SEROTONERGIC DRUGS AND AGGRESSION IN MALE FIREMOUTH CICHLIDS (*CICHLASOMA MEEKI*)

by

C. F. ADAMS

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Department of Zoology

The University of British Columbia
Vancouver, Canada

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ABSTRACT

The objective of this thesis was to examine the role of brain 5-HT in the regulation of mirror-directed biting in male firemouth cichlids (*Cichlasoma meeki*). In experiment 1, treatment with the 5-HT synthesis inhibitor DL-p-chlorophenylalanine methyl ester HCl (PCPA) resulted in a significant increase in mirror-directed biting. In experiment 2, the 5-HT_{1A} agonist (±)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronapthalene HBr (8-OH-DPAT) had no effect on aggression. In experiment 3, the 5-HT_{1A} agonist 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione HCl (buspirone) caused a significant reduction in aggression. In experiment 4, the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) caused a significant reduction in mirror-directed aggression. These findings demonstrate that brain 5-HT inhibits mirror-directed aggression in male firemouth cichlids.

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INTRODUCTION

Brain serotonergic neurons modulate a variety of physiological and behavioral processes (reviewed in Jacobs, 1987). For example, subjective experiences such as anxiety (reviewed in Broekkamp and Jenck, 1989), as well as overt behaviors such as feeding (reviewed in Samanin, 1989) and sex (male behavior reviewed in Ahlenius and Larsson, 1989; female behavior reviewed in Mendelson and Gorzalka, 1989) are all modulated by brain serotonin.

Lagerspetz *et al* (1968) published the first quantitative data suggesting that brain serotonin inhibits intraspecific aggression. These researchers bred aggressive and non-aggressive strains of albino mice, and found that the aggressive mice had a significantly lower mean telencephalic serotonin content as compared to non-aggressive mice. Since this finding, numerous studies have assessed the role of serotonergic neurons in intraspecific aggression, and, as will be made clear below, there is ample evidence that brain serotonergic activity is associated with inhibiting intraspecific agonistic behavior in mammals.

The brain 5-HT system

The neurotransmitter 5-hydroxytryptamine (5-HT), also referred to as serotonin, was first found in the central nervous system (CNS) of mammals by Twarog and Page (1953). Dahlström and Fuxe (1964) demonstrated that the cell bodies of virtually all 5-HT containing neurons in the rat are located in the medulla oblongata, pons and mesencephalon. Two of these cell groups, the nucleus raphe dorsalis and nucleus raphe medianus, send axons to the diencephalon and telencephalon (Fuxe, 1965; Andén *et al*, 1966).

The effect of 5-HT on forebrain neurons is determined to a large degree by the type of receptor that it binds with post-synaptically. Some receptors are rapidly acting ligand-gated ion channels, while others are G-protein linked. When 5-HT binds to G-protein linked receptors, it can have opposite effects, depending on whether the receptor subtype inhibits or stimulates its second messenger system. Furthermore, the different second messenger systems each exert

their own specific physiological effects (Hoyer *et al*, 1994). In other words, the same neurotransmitter can have very different effects, depending on the type of receptor that it binds to post-synaptically. Thus, the use of receptor specific drugs can provide a greater level of physiological detail on how 5-HT modulates behavior.

As will be made clear below, the various 5-HT receptor subtypes tend to occur in different brain regions. This means that receptor specific drugs can also help identify specific brain areas where a behavioral effect of 5-HT may be occurring.

Since Peroutka and Snyder (1979) first demonstrated the existence of two different receptors for serotonin, a plethora of 5-HT receptor subtypes have been identified in the CNS. These include the 5-HT_1 family (5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D}), which inhibit adenylate cyclase activity; the 5-HT_2 family (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}), which stimulate phosphatidyl inositol metabolism; the 5-HT_3 ligand-gated cation channel; and the 5-HT_4 receptor, which stimulates adenylate cyclase activity (all reviewed in Hoyer *et al*, 1994). Cloned receptors include the 5-ht_{1E}, 5-ht_{1F}, 5-ht_{5A}, 5-ht_{5B}, 5-ht_6 and 5-ht_7, and it has been proposed that these receptors retain the lowercase format until they are actually identified in whole tissue (Hoyer *et al*, 1994).

Relevant to this thesis are the 5-HT_{1A} and the 5-HT_2 family of receptor subtypes, which are discussed more fully in the following paragraphs.

The 5-HT_{1A} receptor was first identified in rat brain with tritiated 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) by Gozlan *et al* (1983). Using [^{3}H]-8-OH-DPAT, the distribution of 5-HT_{1A} sites in rat (Marcinkiewicz *et al*, 1984) and human brain (Hoyer *et al*, 1986a) were subsequently described. These authors reported that high densities of 5-HT_{1A} receptors occur in the septum, raphe nuclei, hippocampus and amygdala. De Vivo and Maayani (1985) reported that 5-HT_{1A} receptors act by inhibiting adenylate cyclase activity. This finding was confirmed by Fargin *et al* (1988), who cloned the human 5-HT_{1A} receptor. The rat 5-HT_{1A} receptor has also been cloned, and it is 89% homologous with the human 5-HT_{1A} receptor (Albert *et al*, 1990).

