 1991; Tuinier, Verhoeven, & Van Praag, 1995; Van Praag, 1998; Westergaard, Suomi, Higley, & Mehlman, 1999). Nevertheless, it is important to define high aggression carefully: It may stand for the propensity of an individual to react aggressively in many circumstances (aggression as trait characteristic of an individual); on the other hand, it may refer to the actual performance of aggressive behavior (aggression as a state). In the majority of the studies linking high aggression to low 5-HT, aggression is regarded as trait characteristic.

However, the involvement of 5-HT neurotransmission in controlling the performance of aggressive behavior should be distinguished from the relation between 5-HT and trait aggression. Numerous pharmacological studies have shown that 5-HT_{1A} or 5-HT_{1B} receptor agonist administration reduces aggressive behavior (De Boer, Lesourd, Mocaer, & Koolhaas, 1999; Fish, Faccidomo, & Miczek, 1999; Mos, Olivier, Poth, Van Oorschot, & Van Aken, 1993; Olivier, Mos, Van Oorschot, & Hen, 1995). Receptors of both subtypes exist not only postsynaptically, but also as autoreceptors—the 1A mainly somatodendritic, the 1B at presynaptic terminals. As autoreceptors, they are involved in the negative feedback of the 5-HT system (Bonvento, Scatton, Claustre, & Rouquier, 1992; Evrard et al., 1999; Jolas et al., 1995; Kidd et al., 1993; Pineyro & Blier, 1999). Using S-15535, a 5-HT_{1A} autoreceptor agonist with antagonistic properties for postsynaptic 5-HT_{1A} receptors, De Boer et al. (2000) demonstrated that the antiaggressive effect of 5-HT_{1A} agonists is mediated mainly through autoreceptors. This means that the observed reduction of aggressive behavior is caused by inhibition of serotonergic activity.

The first aim of the present study was to gain further evidence for involvement of somatodendritic 5-HT_{1A} autoreceptors in the antiaggressive action of 5-HT_{1A} agonists. Cell bodies of rostrally projecting 5-HT neurons are located in the dorsal (DR) and median (MR) raphe nuclei. The activity of these neurons can be inhibited by activation of 5-HT_{1A} autoreceptors or gamma aminobutyric acid-A ($GABA_A$) heteroreceptors (Bonvento et al., 1992; Casanovas, Lesourd, & Artigas, 1997; Casanovas, Vilaro, Mengod, & Artigas, 1999; Higgins, Bradbury, Jones, & Oakley, 1988; Jolas et al., 1995; Kidd et al., 1993; Pineyro & Blier, 1999; Tao & Auerbach, 2000; Tao, Ma, & Auerbach, 1996). Therefore, the behavioral effect of local administration of the 5-HT_{1A} agonist alnespirone or the $GABA_A$ agonist muscimol into the DR was studied.

When inhibition of serotonergic transmission reduces aggressive behavior, this suggests that, normally, activation of the 5-HT system is necessary to express aggression. In a second experiment, activation of 5-HT neurons during aggressive behavior was investigated. After a standard resident–intruder (RI) test, *c-fos* expression in 5-HT neurons was examined in rats that showed much

Bea J. van der Vegt, Natasja Lieuwes, Esther H. E. M. van de Wall, Sietse F. de Boer, and Jaap M. Koolhaas, Department of Animal Physiology, University of Groningen, Haren, the Netherlands; Katsunori Kato, Institute of Psychology, University of Tsukuba, Tsukuba, Japan; Luis Moya-Albiol and Sonia Martínez-Sanchis, Area de Psicobiología, Universidad de Valencia, Valencia, Spain.

Correspondence concerning this article should be addressed to Bea J. van der Vegt, Department of Animal Physiology, University of Groningen, Biological Centre, P.O. Box 14, 9750 AA Haren, the Netherlands. E-mail: b.j.van.der.vegt@biol.rug.nl

aggressive behavior during the test (high aggressive), and in rats that were hardly aggressive (low aggressive). Another group of high-aggressive rats that was not given a preceding RI test was used to control for possible basal differences between high- and low-aggressive rats.

Method

Subjects and Housing

For these studies, male Wild type Groningen rats (*Rattus norvegicus*) were used, because these rats exhibit a rich social behavior, including aggressive behavior, as well as a high level of individual variation in genetics, behavior, and physiology. Their ancestors were originally caught in the wild, and the strain has been randomly bred in our laboratory for approximately 20 generations.

The rats had free access to food and water throughout the experiments and were housed in climate-controlled rooms under a 12:12-hr light–dark cycle. All experiments were performed during the dark phase. After weaning, at the age of 23 days, rats were housed in groups of 6 males in clear Plexiglas cages (55 cm long × 35 cm wide × 20 cm high) until they were tested for offensive aggression in a standardized RI paradigm at 4.5 months of age. Each rat was housed in a home cage (80 cm long × 55 cm wide × 40 cm high), together with a sterilized female, to stimulate territorial behavior and prevent social isolation. Body weights of the rats ranged initially from 370 to 450 g, and up to 500 g at the end of the experiments. The experiments were approved by the animal experiments committee of the University of Groningen.

Aggression Tests

After 1 week of habituation to the new home cage, four aggression tests were performed on consecutive days. For each test, the female was removed 30 min in advance, and an unfamiliar male conspecific (Wistar) was introduced into the home cage of the experimental rat. For 3 days, the attack latency time (ALT) was scored, with the test being terminated shortly after occurrence of an attack or after a maximum test duration of 10 min. During the fourth test, the full behavioral profile was recorded for 10 min with The Observer (Noldus Information Technology, Wageningen, the Netherlands, Version 3.0). The following behavioral elements were scored: aggressive behavior (clinch, threat, offensive upright, keep down, chase), social behavior (investigate opponent, sniff in anogenital region, social groom, mount), exploratory behavior (explore environment, rear), immobility, and groom (see Koolhaas, Schuurman, & Wiepkema, 1980, for a more detailed description of agonistic behavior).

Local Administration

For this experiment, rats were selected with a moderate to high level of aggression, that is, they attacked in each of the four tests and exhibited aggressive behavior for at least 30% of the time in the fourth test. Guide cannulas (22-gauge stainless steel cannulas, C313; Plastics One, Roanoke, VA) were implanted under general anesthesia with a halothane/nitrous oxide/oxygen mixture; procaine was administered subcutaneously at the place of incision for local analgesia because of the sensitivity of the skull membranes. The guides were placed stereotactically, according to the brain map of Pellegrino and Cushman (1967; tooth bar + 5 mm), for infusion into the DR nucleus ($n = 16$) at 6.0 mm posterior to bregma and 1.4 mm lateral of the midline, under a 12° lateral angle, and 6.6 mm ventral of the dura mater. For control infusion into the aqueduct (AD; $n = 11$) guides were placed at 6.6 mm posterior to bregma and 1.4 mm lateral of the midline, under a 12° lateral angle, and 5.5 mm ventral of the dura mater (see Figure 1). Increasing the height of the tooth bar and implanting at a lateral angle were necessary to avoid damaging the sinus. Rats were allowed to recover for at least 2 weeks. Each rat was housed in a cage (80 cm long × 55 cm wide × 40 cm high), singly for the first days of recovery, then together with a sterilized female.

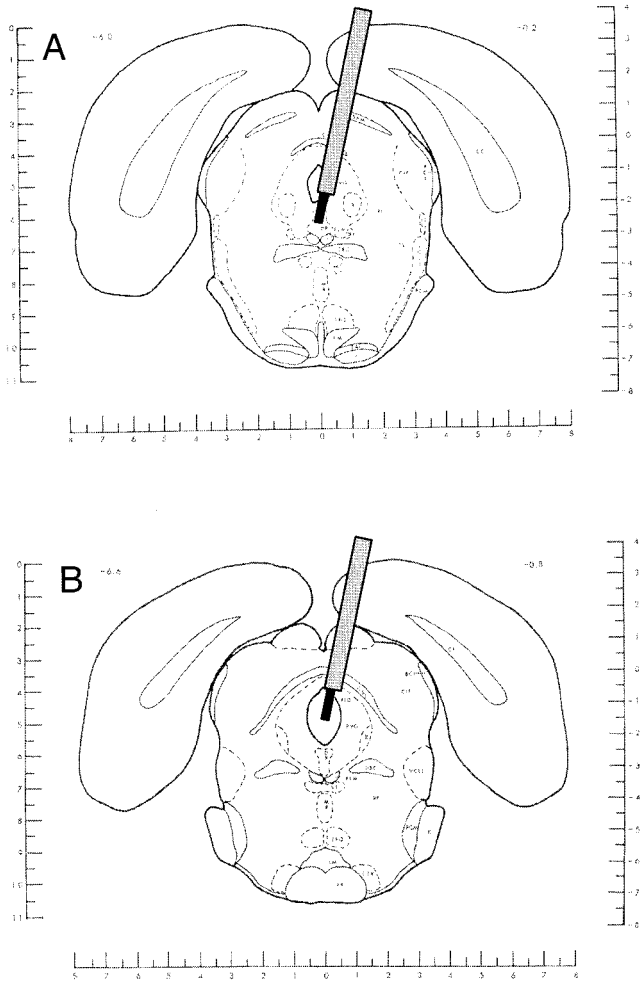

Figure 1. Pictures from the brain map of Pellegrino and Cushman (1967) depicting the location of the cannulas in (A) the dorsal raphe at 6.0 mm posterior to bregma and (B) the aqueduct at 6.6 mm posterior to bregma. From *A Stereotaxic Atlas of the Rat Brain*, by L. J. Pellegrino and A. J. Cushman, Copyright 1967 by Appleton-Century-Crofts. Reprinted with permission of Kluwer Academic.

To determine the effect of local administration of drugs on aggressive behavior, rats were tested twice a week in a standard 10-min RI test, as described in the previous section. In the first test, basal levels of aggression were measured, and in a second test 2 days later, rats received an infusion prior to the aggression test. The rat was taken from the home cage, and an infusion cannula was placed in the guide, protruding below its tip for 1 mm. Through the infusion cannula, connected to a Hamilton (Reno, NV) syringe with a tube (i.d. = 0.28 mm), 0.5 μl fluid was infused in 7 min with an infusion pump. One minute after the end of infusion, the infusion cannula was taken out, the dummy was replaced, and the rat was placed back in its home cage. Fifteen minutes after the start of infusion, an aggression test was performed. The following infusions were given in random order: vehicle (0.5 μl), the 5-HT_{1A} agonist alnespirone (25 μg/0.5 μl), or the $GABA_A$ agonist muscimol (50 ng/0.5 μl).

Alnespirone has been used as a 5-HT_{1A} agonist for its selective antiaggressive effect (De Boer et al., 1999). Doses were chosen on the basis of published results from studies of drug administration into the brain, which showed inhibition of 5-HT transmission by local DR administration of

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

muscimol (1–100 ng; Higgins et al., 1988; Nishikawa & Scatton, 1985) or 8-OH-DPAT (5-HT_{1A} agonist, 0.1–5 μg; Bonvento et al., 1992; Jolas et al., 1995). No data were available on DR administration of alnespirone, but 5-HT transmission can be inhibited by systemic administration of 8-OH-DPAT (0.025–0.300 mg/kg) or alnespirone (0.1–3.0 mg/kg; Casanovas et al., 1997), indicating that a higher dose of alnespirone is required. In a pilot experiment, 5 μg alnespirone was administered into the DR, causing a small, nonsignificant decrease in aggressive behavior (control: 38.86% $\pm$ 2.65% of the time, alnespirone-treated: 33.72% $\pm$ 2.22%) and an almost-significant increase in ALT (control: 64 $\pm$ 9 s, alnespirone-treated: 225 $\pm$ 57 s; *t* test: $p = .07$, $n = 5$). Therefore a 5-fold higher dose of 25 μg was applied in this experiment, which was performed in different cohorts.

Alnespirone ((+)-S-20499-2 hydrochloride; Lot No. 48647, molecular weight 479) was provided by Institut de Recherches Internationales Servier (France). Muscimol (5-aminomethyl-3-hydroxyisoxazole; Lot No. 6/11143, molecular weight 114.10) was obtained from Tocris (Bristol, UK). Both drugs were dissolved in ultrapure water (vehicle solution).

c-fos Expression

In order to compare the effects of a differential amount of aggressive behavior, rats were tested for aggression as described in the section *Aggression Tests*, and high-aggressive and low-aggressive rats were selected (see Results section; $n = 7$ and 6 rats, respectively). They were perfused 2 hr after the start of a standard RI test. A control group of high-aggressive rats ($n = 7$) was perfused without a preceding aggression test. All subjects were housed in the same room and taken in random order. They were perfused under deep anesthesia (1 ml pentobarbital, intraperitoneally) with heparinized saline (10 ml [4000 IU] heparin/1 L saline) for 1 min, followed by 4% (wt/vol) paraformaldehyde in 0.1 M phosphate buffer containing 0.1% magnesium sulfate (pH 7.4). Brains were removed; post-fixed overnight in 4% paraformaldehyde solution at 4 °C; and stored in 0.1 M phosphate-buffered saline (PBS), containing 0.1% azide, at 4 °C. They were cryoprotected by storage in 30% (wt/vol) sucrose solution (0.1 M phosphate buffer + 0.1% azide) for 48–72 hr, and sliced in coronal sections of 30 μm on a cryostat microtome. Free-floating sections were immunostained for 5-HT and *c-fos*.