The 5-HT_{2A} receptor was first identified in rat brain with [^{3}H]ketanserin by Leysen *et*

al (1982). Using [^{3}H]ketanserin, the distribution of 5-HT$_{2A}$ sites in rat (Pazos *et al*, 1985) and human brain (Hoyer *et al*, 1986b) were subsequently described. These authors reported that high densities of 5-HT$_{2A}$ receptors occur in the cortex, olfactory system, hypothalamus and basal ganglia. Conn and Sanders-Bush (1984) reported that 5-HT$_{2A}$ receptors act by stimulating phosphatidylinositol turnover. This finding was confirmed by Pritchett *et al* (1988), who cloned the rat 5-HT$_{2A}$ receptor. The hamster (Chambard *et al*, 1990) and human (Saltzman *et al*, 1991) 5-HT$_{2A}$ receptors have also been cloned, and the latter is 87% homologous with the rat 5-HT$_{2A}$ receptor.

The 5-HT$_{2B}$ receptor was first identified by Foguet *et al* (1992), who isolated a cDNA clone for a serotonin receptor that stimulates phosphatidylinositol turnover in rat stomach fundus. The mouse (Loric *et al*, 1992) and human (Schmuck *et al*, 1994) 5-HT$_{2B}$ receptors have also been cloned, and the latter is 82% homologous with the rat 5-HT$_{2B}$ receptor. A selective ligand has only recently been developed (Forbes *et al*, 1995), so the brain distribution has yet to be described. Until such information becomes available, it is worthwhile to note that 5-HT$_{2B}$ mRNA has been found in the brain of mouse (Loric *et al*, 1992), cat and monkey (Helton *et al*, 1994) and human (Schmuck *et al*, 1994).

The 5-HT$_{2C}$ receptor was first identified in pig choroid plexus with [^{3}H]mesulergine by Pazos *et al* (1984). Using [^{3}H]mesulergine, the distribution of 5-HT$_{2C}$ sites in rat (Pazos and Palacios, 1985) and human brain (Hoyer *et al*, 1986b) were subsequently described. These authors reported that the highest densities of 5-HT$_{2C}$ receptors occur in the choroid plexus. Conn *et al* (1986) reported that 5-HT$_{2C}$ receptors act by stimulating phosphatidylinositol turnover. This finding was confirmed by Julius *et al* (1990), who cloned the rat 5-HT$_{2C}$ receptor. The mouse (Yu *et al*, 1991) and human (Saltzman *et al*, 1991) 5-HT$_{2C}$ receptors have also been cloned. Their homology with the rat 5-HT$_{2C}$ receptor is 97% and 90%, respectively.

Defining aggression

The type of aggression that I will deal with in this thesis are attack behaviors that a male

directs toward a conspecific male. This type of aggression has been referred to as conspecific attack by Blanchard and Blanchard (1977) and offensive aggression by Adams (1979).

Blanchard and Blanchard (1977) described the behavior of male rats toward an unfamiliar conspecific male introduced into the colony. The resident male approaches and sniffs the intruder. This is followed by piloerection of the hair on the back and shoulders, as well as chattering of the teeth. The resident then bites the head or back of the intruder. The intruder then engages in one of two defensive responses to this attack: lying on the back or the defensive upright posture. If the intruder tries to defend his back regions with the "on back" posture, the resident stands "on top" and attempts to deliver more bites (the fact that the resident continues to bite illustrates that this "on back" posture does not serve as a submissive signal to inhibit further attack). On the other hand, if the intruder tries to maintain a distance from the resident with defensive "boxing," the resident will either assume the offensive upright posture and try to knock the intruder onto its back, or else initiate a lateral attack. A lateral attack is the more common strategy. In this case the resident rises up on its toes, approaches laterally and uses its hind leg or flank to push at the intruder. Although the intruder may flee in response to this pushing (in which case it is chased), it often falls on its back, and then the resident stands on top and attempts to deliver bites. Eventually the resident male tires and the attacks cease. Sudden movement on the part of the intruder, however, usually elicits renewed attacks. In conclusion, it should be noted that these behaviors are seen both in the laboratory and in the wild.

Adams (1979) reviewed numerous stimulation, lesion and recording studies of aggressive behavior in rats. Based on this analysis, he proposed that two different neural "circuits" underlie the defensive behaviors displayed by intruder rats and the offensive behaviors displayed by resident rats. According to this author, the defensive "circuit" is as follows: motivating stimuli activate the "defense zone" of the amygdala, pretectum (moving visual stimuli) and auditory, pain and tactile pathways. These signals then converge in the midbrain central gray, which in turn initiates motor patterns such as fleeing, defensive upright and freezing. In contrast, the offensive "circuit" is as follows: motivating stimuli activate the

corticomedial amygdala, olfactory tubercle or anterior olfactory nucleus, and septum. These signals are then conveyed by the median forebrain bundle through the lateral hypothalamus to the midbrain, which in turn initiates motor patterns such as approach, lateral attack, offensive upright and chasing.

As might be expected, numerous studies since Adams (1979) review have refined the circuit for offensive behavior. Although these points will be detailed in my discussion, I have described Adams (1979) proposed circuit at this point to illustrate that specific motor patterns are associated with different neural substrates. As the objective of this thesis is to examine the role of a brain neurotransmitter system in aggressive behavior, I will use Adams (1979) categorization of aggression throughout this thesis. I will also only review papers that address offensive aggression in rats, or attack behaviors in other species.

Effects of depleting brain 5-HT on offensive aggression in male mammals

PCPA

Koe and Weissman (1966) found that *p*-chlorophenylalanine (PCPA) decreases brain 5-HT without reducing catecholamine levels. In rats, maximal depletion occurs 3 days after injection, when whole brain levels of 5-HT are only 7 ± 1% of controls. The authors suggested that PCPA acts by blocking tryptophan hydroxylase, the first of two enzymes that converts the essential amino acid tryptophan to 5-HT (Lovenberg *et al*, 1967). This was subsequently confirmed by Jéquier *et al* (1967).

Effects of PCPA on aggression in male monkeys

Raleigh *et al* (1980) gave intraperitoneal injections of PCPA (80 mg/kg/day) to group-housed male vervet monkeys (*Cercopithecus aethiops sabaeus*). The frequency of threatening, hitting, chasing and biting was recorded over a 2 week period. The result was that PCPA-treated monkeys showed a significantly higher frequency of all aggressive behaviors as compared to saline-injected controls.

Effects of PCPA on fighting in male mice

Welch and Welch (1969) reported a complete inhibition of fighting in PCPA-treated pairs of mice observed 100 minutes post-injection, while at 5 hours post-injection there was no difference between PCPA-treated pairs and saline-treated pairs. These findings should be viewed with caution, as a complete inactivation of tryptophan hydroxylase does not occur until 24 hours after injection (Koe and Weissman, 1966).

Rolinski (1975) also reported that PCPA exerted an anti-aggressive effect, although the significance of this finding is unclear, given the fact that there is no mention of the time after injection when aggression was tested.

Matte and Tornow (1978) gave intraperitoneal injections of PCPA (350 mg/kg) to male wild mice (*Mus musculus*) that had been isolated for 3 weeks. Twenty-four hours later, two mice that had received the same treatment were placed in a neutral cage. The result was that PCPA-treated pairs showed a significantly higher total duration of fighting time, as well as a significantly lower latency to fight, as compared to saline-injected pairs.

Effects of PCPA on offensive aggression in male rats

Sheard (1973) reported that resident rats bit or clawed PCPA-treated intruders for a significantly greater percentage of time than saline-injected intruders. The significance of this finding is unclear, given the fact that the intruder rat was treated with PCPA, rather than the focal animal.

Vergnes *et al* (1986) gave intraperitoneal injections of PCPA (375 mg/kg) to individually-housed male Wistar rats. Three days later a weight matched male was introduced. The result was that PCPA-treated residents showed a significantly higher frequency and duration of lateral attack, as well as a significantly higher frequency of offensive upright, as compared to saline-injected controls. Defensive behaviors were not affected.

Effects of 5-HT receptor agonists on offensive aggression in male rodents

8-OH-DPAT

Arvidsson *et al* (1981) found that 8-OH-DPAT is a centrally active 5-HT agonist, and Middlemiss and Fozard (1983) subsequently reported that it is selective for 5-HT_{1A} sites.

Effects of 8-OH-DPAT on offensive aggression in male mice

McMillen *et al* (1987) reported that 8-OH-DPAT caused a dose-dependent increase in attack latency. White *et al* (1991) reported that 8-OH-DPAT reduced total fighting time. Unfortunately, no measure of behavioral sedation was reported in either of these papers.

Olivier *et al* (1989) gave subcutaneuous injections of 8-OH-DPAT (0.05, 0.25, 1.25 and 6.25 mg/kg) to male albino mice that had been isolated for 3 weeks. Thirty minutes later a treated mouse was placed in a neutral cage with a group-housed male. The result was that 8-OH-DPAT-treated mice showed a significant, dose-dependent decrease in the mean frequency of all aggressive behaviors as compared to saline-injected controls. There was also a significant increase in inactivity (i.e., immobile and immobile alert) at all but the lowest dose.

Effects of 8-OH-DPAT on offensive aggression in male rats

Mos *et al* (1992) gave subcutaneuous injections of 8-OH-DPAT (0.05, 0.1 and 0.2 mg/kg) to male Wistar and TMD-S3 rats individually-housed with a sterilized female. Thirty minutes prior to injection the female was removed, and 30 minutes after injection a male Wistar rat was introduced. The result was that 8-OH-DPAT-treated residents showed a significant, dose-dependent decrease in the time spent on all offensive behaviors at 0.1 and 0.2 mg/kg, as compared to saline-injected controls. There was also a significant increase in inactivity at 0.1 and 0.2 mg/kg.

Buspirone

Peroutka (1985) reported that buspirone is a selective agonist for brain 5-HT_{1A} sites.

Effects of buspirone on fighting in male mice

McMillen *et al* (1987) reported that buspirone caused a dose-dependent increase in

attack latency. White *et al* (1991) reported that buspirone reduced total fighting time. Unfortunately, no measure of behavioral sedation was reported in either of these papers.

Olivier *et al* (1989) gave intraperitoneal injections of buspirone (0.3, 1.0, 3.0 and 10.0 mg/kg) to male albino mice that had been isolated for 3 weeks. Thirty minutes later a treated mouse was placed in a neutral cage with a group-housed male. The result was that buspirone-treated mice showed a significant, dose-dependent decrease in the mean frequency of all aggressive behaviors at the three highest doses, as compared to saline-injected controls. Inactivity was not affected.

Effects of buspirone on offensive aggression in male rats

Olivier *et al* (1984) gave intraperitoneal injections of buspirone (2.0, 4.0 and 8.0 mg/kg) to male Wistar and Wezob rats individually-housed with a sterilized female. Thirty minutes prior to injection the female was removed, and 30 minutes after injection a male Wistar rat was introduced. The result was that buspirone-treated residents showed a dose-dependent decrease in the mean duration and mean frequency of all offensive behaviors as compared to saline-injected controls. There was also a dose-dependent increase in inactivity.