In order to identify the 5-HT neurons, sections were incubated—after preincubation in 5% normal goat serum (NGS)—with the primary antibody (1:200 rabbit anti-5-HT; Zymed, San Francisco, CA) for 2 hr at room temperature and 2 days at 4 °C, followed by incubation with biotinylated goat anti-rabbit immunoglobulin G (1:200, Zymed) for 2 hr at room temperature—all in 0.01 M PBS (containing 0.05% Tween and 1.00% NGS, and 0.10% azide for periods longer than 1 day), with intermittent rinsing in PBS/Tween. Then, sections were incubated with streptavidin-alkaline phosphatase (1:100, Zymed, in 0.05 M Tris-buffered saline) for 1 hr at room temperature. Subsequently, the sections were colored for a maximum of 30 min in 300 μl of Fuchsin solution (5 g Fuchsin in 100 ml 2 N HCl, 150 μl 4% sodium nitrite, 60 ml 0.05 M Tris buffer, 60 μl 1 M levamisol, and 2 ml naphthol-AS-BI-phosphate solution [18 mg in 2 ml dimethylformamide]; pH 8.0–8.4).

Then sections were stained for *c-fos*: After pretreatment with 0.3% H_2O_2 and preincubation in 5% NGS, sections were first incubated with primary antibody rabbit anti-Fos (1:8000; Santa Cruz Biotechnology, Santa Cruz, CA) for 2 days, followed by a biotinylated goat anti-rabbit immunoglobulin G (1:200, Zymed) for 2 hr, and streptavidin-horseradish peroxidase complex (1:200; Zymed) for 1 hr—all in 0.01 M PBS, with intermittent rinsing, also in 0.01 M PBS. After rinsing in 0.05 M Tris HCl, sections were incubated with nickel-diaminobenzidine for 5 min, and the coloring reaction was started by adding 1% H_2O_2 and stopped by rinsing with Tris HCl after 15 min.

Sections were mounted on slides. *c-fos*-positive cells, 5-HT-positive neurons, and double-labeled neurons in the DR and MR were counted manually within a 1,000 $\times$ 1,000-μm grid at a magnification of 200 in two sections per rat, by an experimenter who was unaware of the subject's identity. The location and relative size of the analyzed areas are indicated in Figure 2.

Statistical Analysis

Data from the local administration experiment were tested with a repeated measures analysis of variance (ANOVA) for the behavioral categories and ALT separately, with test (basal vs. infusion) and treatment (vehicle–alnespirone–muscimol) as within-subject variables and group (DR or AD) as a between-subjects variable. Post hoc pairwise comparisons (least significant difference [LSD] test) were done on the basis of estimated marginal means.

Behavioral data from the *c-fos* expression experiment were analyzed with ANOVA: For confirmation of the intended differences and similarities in aggressiveness between the experimental groups, the behavioral data from the selection tests were statistically analyzed after division. For ALT data, a repeated measures ANOVA was used, with test (1–4) as a within-subject variable and group (high-aggressive–low-aggressive–control) as a between-subjects variable, followed by post hoc pairwise comparisons (LSD). The behavioral categories of the fourth selection test were analyzed separately with group (high-aggressive–low-aggressive–control) as a between-subjects variable, followed by a post hoc LSD test. For analysis of the test before perfusion, a repeated measures ANOVA was used for the ALT (the high- and low-aggressive group as a between-subjects variable and five tests as a within-subject variable), followed by pairwise comparisons; a two-way ANOVA was used for the behavioral categories (two tests as within-subject variable and the high- and low-aggressive group as between-subjects variable). The results of staining were analyzed with a *t* test for independent samples.

For all tests, the software package SPSS was used (Version 10.0.5; SPSS, Chicago, IL); in all cases, differences were regarded as significant at $p < .05$. Data are expressed as means ($\pm$ *SEM*).

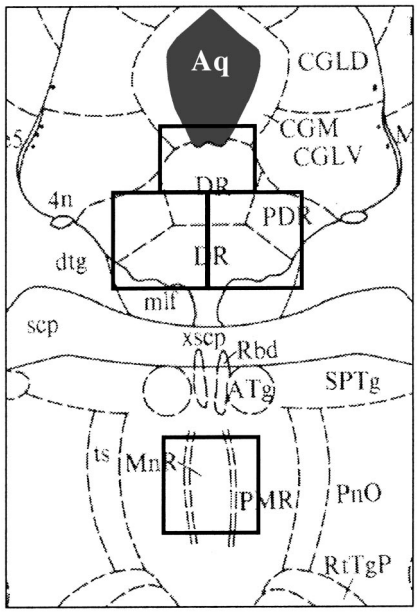

Figure 2. Location and relative size of the areas in which serotonin- and *c-fos*-positive cells were counted in the dorsal raphe nucleus (DR) and median raphe nucleus (MnR), approximately 8 mm posterior to bregma. Reprinted from *The Rat Brain in Stereotaxic Coordinates*, 2nd ed., G. Paxinos and C. Watson, Figure 50, Copyright 1986, with permission from Elsevier.

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

Results

Local Administration

Histological verification of the location of infusion sites showed that in 2 cases, the tip of the DR cannula was not in a proper position. Thus, data from these rats were excluded from the results. In all other cases, cannulas were correctly placed.

Administration of alnespirone or muscimol into the DR lowered aggressive behavior significantly, measured as a decrease in time spent on aggressive behaviors and an increased ALT, whereas vehicle administration did not affect aggressive behavior (see Figure 3). Infusing vehicle or alnespirone into the AD had no effect on aggression, but infusing muscimol lowered aggression in the AD group as well. Analyzing the percentage of time spent on aggressive behavior with ANOVA resulted in a significant test effect, $F(1, 23) = 27.32$, $p < .01$. Pairwise comparisons showed that there were no differences in basal values between groups or between the different treatments. There were differences between the test after infusion compared with the preceding basal test in the DR group for alnespirone ($p = .02$) and muscimol ($p < .01$), and in the AD group for muscimol ($p < .01$). Analysis of the ALT resulted in an effect of test, $F(1, 23) = 8.14$, $p < .01$, and a Test × Treatment interaction, $F(2, 46) = 7.52$, $p < .01$. Pairwise comparisons revealed no differences in basal values between groups or between different treatments, whereas the infusion test differed significantly from the basal test after alnespirone ($p = .03$) and muscimol ($p = .02$) administration into the DR, and after muscimol ($p = .03$) administration into the AD.

In Figure 4, the effect of drug administration on all behavioral categories is shown. Data were analyzed per category. For social behavior, there was a main effect of test, $F(1, 23) = 10.16$, $p < .01$, and a Test × Treatment interaction, $F(2, 46) = 3.94$, $p = .03$. There were no differences in basal values between groups or between different treatments; however, the infusion test differed significantly from the basal test after alnespirone ($p = .006$) and muscimol ($p = .012$) administration in the AD group. For exploratory behavior, main effects of test, $F(1, 23) = 11.28$, $p < .01$; group, $F(1, 23) = 7.11$, $p = .01$; and a Test × Group interaction, $F(1, 23) = 7.04$, $p = .01$, were found. The basal values differed only for the vehicle test between the groups ($p < .05$); values after the infusions also differed between groups (vehicle: $p = .03$, alnespirone: $p < .01$, muscimol: $p < .05$), and the infusion test was significantly different from the basal test after muscimol infusion into the DR ($p < .01$). For immobility, no main effects were found. For grooming, there was a test effect, $F(1, 23) = 4.62$, $p < .05$. There were no differences between basal values, but muscimol administration in the DR caused a difference in percentage of time spent grooming compared with the basal test ($p = .03$).

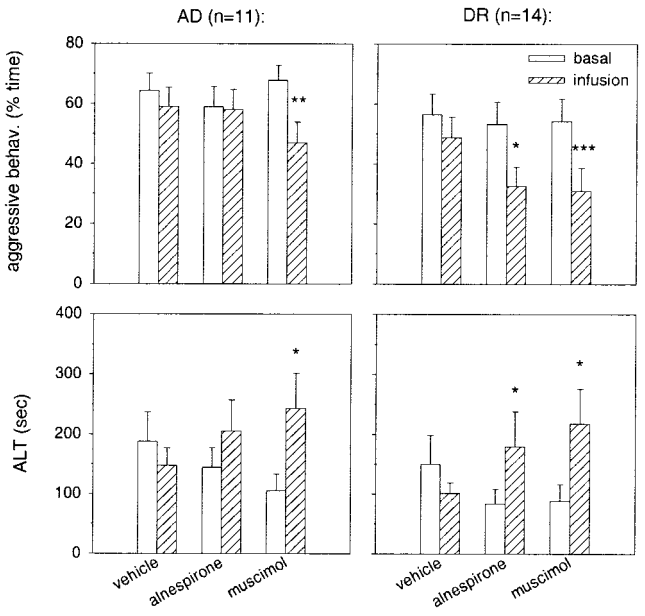

Figure 3. Effect of administration of drugs into the dorsal raphe (DR) or aqueduct (AD; as control) on aggressive behavior (behav.), measured as the mean (± *SEM*) percentage of the time spent on aggressive behavior and attack latency time (ALT). Least significant difference test: * $p < .05$, ** $p < .01$, *** $p < .001$.

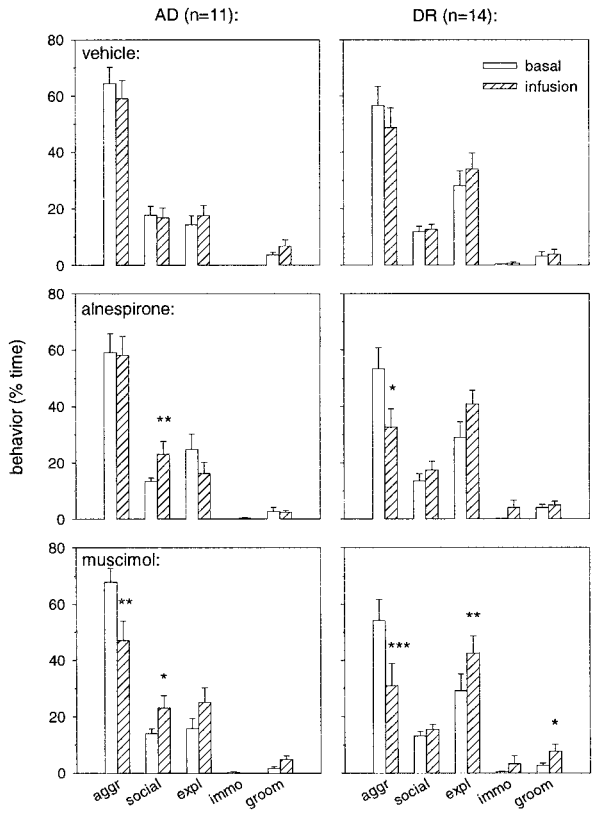

Figure 4. Effect of administration of drugs into the dorsal raphe (DR) or aqueduct (AD; as control) on the total behavioral profile, measured as the mean (± *SEM*) percentage of time spent in the following behaviors: aggressive behavior (aggr), social behavior (social), exploratory behavior (expl), immobility (immo), and grooming (groom). Least significant difference test: * $p < .05$, ** $p < .01$, *** $p < .001$.

c-fos Expression

Rats were selected for this experiment on the basis of the behavior during four consecutive RI tests. High- and low-aggressive rats were selected, and Figure 5 shows the ALTs of the different groups, as well as the full behavioral profile during the