Similarly, Mos *et al* (1992) gave intraperitoneal injections of buspirone (2.0, 4.0 and 8.0 mg/kg) to male Wistar and TMD-S3 rats individually-housed with a sterilized female. Thirty minutes prior to injection the female was removed, and 30 minutes after injection a male Wistar rat was introduced. The result was that buspirone-treated residents showed a significant, dose-dependent decrease in the time spent on all offensive behaviors as compared to saline-injected controls. There was also a significant increase in inactivity at 4.0 and 8.0 mg/kg.

DOI

Shannon *et al* (1984) demonstrated that 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) is a selective agonist for brain 5-HT_{2A} sites. Appel *et al* (1990) subsequently reported that DOI also has a high affinity for brain 5-HT_{2C} sites. Although the

binding of this compound to brain 5-HT_{2B} sites has yet to be assessed, it is worthwhile to note that it does have a high affinity for 5-HT_{2B} receptors in rat stomach fundus (Wainscott *et al*, 1992).

Effects of DOI on fighting in male mice

Sánchez *et al* (1993) state that "DOI has an antiaggressive effect but only at a high dose" in male mice. Unfortunately the authors do not report the dose(s) that produced this effect, or if there was a concomitant increase inactivity.

Effects of DOI on offensive aggression in male rats

Mos *et al* (1992) gave intraperitoneal injections of DOI (0.25, 0.5 and 1.0 mg/kg) to male Wistar and TMD-S_3 rats individually-housed with a sterilized female. Thirty minutes prior to injection the female was removed, and 30 minutes after injection a male Wistar rat was introduced. The result was that DOI-treated residents showed a significant, dose-dependent decrease in the time spent on all offensive behaviors as compared to saline-injected controls. There was also a significant increase in inactivity at all doses.

The brain 5-HT system and offensive behavior in male mammals

PCPA has been shown to increase aggression in group-housed monkeys (Raleigh *et al*, 1980) and isolated mice (Matte and Tornow, 1978). Furthermore, PCPA increases offensive, but not defensive behaviors in isolated rats (Vergnes *et al*, 1986). Because PCPA selectively depletes brain 5-HT in mammals (Koe and Weissman, 1966), these studies suggest that brain 5-HT inhibits offensive behaviors in male mammals, in both laboratory and more "natural" settings. This hypothesis is supported by work with 5-HT receptor agonists.

Work in rodents suggests that serotonergic inhibition of offensive behavior is mediated by 5-HT_{1A} receptors (McMillen *et al*, 1987; McMillen *et al*, 1988; Olivier *et al*, 1989; White *et al*, 1991; Mos *et al*, 1992). Nevertheless, it is important to note that only a low dose of 8-OH-DPAT (0.05 mg/kg) reduces offensive behavior in rats and mice, while higher doses produce

an overall behavioral sedation (Olivier *et al*, 1989; Mos *et al*, 1992). This is in contrast with the species-specific effects of buspirone. While only a low dose of buspirone (2.0 mg/kg) selectively reduces offensive behavior in rats (Mos *et al*, 1992), aggression is dose-dependently reduced in mice without a concomitant increase in inactivity (Olivier *et al*, 1989). These findings illustrate the importance of monitoring inactivity and immobility when administering 5-HT receptor agonists, as behavioral sedation may be produced at different doses in different species.

The role of 5-HT_2 receptors in offensive behavior must be considered tentative at this time. Although DOI produces an overall behavioral sedation in rats (Mos *et al*, 1992), the drug may selectively reduce aggression in mice (Sánchez *et al*, 1993).

The brain 5-HT system in Teleost fish

Serotonin cell bodies and their projections were first described in the brain of *Lepomis gibbosus* by Parent *et al* (1978). Since this initial description, the brain 5-HT system has been mapped in a number of other Teleost species, including *Myoxocephalus scorpius* (Watson, 1980), *Carassius auratus* (Kah and Chambolle, 1983), *Perca flavescens* (Parent, 1983), *Gasterosteus aculeatus* (Ekström and van Veen, 1984), *Oncorhynchus mykiss* (Frankenhuis-van den Heuvel and Nieuwenhuys, 1984), *Xiphophorus maculatus* (Margolis-Kazan *et al*, 1985), *Oncorhynchus nerka* (Ekström and Ebbesson, 1989), *Dicentrarchus labrax* (Batten *et al*, 1993) and *Micropogonias undulatus* (Khan and Thomas, 1993).

Unfortunately there has been little work on 5-HT receptor subtypes in the Teleost brain. Autoradiographic information is limited to a few sentences in Palacios and Dietl (1988), who do not even mention what species they investigated. Nevertheless, they report the labeling of 5-HT_{1A} sites in fish brain with [^{3}H]8-OH-DPAT. On the other hand, they saw no labeling with either [^{3}H]ketanserin or [^{3}H]mesulergine, suggesting an absence of central 5-HT_{2A} and 5-HT_{2C} receptors, respectively.

Pharmacological information is also limited. Hensley and Cohen (1992) found that 5-HT creatinine sulfate decreased maintained activity of on-center ganglion cells and increased

maintained activity of off-center ganglion cells in the goldfish retina. 8-OH-DPAT mimicked this effect at off-center ganglion cells, while methysergide, a non-selective 5-HT_2 antagonist (Schmidt and Peroutka, 1989), blocked the effects of 5-HT at both on- and off-center ganglion cells. This suggests that the action of 5-HT at on-center ganglion cells occurs via a 5-HT_2 receptor subtype.