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

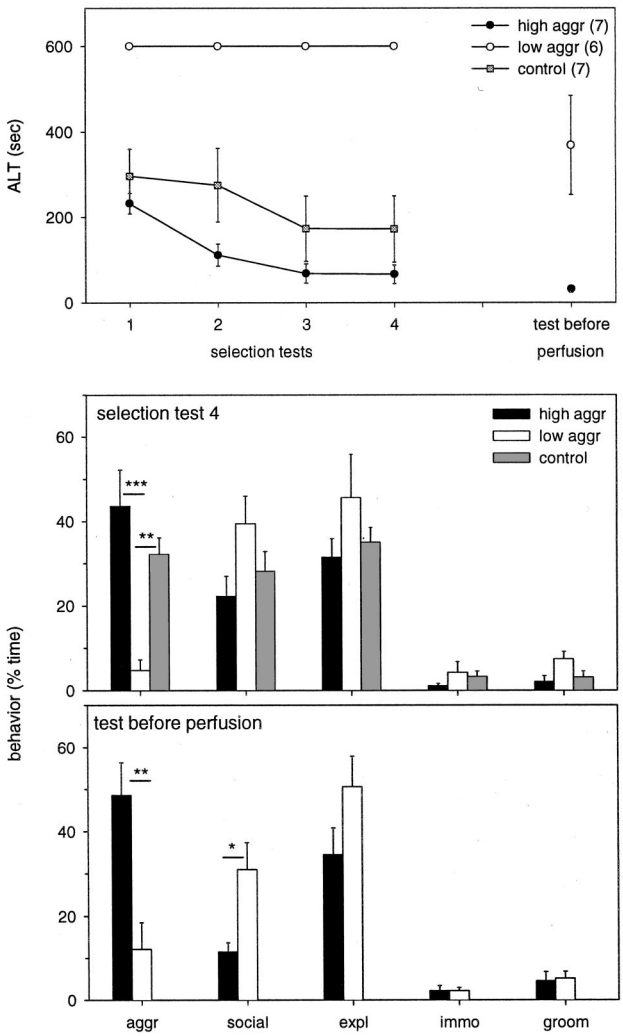

Figure 5. Behavior of the high-aggressive (aggr), low-aggressive, and control rats in the *c-fos* expression experiment. On the basis of the attack latency time (ALT) in four consecutive resident–intruder tests and the full behavioral profile during the fourth test, rats were selected for the different groups. The behaviors and ALT during the test before perfusion are also shown for the high- and low-aggressive rats. The observed behavioral elements were classified in the following categories: aggressive behavior (aggr), social behavior (social), exploratory behavior (expl), immobility (immo), and grooming (groom). Data are means (± *SEM*); numbers in parentheses are the number of rats in each group. Least significant difference test: * $p < .05$, ** $p < .01$, *** $p < .001$.

fourth test. Low-aggressive rats did not attack and showed only very little aggressive behavior (4.79% ± 2.53% of the time). The high-aggressive and control rats, on the other hand, did attack, had lower ALTs, and were much more aggressive (43.60% ± 8.61% and 32.17% ± 3.97% of the time, respectively). Analysis of the ALT results with a repeated measures ANOVA resulted in a significant effect of test, $F(3, 51) = 14.17$, $p < .01$; group, $F(1, 17) = 146.76$, $p < .01$; and a Test × Group interaction, $F(6, 51) = 4.34$, $p < .01$. Post hoc pairwise comparisons showed that the low-aggressive rats differed significantly from the high-aggressive and control rats in all four tests ($p < .01$), and that only in Test 2 did the high-aggressive rats differ from the control rats ($p < .05$). During the fourth test, there was a difference between groups only for aggressive behavior: ANOVA, significant effect of group, $F(2, 17) = 11.38$, $p < .01$. The amount of aggressive behavior of the low-aggressive rats was less than that of the high-aggressive and control rats (post hoc LSD test: ps $< .01$), whereas the last two groups did not differ significantly.

Before perfusion, only the high- and low-aggressive rats were tested again in an RI test. Although some of the low-aggressive rats did attack, there was still a very clear difference in the level of aggression between the groups: The ALT was longer in the low-aggressive group, $p < .01$ in the pairwise comparison after ANOVA, with an effect of test, $F(4, 44) = 10.04$, $p < .01$; group, $F(1, 11) = 250.32$, $p < .01$; and a Test × Group interaction, $F(4, 44) = 3.49$, $p = .02$. The low-aggressive group also spent a smaller amount of time on aggressive behavior, $p < .01$ in the pairwise comparison after ANOVA, with a significant effect of group, $F(1, 11) = 17.37$, $p < .01$, but a larger amount on social behavior, $p = .01$, after a group effect in the ANOVA, $F(1, 11) = 5.10$, $p < .05$. There were no differences in behavior found within groups between the test before perfusion and the fourth selection test. Only the ALT in the low-aggressive group was decreased ($p = .01$).

The total number of 5-HT neurons within the analyzed areas (Figure 2), the percentage of these 5-HT neurons expressing *c-fos*, and the number of non-5-HT cells expressing *c-fos* are shown in Figure 6. Within the DR and the MR, there were no differences between groups in the number of 5-HT neurons. A higher percentage of these neurons was activated in the high-aggressive rats after the RI test: in the DR compared with low-aggressive or control rats (*t* test: $p < .01$ and $p = .01$, respectively), and in the MR compared with the low-aggressive rats (*t* test: $p < .05$). There were no significant differences in the number of non-5-HT neurons expressing *c-fos*.

Discussion

The main results of this study are, first, that aggressive behavior is inhibited by administration of a 5-HT_{1A} agonist or $GABA_A$ agonist in the DR; and second, that there is an increased activation of 5-HT neurons during aggressive behavior.

The aim of the first experiment was to pharmacologically inhibit 5-HT neuronal activity and to examine the influence on (aggressive) behavior. For this purpose, the 5-HT_{1A} agonist alnespirone was given within the DR to activate specifically 5-HT_{1A} somatodendritic autoreceptors. This results in an inhibition of 5-HT neurons (Bonvento et al., 1992; Casanovas et al., 1997, 1999; Higgins et al., 1988; Jolas et al., 1995; Kidd et al., 1993; Pineyro & Blier, 1999; Tao & Auerbach, 2000). The activity of 5-HT neurons is controlled by $GABA_A$ heteroreceptors as well (Higgins et al., 1988; Pineyro & Blier, 1999; Nishikawa & Scatton, 1985; Tao & Auerbach, 2000; Tao et al., 1996). The $GABA_A$ agonist muscimol was also administered to reach the same physiological effect via different pharmacological routes. Both treatments resulted in a decrease in aggressive behavior, measured as an increased ALT and a decreased percentage of the time the rats spent in aggressive behaviors.

The effects on behavior of locally administered drugs are specific: The reduction in aggressive behavior was compensated for by an increase in other behavioral elements, like social or exploratory behaviors, without an increase in immobility. And because

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

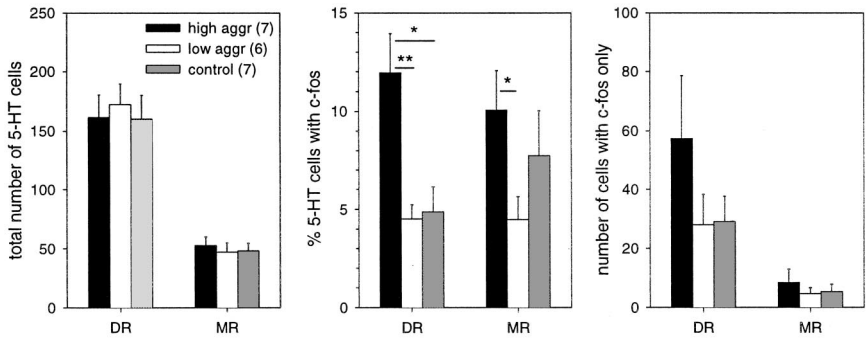

Figure 6. Total number of serotonin (5-HT) neurons, percentage of these 5-HT neurons expressing *c-fos*, and number of non-5-HT neurons expressing *c-fos* in the dorsal raphe (DR) and medial raphe (MR) of high-aggressive (aggr), low-aggressive, and control rats. Data are means (± *SEM*); numbers in parentheses are the number of rats in each group. Independent sample *t* test: * $p < .05$, ** $p < .01$.