Both of these papers demonstrate the existence of 5-HT_{1A} receptors in fish brain. On the other hand, the apparent absence of 5-HT_{2A} and 5-HT_{2C} sites in fish brain, along with the fact that methysergide has a high affinity for 5-HT_{2B} receptors (Foguet *et al*, 1992; Wainscott *et al*, 1993), suggests the existence of 5-HT_{2B} receptors in the fish CNS.

Evidence for serotonergic inhibition of aggression in male Teleosts

Imipramine increases available 5-HT by blocking its re-uptake (Langer *et al*, 1980). Avis and Peeke (1979) dissolved imipramine in the tank water of male convict cichlids (*Cichlasoma nigrofasciatum*) who had been isolated for 1 week. Twenty minutes later a male conspecific confined to a glass tube was introduced. The result was that imipramine-treatment resulted in a significantly reduced median display frequency.

Maler and Ellis (1987) gave ventricular injections of 5-HT to male *Apteronotus leptorhynchus*, a weakly electric fish. Five minutes later the fish were presented with a simulated electric organ discharge (EOD) of a male conspecific. The result was that 5-HT-treated fish had a significantly lower frequency of aggressive EOD "chirping" as compared to carrier-injected controls.

Behavioral ecology of firemouth cichlids

Cichlasoma meeki, commonly known as the firemouth cichlid, is a member of the family Cichlidae (Pisces). Hasse (1981) gives a detailed anatomical description of the species and resolves previous taxonomic disputes.

Firemouths are found in Central America. Geographically, their range extends from the Yucatán Peninsula in the north, to northern Guatemala in the south (Hasse, 1981); from the

Mexican state of Tabasco in the west, to the Caribbean in the east (Neil, 1984). They tend to occupy rocks and vegetation in the shallow and/or inshore areas of lakes, rivers and lagoons. They are diurnal, with activity peaking in the morning. Invertebrates constitute their major food source (Neil, 1984).

Firemouths spawn throughout the year. Territories are established by solitary males, who subsequently attract a mate, or by wandering pairs that have spawned together in the past. Rocky crevices are the preferred spawning site (Neil, 1984). The pair make alternate passes over the substrate when spawning (Baerends and Baerends-van Roon, 1950).

Territories are maintained for 7 to 10 days following spawning. The female tends to stay extremely close to the spawning site, while the male defends an area (about 1 m in diameter) around the site from other fish (Neil, 1984). Once the fry are free-swimming the parents defend a mobile territory around the young as they forage. Parental care lasts approximately 3 months (Neil, 1984).

Aggression of a resident cichlid toward an intruder typically occurs as follows. The median fins are erected as the fish approaches the intruder. As the distance between the fish closes the gill covers and branchiostegal membrane are also erected. The resident swims up to the intruder, displaying laterally. This is often accompanied by tailbeating. As the encounter escalates the fish switch to a frontal orientation and rush at each other while still displaying. The fish try to evade the rushes of their opponent by turning quickly, and this leads to the two fish rapidly circling each other head-to-tail. They attempt to tailbeat and butt while circling. Finally, the two fish grip each other by the jaws and push/pull vigorously. This mouth-fighting can go on for a considerable amount of time. At some point one of the fish suddenly flees (Baerends and Baerends-van Roon, 1950).

Thesis objectives

Although PCPA has been shown to selectively deplete brain 5-HT in fish (Johnston *et al*, 1993; Winberg *et al*, 1993; Adams, Drew and Ebbesson, unpublished data), no study has reported the effects of this drug on the aggressive behavior of fish. Furthermore, as no study

has assessed the physiological control of aggression in firemouths, I decided that my preliminary objective would be to determine whether PCPA increases aggression in males of this species. Then I would move on to my main objective, which was to determine which 5-HT receptors mediate the effects of serotonin on aggression in male firemouths. I limited this portion of my experimental work to the 5-HT_{1A} receptor, which has been identified in fish brain (Palacios and Dietl, 1988), and the 5-HT_2 receptors, whose presence has been suggested in the fish CNS (Hensley and Cohen, 1992).

METHODS AND RESULTS

General methods

Animals

Juvenile firemouth cichlids (*Cichlasoma meeki*) were purchased from Delta Aquatics (Richmond, B. C.). The appearance of the genital papilla allowed separation of males and females into two stock tanks as individuals reached sexual maturity. Fish were fed once daily on frozen red worms. Water temperature was maintained at 26 ± 1° C. Photoperiod was 12L:12D.

Procedure

Individual males were isolated in a 55 l holding tank prior to testing. While in this tank the fish could interact with 1 or 2 other males in neighboring tanks. After 6 days in the holding tank the fish was transferred to an observation tank.

The observation tanks were constructed from 102 l aquaria. Sliding door tracks were mounted to the sides to provide a guide for a black Plexiglas partition. This partition could be quietly lifted by means of a simple string-pulley system, thus revealing a mirror. Each tank was also provided with a shelter for the fish. The shelter was arranged so that the fish could see the partition, and the fish could be observed from the front of the tank at all times.

After 24 hours in the observation tank, the fish was presented with the mirror for 15 minutes. If it reacted aggressively to the mirror, the total number of bites was recorded for 5 minutes and then the observation was terminated. If the fish did not react aggressively to the mirror it was scored not aggressive (NA). The fish was then assigned to saline or drug treatment by coin toss and received an appropriate injection. The same mirror presentation procedure was used 24 hours post-injection for experiment 1, and 30 minutes post-injection for all other experiments.

Behaviors were recorded with an RCA Pro 843 camcorder. At the start of each session I would begin videotaping, lift the partition and then leave the room. The fish were observed

on a monitor and the number of bites were later tallied using The Observer (Noldus).

Behaviors

A bite was defined as the ramming of the mirror with an open mouth (Baerends and Baerends-van Roon, 1950). This was the aggressive behavior of interest in all experiments.

Reliable recording of the duration of displaying (Baerends and Baerends-van Roon, 1950) was difficult, and so this behavior was only used to start the 5 minute recording period on occasions when it preceded the first bite. Other behaviors, such as head down (Neil, 1984) and tail beating (Baerends and Baerends-van Roon, 1950), occurred so infrequently that they did not warrant recording.

The occurrence of an overall behavioral sedation in experiments 2, 3 and 4 was limited to noting when a fish did not move more than one body length during the observation.

Drug administration

Fish were netted and placed on a table. While on the table they were left in the net to restrain them during the injection procedure. The injection site was located above the genital papilla. The needle (30G) was run into the intraperitoneal cavity, where it was held in place for approximately 20 seconds immediately following the injection, thus insuring proper distribution of its contents. The fish was then returned to its tank. The total time the fish was out of the water was never more than 30 seconds. All fish were handled with rubber gloves.

Statistics

Although the results for each experiment are expressed graphically as the mean number of bites ± SEM, significance levels were calculated with a Mann-Whitney test.

A Wilcoxon test was also used to compare the number of pre- and post-treatment bites within each group.

Experiment 1:

Effects of a 5-HT synthesis inhibitor (PCPA) on firemouth aggression

Objective

The purpose of this experiment was to test the hypothesis that brain 5-HT inhibits mirror-directed biting in male firemouths.

Drug treatment

DL-p-chlorophenylalanine methyl ester HCl (PCPA) was purchased from Sigma Chemical Co. (St. Louis, MO). Preliminary observations indicated that a dose of 0.3 mg/g produced an obvious increase in aggression 24 hours post-injection. Fish were also tested at 48 and 72 hours post-injection, but the changes in aggression were not as dramatic at those times. Furthermore, because twice as many fish could be observed at 24 hours intervals, I decided that the post-treatment observations would be done 24 hours after injection.

50.0 mg of PCPA was dissolved in 1.0 ml of 0.9% saline. The dose each fish received was 0.3 mg/g. Control fish received an equivalent amount of saline.

Results

Results are expressed in Figure 1 as the mean number of bites ± SEM. While there was no difference between the groups during the pre-treatment observations ($P = 0.222$), PCPA-treated firemouths showed a significantly greater number of bites as compared to saline-injected controls ($P < 0.05$).

A comparison of the number of pre- and post-treatment bites for the control group shows no significant difference ($P = 0.394$), while the same comparison for the PCPA group shows a significantly greater number of bites post-treatment ($P < 0.001$).

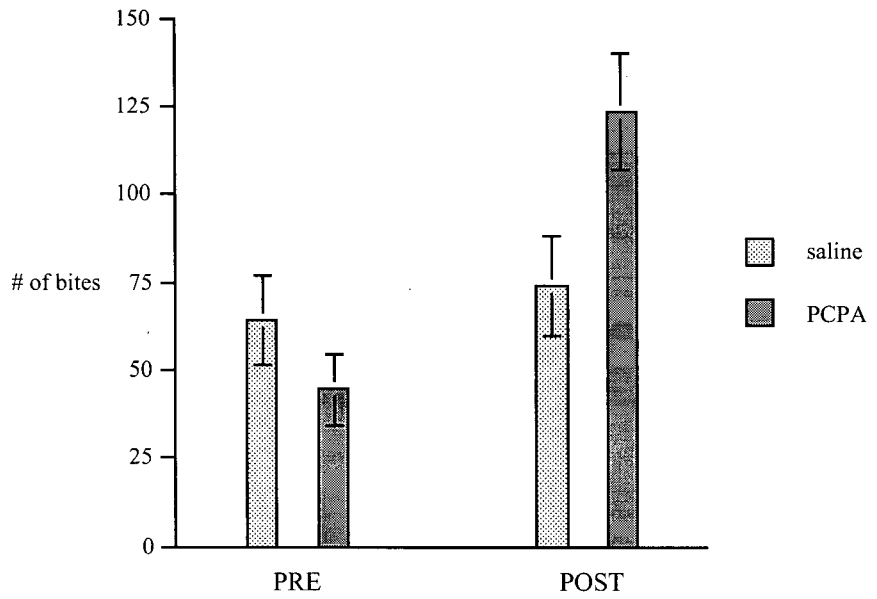

Figure 1. Mean number of mirror-directed bites ± SEM for saline (N = 17) and PCPA (N = 19) treated firemouths, pre- and post-treatment.

Table 1. Number of mirror-directed bites for saline- and PCPA-treated firemouths.

PRE	TREATMENT	POST	CHANGE
NA*	saline	20	+ 20
99	saline	92	- 7
NA*	saline	84	+ 84
NA*	saline	2	+ 2
112	saline	162	+ 50
102	saline	28	- 74
NA	saline	NA†	0
0	saline	0	0
44	saline	42	- 2
6	saline	63	+ 57
107	saline	159	+ 52
139	saline	149	+ 10
96	saline	65	- 31
90	saline	62	- 28
116	saline	167	+ 51
75	saline	118	+ 43
106	saline	38	- 68
45	PCPA	141	+ 96
12	PCPA	43	+ 31
NA*	PCPA	80	+ 80
67	PCPA	106	+ 39
92	PCPA	72	- 20
NA	PCPA	141	+ 141
25	PCPA	78	+ 53
NA†	PCPA	191	+ 191
72	PCPA	49	- 23
0	PCPA	67	+ 67
114	PCPA	170	+ 56
17	PCPA	153	+ 136
145	PCPA	190	+ 45
65	PCPA	116	+ 51
66	PCPA	155	+ 89
49	PCPA	65	+ 16
NA†	PCPA	2	+ 2
73	PCPA	272	+ 199
NA†	PCPA	252	+ 252

NA = no aggression
0 = displayed toward the mirror but did not bite
* = did not move greater than one body length
† = under the shelter for the entire observation

Experiment 2:

Effects of a 5-HT_{1A} agonist (8-OH-DPAT) on firemouth aggression

Objective

The purpose of this experiment was to test the hypothesis that serotonergic inhibition of mirror-directed biting in male firemouths is mediated by 5-HT_{1A} receptors.