the vehicle administration had no influence, we may conclude that the procedure itself did not affect behavior. Another factor to keep in mind when interpreting results of local administration experiments is that the effects of drugs may be mediated in brain areas other than where they are administered, for instance, by diffusion of the drug through the brain tissue (Bonvento et al., 1992; Jolas et al., 1995). In the control group of this experiment, drugs were administered into the AD, close to the location of the DR. Via the AD, the drugs could easily disperse, and this was the most likely route for accidental leakage from the DR. If spreading of the drug had taken place in the experimental group, and the observed effects consequently been mediated by another brain region, then we would have expected to see the same effects in the control group. However, alnespirone inhibited aggressive behavior when applied within the DR, but not in the AD. From this we may conclude that the mechanism by which alnespirone exerts its antiaggressive effect is the 5-HT_{1A} autoreceptor. Muscimol had an antiaggressive effect as well, not only when given within the DR, but also when given in the AD. Compared with the results of alnespirone administration, it is likely that in the DR group the infused drugs acted within this nucleus. Moreover, $GABA_A$ receptors are found on the cell bodies and dendrites of 5-HT neurons in the raphe nuclei (Gao, Fritschy, Benke, & Mohler, 1993), but none, or only very few, are found on 5-HT terminals (Mennini, Gobbi, & Romandini, 1986). Tao et al. (1996, 2000) showed that administration of the $GABA_A$ agonist muscimol within the DR caused a diminished release of 5-HT, whereas administration in a 5-HT target area had no effect on the release. The authors concluded that GABA has a tonic inhibitory control function on the 5-HT system via somatodendritic $GABA_A$ heteroreceptors. So, although alternatives cannot fully be excluded, it is likely that the behavioral effects of muscimol administered within the DR are (at least partly) mediated by activation of $GABA_A$ receptors on 5-HT neurons. One possible explanation for the action of muscimol administered into the AD is that the same behavioral effect is attained via other, non-5-HT neuronal pathways, as the GABA-ergic system is a very widespread inhibitory system in the central nervous system. Depaulis and Vergnes (1983) found increased aggression after intracerebroventricular administration of a $GABA_A$ agonist and decreased aggression after administration of an antagonist. This indicates a complex relationship between aggressive behavior and the GABA-ergic system, partly via the 5-HT system, partly apart from it. Altogether, we can conclude that inhibition of 5-HT neuronal activity, via differential pharmacological routes, causes a decrease in aggressive behavior.

The second experiment aimed to test whether there is an activation of 5-HT neurons during the performance of aggressive behavior. The results demonstrate that after a confrontation with an intruder rat in a standardized RI test, there was a stronger activation in the rats that showed a large amount of aggressive behavior, compared with those that showed only a little aggressive behavior. An increased *c-fos* expression could be the result of behavioral activation in general. The low-aggressive rats were also behaviorally activated during the test, as can be seen in high scores for social and exploratory behaviors, but were less aggressive. It is impossible to match the groups for an exactly equal level of activity or to use a control group solely for physical activity. The influence of physical effort involved in the performance of aggressive behavior on enhanced *c-fos* expression cannot be excluded, and may even be very likely to have contributed (Jacobs & Fornal, 1999). However, we may conclude that it is the performance of aggressive behavior that is associated with increased *c-fos* expression in 5-HT neurons.

In order to obtain a differential level of aggression in the test before perfusion, we had to select high- and low-aggressive rats beforehand. To exclude a bias of a basal difference between these rats, a control group of high-aggressive rats was used, which was not confronted with an intruder. The difference between high-aggressive rats with and without a preceding RI test demonstrates that it is the performance of aggressive behavior that caused the increased *c-fos* expression. Differences between groups were most prominent in the DR, compared with the MR. This may have been caused by the higher number of 5-HT neurons counted in the DR, which makes differences in percentages clearer. There may also be a differential involvement of DR or MR 5-HT neurons in the regulation of aggressive behavior: In general, the involvement of 5-HT neurons within the DR is widely accepted, whereas the precise role of MR 5-HT neurons is less clear. Further investigations will be needed to elucidate these differences.

Although in the present experiment no controls for cross-reactivity between the *c-fos* and 5-HT antisera were conducted, it is unlikely that this could have seriously distorted the current observations: The 5-HT cells and nuclei expressing *c-fos* were counted by one investigator who was unaware of the identity of the

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

subjects. The results, as presented in Figure 6, indicate there was a clear staining of 5-HT neurons with or without *c-fos*. And the percentage of activated 5-HT neurons in this experiment (maximum = 12%) corresponds to the findings of Delville, De Vries, and Ferris (2000), who studied 5-HT neuronal activation in relation to agonistic behavior in hamsters.

The finding of decreased aggression after local drug application is in line with other studies describing administration of 5-HT_{1A} or 5-HT_{1B} agonists, systemically (De Almeida, Nikulina, Faccidomo, Fish, & Miczek, 2001; De Boer et al., 1999, 2000; Fish et al., 1999; Olivier et al., 1995; Sijbesma et al., 1991), locally into the DR (Mos et al., 1993), or intracerebroventricularly (De Almeida & Lucion, 1994; Mos, Olivier, Poth, & Van Aken, 1992). In most cases, this is explained as being mediated by postsynaptic receptors, for example, based on studies with neurotoxic destruction of 5-HT neurons (De Almeida et al., 2001; Sijbesma et al., 1991). However, this manipulation is nonspecific, and data are difficult to interpret. In general, it is hard to distinguish autoreceptor- and postsynaptic receptor-mediated processes in an experimental approach. A complicating side effect of administration of many 5-HT_1 agonists is their sedative effect and induction of the so-called "5-HT behavioral syndrome" (De Boer et al., 1999; Higgins & Elliott, 1991; Higgins et al., 1988; Olivier et al., 1995). Mos et al.'s (1993) conclusion that the antiaggressive effect of intraraphe administration of 5-HT_1 agonists is mediated by the autoreceptors also reflects a nonspecific reduction in aggression. We used the 5-HT_{1A} agonist alnespirone for its selective antiaggressive effect; that is, it does not induce immobility (De Boer et al., 1999). In this experiment, the reduction in aggressive behavior was not accompanied by an enhanced immobility but was compensated for by increased social and nonsocial exploration. From these data and previous findings (De Boer et al., 2000), we may conclude that the selective antiaggressive effect is mediated by 5-HT_{1A} autoreceptors, and thereby, inhibition of 5-HT neurons.