Drug treatment

(±)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronapthalene HBr (8-OH-DPAT) was purchased from Research Biochemicals International (Natick, MA). Preliminary observations indicated that doses of 0.1 and 0.2 μg/g had no obvious effect on aggression. Higher doses (0.4, 0.8, 1.6 or 3.2 μg/g) resulted in a number of abnormal physical and behavioral effects: the edges of the fins started to fray and certain portions of their bodies appeared to be "rotting," and these effects persisted 24 hours later. The fish who received 3.2 μg/g had trouble swimming 30 minutes post-injection. It alternated between splashing about at the surface of the water and standing motionless in a vertical posture. Thus, it became clear that the data collected at 0.2 μg/g would have to be used for this experiment.

1.0 mg of 8-OH-DPAT was dissolved in 5.0 ml of heated 0.9% saline. The dose each fish received was 0.2 μg/g. Control fish received an equivalent amount of saline/NaBR (Mendelson and Gorzalka, 1986)

Results

Results are expressed in Figure 2 as the mean number of bites ± SEM. There was no difference between the groups during the pre-treatment observations ($P = 1.000$). There was also no difference between 8-OH-DPAT- and saline-injected controls ($P = 0.291$).

A comparison of the number of pre- and post-treatment bites for the control group shows no significant difference ($P = 0.722$). The same comparison for the 8-OH-DPAT group also shows no significant difference ($P = 0.965$).

No fish were sedated by 8-OH-DPAT-treatment (Table 2).

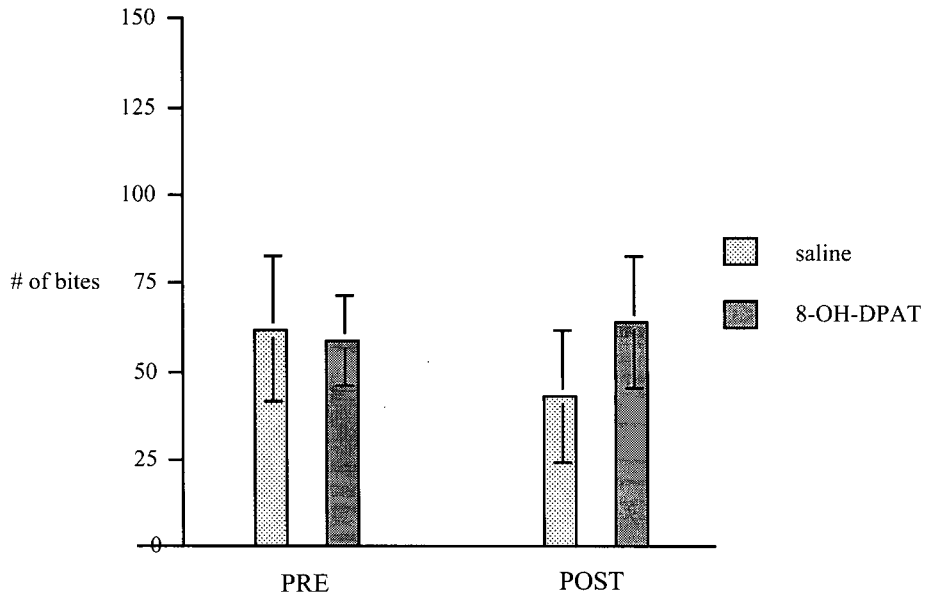

Figure 2. Mean number of mirror-directed bites ± SEM for saline (N = 10) and 8-OH-DPAT (N = 11) treated firemouths, pre- and post-treatment.

Table 2. Number of mirror-directed bites for saline- and 8-OH-DPAT-treated firemouths.

PRE	TREATMENT	POST	CHANGE
13	saline	22	+ 9
157	saline	173	+ 16
67	saline	2	- 65
68	saline	12	- 56
0	saline	49	+ 49
156	saline	6	- 150
21	saline	31	+ 10
8	saline	3	- 5
130	saline	131	+ 1
NA†	saline	NA*	0
91	8-OH-DPAT	100	+ 9
139	8-OH-DPAT	60	- 79
NA*	8-OH-DPAT	1	+ 1
NA*	8-OH-DPAT	30	+ 30
52	8-OH-DPAT	181	+ 129
57	8-OH-DPAT	135	+ 78
68	8-OH-DPAT	17	- 51
41	8-OH-DPAT	16	- 25
45	8-OH-DPAT	7	- 38
46	8-OH-DPAT	22	- 24
108	8-OH-DPAT	135	+ 27

NA = no aggression
0 = displayed toward the mirror but did not bite
* = did not move greater than one body length
† = under the shelter for the entire observation

Experiment 3:

Effects of a second 5-HT$_{1A}$ agonist (buspirone) on firemouth aggression

Objective

As the results of the previous experiment may have been due to pharmacological effects unique to 8-OH-DPAT, this experiment was conducted to re-test the hypothesis that serotonergic inhibition of mirror-directed biting in male firemouths is mediated by 5-HT$_{1A}$ receptors.