This is in agreement with the observed activation of 5-HT neurons during the performance of aggressive behavior. Other studies also describe an increased *c-fos* mRNA expression in the DR after an aggressive encounter (Kollack-Walker, Watson, & Akil, 1997; Stork, Welzl, Cremer, & Schachner, 1997). And Delville et al. (2000) reported an enhanced activation of 5-HT neurons after a fight, although they explain this finding in terms of physical activity, because 5-HT is thought to inhibit aggression. This neuronal activation does not seem to be specific for offensive aggressive behavior; the same circuitry (or parts of it) may also be activated with the performance of other behaviors. Nevertheless, all results suggest an activation of 5-HT neurons. Direct measurement of changes in neurotransmission related to behavior has been attempted. The results of Van Erp and Miczek (2000)—measured by microdialysis—of a decreased 5-HT level in the prefrontal cortex following an aggression test seem to contradict our results. Unfortunately, this technique is also restricted in its limited time resolution, as already mentioned by the authors. Changes in neurotransmission related to behavior probably occur in a very short time span, of seconds rather than minutes (Jacobs & Fornal, 1999). It may therefore not be justified to match neurotransmitter levels measured by microdialysis directly to anticipation, initiation, execution, or termination of specific behaviors. The *c-fos* expression studies alone do not allow a differentiation between the performance of behavior or the recovery, as brains are studied 1–2 hr after the behavior has taken place. A combination of these results leads to the hypothesis of a short-lasting activation of 5-HT neurotransmission during the initiation and/or performance of aggressive behavior, followed by an inhibition.

Previous experiments in our laboratory with high- and low-aggressive mice and rats demonstrated an increased sensitivity of postsynaptic 5-HT_{1A} receptors in subjects with a high trait aggression, compared to those with a low trait aggression. This could be due to a tonically lower 5-HT transmission in these high-aggressive animals, in line with the general view on 5-HT and aggression as mentioned in the introduction (Van der Vegt et al., 2001). On the other hand, the present results make clear that this cannot automatically be extrapolated to the involvement of 5-HT transmission in aggressive behavior.

In summary, the results of both experiments described in this study demonstrate that with performance of aggressive behavior, 5-HT neuronal activity is increased, and that preventing this activation inhibits the performance of aggressive behavior. This leads to the conclusion that, although high trait aggression may be related to a diminished functioning of the central 5-HT system, the overt expression of aggressive behavior is accompanied by an increase in activity of this system.

References

Bonvento, G., Scatton, B., Claustre, Y., & Rouquier, L. (1992). Effect of local injection of 8-OH-DPAT into the dorsal or median raphe nuclei on extracellular levels of serotonin in serotonergic projection areas in the rat brain. *Neuroscience Letters, 137,* 101–104.

Casanovas, J. M., Lesourd, M., & Artigas, F. (1997). The effect of the selective 5-HT1A agonists alnespirone (S-20499) and 8-OH-DPAT on extracellular 5-hydroxytryptamine in different regions of rat brain. *British Journal of Pharmacology, 122,* 733–741.

Casanovas, J. M., Vilaro, M. T., Mengod, G., & Artigas, F. (1999). Differential regulation of somatodendritic serotonin 5-HT1A receptors by 2-week treatments with the selective agonists alnespirone (S-20499) and 8-hydroxy-2-(di-n-propylamino)tetralin: Microdialysis and autoradiographic studies in rat brain. *Journal of Neurochemistry, 72,* 262–272.

Coccaro, E. F. (1989). Central serotonin and impulsive aggression. *British Journal of Psychiatry* (Suppl.), 52–62.

Coccaro, E. F., & Astill, J. L. (1990). Central serotonergic function in parasuicide. *Progress in Neuropsychopharmacology and Biological Psychiatry, 14,* 663–674.

De Almeida, R. M. M., & Lucion, A. B. (1994). Effects of intracerebroventricular administration of 5-HT receptor agonists on the maternal aggression of rats. *European Journal of Pharmacology, 264,* 445–448.

De Almeida, R. M. M., Nikulina, E. M., Faccidomo, S., Fish, E. W., & Miczek, K. A. (2001). Zolmitriptan, a 5-$HT_{1B/D}$ agonist, alcohol, and aggression in mice. *Psychopharmacology, 157,* 131–141.

De Boer, S. F., Lesourd, M., Mocaer, E., & Koolhaas, J. M. (1999). Selective antiaggressive effects of alnespirone in resident-intruder test are mediated via 5-hydroxytryptamine1A receptors: A comparative pharmacological study with 8-hydroxy-2-dipropylaminotetralin, ipsapirone, buspirone, eltoprazine, and WAY-100635. *Journal of Pharmacology and Experimental Therapeutics, 288,* 1125–1133.

De Boer, S. F., Lesourd, M., Mocaer, E., & Koolhaas, J. M. (2000). Somatodendritic 5-HT(1A) autoreceptors mediate the antiaggressive actions of 5-HT(1A) receptor agonists in rats: An ethopharmacological study with S-15535, alnespirone, and WAY-100635. *Neuropsychopharmacology, 23,* 20–33.

Delville, Y., De Vries, G. J., & Ferris, C. F. (2000). Neural connections of the anterior hypothalamus and agonistic behavior in golden hamsters. *Brain Behavior and Evolution, 55,* 53–76.

Depaulis, A., & Vergnes, M. (1983). Induction of mouse-killing in the rat

by intraventricular injection of a GABA-agonist. *Physiology & Behavior, 30,* 383–388.

Evrard, A., Laporte, A. M., Chastanet, M., Hen, R., Hamon, M., & Adrien, J. (1999). 5-HT1A and 5-HT1B receptors control the firing of serotoninergic neurons in the dorsal raphe nucleus of the mouse: Studies in 5-HT1B knock-out mice. *European Journal of Neuroscience, 11,* 3823–3831.

Fish, E. W., Faccidomo, S., & Miczek, K. A. (1999). Aggression heightened by alcohol or social instigation in mice: Reduction by the 5-HT(1B) receptor agonist CP-94,253. *Psychopharmacology (Berlin), 146,* 391–399.

Gao, B., Fritschy, J. M., Benke, D., & Mohler, H. (1993). Neuron-specific expression of GABAA-receptor subtypes: Differential association of the alpha 1- and alpha 3-subunits with serotonergic and GABAergic neurons. *Neuroscience, 54,* 881–892.

Higgins, G. A., Bradbury, A. J., Jones, B. J., & Oakley, N. R. (1988). Behavioural and biochemical consequences following activation of 5HT1-like and GABA receptors in the dorsal raphe nucleus of the rat. *Neuropharmacology, 27,* 993–1001.

Higgins, G. A., & Elliott, P. J. (1991). Differential behavioural activation following intra-raphe infusion of 5-HT1A receptor agonists. *European Journal of Pharmacology, 193,* 351–356.

Higley, J. D., King, S. T., Hasert, M. F., Champoux, M., Suomi, S. J., & Linnoila, M. (1996). Stability of interindividual differences in serotonin function and its relationship to severe aggression and competent social behavior in rhesus macaque females. *Neuropsychopharmacology, 14,* 67–77.

Jacobs, B. L., & Fornal, C. A. (1999). Activity of serotonergic neurons in behaving animals. *Neuropsychopharmacology, 21,* 9S–15S.

Jolas, T., Schreiber, R., Laporte, A. M., Chastanet, M., De Vry, J., Glaser, T., et al. (1995). Are postsynaptic 5-HT1a receptors involved in the anxiolytic effects of 5-HT1a receptor agonists and in their inhibitory effects on the firing of serotonergic neurons in the rat? *Journal of Pharmacology and Experimental Therapeutics, 272,* 920–929.

Kavoussi, R., Armstead, P., & Coccaro, E. (1997). The neurobiology of impulsive aggression. *Psychiatric Clinics of North America, 20,* 395–403.

Kidd, E. J., Haj-Dahmane, S., Jolas, T., Lanfumey, L., Fattaccini, C. M., Guardiola-Lemaitre, B., et al. (1993). New methoxy-chroman derivatives, 4[N-(5-methoxy-chroman-3-yl)N-propylamino]butyl-8-azaspiro-(4,5)-decane-7,9-dione [(+/−)-S 20244] and its enantiomers, (+)-S 20499 and (−)-S 20500, with potent agonist properties at central 5-hydroxytryptamine1A receptors. *Journal of Pharmacology and Experimental Therapeutics, 264,* 863–872.

Kollack-Walker, S., Watson, S. J., & Akil, H. (1997). Social stress in hamsters: Defeat activates specific neurocircuits within the brain. *Journal of Neuroscience, 17,* 8842–8855.

Koolhaas, J. M., Schuurman, T., & Wiepkema, P. R. (1980). The organization of intraspecific agonistic behaviour in the rat. *Progress in Neurobiology, 15,* 247–268.

Linnoila, V. M., & Virkkunen, M. (1992) Aggression, suicidality, and serotonin. *Journal of Clinical Psychiatry,* 53(Suppl. 10), 46–51.

Mehlman, P. T., Higley, J. D., Faucher, I., Lilly, A. A., Taub, D. M., Vickers, J., et al. (1994). Low CSF 5-HIAA concentrations and severe aggression and impaired impulse control in nonhuman primates. *American Journal of Psychiatry, 151,* 1485–1491.

Mennini, T., Gobbi, M., & Romandini, S. (1986). Localization of GABAA and GABAB receptor subtypes on serotonergic neurons. *Brain Research, 371,* 372–375.

Mos, J., Olivier, B., Poth, M., & van Aken, H. (1992). The effects of intraventricular administration of eltoprazine, 1-(3-trifluoromethylphenyl)piperazine hydrochloride and 8-hydroxy-2-(di-n-propylamino)-tetralin on resident intruder aggression in the rat. *European Journal of Pharmacology, 212,* 295–298.

Mos, J., Olivier, B., Poth, M., Van Oorschot, R., & Van Aken, H. (1993). The effects of dorsal raphe administration of eltoprazine, TFMPP and 8-OH-DPAT on resident intruder aggression in the rat. *European Journal of Pharmacology, 238,* 411–415.

Nishikawa, T., & Scatton, B. (1985). Inhibitory influence of GABA on central serotonergic transmission. Raphé nuclei as the neuroanatomical site of the GABAergic inhibition of cerebral serotonergic neurons. *Brain Research, 331,* 91–103.

Olivier, B., Mos, J., Van Oorschot, R., & Hen, R. (1995). Serotonin receptors and animal models of aggressive behavior. *Pharmacopsychiatry, 28*(Suppl. 2), 80–90.

Paxinos, G., & Watson, C. (1986). *The rat brain in stereotaxic coordinates* (2nd ed.). North Ryde, Australia: Academic Press.

Pellegrino, L. J., & Cushman, A. J. (1967). *A stereotaxic atlas of the rat brain.* New York: Appleton-Century-Crofts.

Pineyro, G., & Blier, P. (1999). Autoregulation of serotonin neurons: Role in antidepressant drug action. *Pharmacology Review, 51,* 533–591.

Popova, N. K., Kulikov, A. V., Nikulina, E. M., Kozlachkova, E. Y., & Maslova, G. B. (1991). Serotonin metabolism and serotonergic receptors in Norway rats selected for low-aggressiveness to man. *Aggressive Behavior, 17,* 207–213.

Sijbesma, H., Schipper, J., De Kloet, E. R., Mos, J., Van Aken, H., & Olivier, B. (1991). Postsynaptic 5-HT1 receptors and offensive aggression in rats: A combined behavioural and autoradiographic study with eltoprazine. *Pharmacology Biochemistry and Behavior, 38,* 447–458.

Stork, O., Welzl, H., Cremer, H., & Schachner, M. (1997). Increased intermale aggression and neuroendocrine response in mice deficient for the neural cell adhesion molecule (NCAM). *European Journal of Neuroscience, 9,* 1117–1125.

Tao, R., & Auerbach, S. B. (2000). Regulation of serotonin release by GABA and excitatory amino acids. *Journal of Psychopharmacology, 14,* 100–113.

Tao, R., Ma, Z., & Auerbach, S. B. (1996). Differential regulation of 5-hydroxytryptamine release by GABAA and GABAB receptors in midbrain raphe nuclei and forebrain of rats. *British Journal of Pharmacology, 119,* 1375–1384.

Tuinier, S., Verhoeven, W. M., & Van Praag, H. M. (1995). Cerebrospinal fluid 5-hydroxyindolacetic acid and aggression: A critical reappraisal of the clinical data. *International Clinical Psychopharmacology, 10,* 147–156.

Van der Vegt, B. J., De Boer, S. F., Buwalda, B., De Ruiter, A. J., De Jong, J. G., & Koolhaas, J. M. (2001). Enhanced sensitivity of postsynaptic serotonin-1A receptors in rats and mice with high trait aggression. *Physiology & Behavior, 74,* 205–211.

Van Erp, A. M., & Miczek, K. A. (2000). Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. *Journal of Neuroscience, 20,* 9320–9325.

Van Praag, H. M. (1998). Anxiety and increased aggression as pacemakers of depression. *Acta Psychiatrica Scandinavica, 393*(Suppl. 393), 81–88.

Westergaard, G. C., Suomi, S. J., Higley, J. D., & Mehlman, P. T. (1999). CSF 5-HIAA and aggression in female macaque monkeys: Species and interindividual differences. *Psychopharmacology (Berlin), 146,* 440–446.

Received July 25, 2002
Revision received December 18, 2002
Accepted February 12, 2003 ■

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.