Drug treatment

8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione HCl (buspirone) was purchased from Research Biochemicals International (Natick, MA). Preliminary observations at a dose of 2.0 μg/g revealed a tendency for the fish to swim at the surface of the water, which is atypical behavior for this species. Reducing the dose to 1.0 μg/g appeared to eliminate this behavior.

1.0 mg of buspirone was dissolved in 1.0 ml of 0.9% saline. The dose each fish received was 1.0 μg/g. Control fish received an equivalent amount of saline.

Results

Results are expressed in Figure 3 as the mean number of bites ± SEM. While there was no difference between the groups during the pre-treatment observations ($P = 0.421$), buspirone-treated firemouths showed a significantly lower number of bites as compared to saline-injected controls ($P < 0.05$).

A comparison of the number of pre- and post-treatment bites for the control group shows no significant difference ($P = 0.359$), while the same comparison for the buspirone group shows a significantly lower number of bites post-treatment ($P < 0.05$).

No fish were sedated by buspirone-treatment (Table 3).

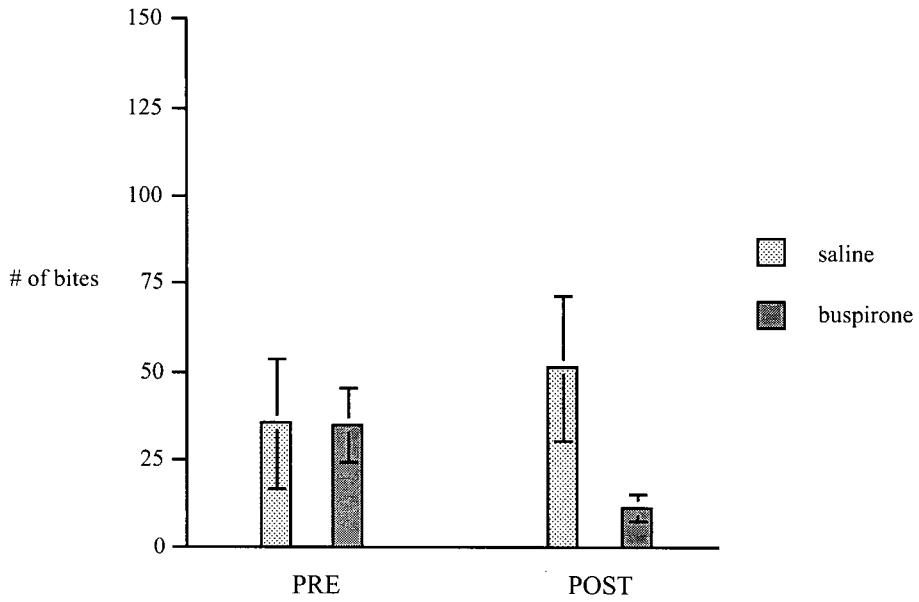

Figure 3. Mean number of mirror-directed bites ± SEM for saline (N = 10) and buspirone (N = 10) treated firemouths, pre- and post-treatment.

Table 3. Number of mirror-directed bites for saline- and buspirone-treated firemouths.

PRE	TREATMENT	POST	CHANGE
18	saline	25	+ 7
0	saline	30	+ 30
NA	saline	50	+ 50
NA†	saline	111	+ 111
90	saline	22	- 68
37	saline	10	- 27
11	saline	2	- 8
183	saline	215	+ 32
12	saline	4	- 8
NA*	saline	40	+ 40
78	buspirone	3	- 75
NA	buspirone	7	+ 7
35	buspirone	NA	- 35
NA†	buspirone	9	+ 9
15	buspirone	2*f*	- 13
100	buspirone	20	- 80
26	buspirone	18	- 8
46	buspirone	37	- 9
45	buspirone	15	- 30
2	buspirone	2*f*	0

NA = no aggression
0 = displayed toward the mirror but did not bite
* = did not move greater than one body length
† = under the shelter for the entire observation
f = swam to surface at least once during the observation

Experiment 4:

Effects of a 5-HT_2 agonist (DOI) on firemouth aggression

Objective

The purpose of this experiment was to test the hypothesis that serotonergic inhibition of mirror-directed biting in male firemouths is mediated by 5-HT_2 receptors.

Drug treatment

1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) was purchased from Research Biochemicals International (Natick, MA). Preliminary observations indicated that a dose of 0.5 μg/g had no obvious effect on aggression. Searching for a potentially higher dose to use, I injected several fish with either 1.0, 2.0, 4.0, 8.0 or 16.0 μg/g. The fish that received 8.0 and 16.0 μg/g showed a number of abnormal physical and behavioral effects: blackened eyes, continuous erection of all fins, a medium gray coloration and the display of the caudal 4 vertical "bars." This altered appearance made these two fish easy to identify when returned to their stock tanks 1 hour post-injection. At this time "frayed fins" and "body rotting" appeared. They also began chasing the other fish in the tank incessantly. Both the physical and behavioral effects persisted 24 hours later. The fish that received 4.0 μg/g had erect fins 30 minutes post-injection, but appeared normal when returned to the stock tank after 1 hour. The fish that received 1.0 and 2.0 μg/g showed no symptoms. Thus I decided to use a dose of 2.0 μg/g.

1.0 mg of DOI was dissolved in 1.0 ml of 0.9% saline. The dose each fish received was 2.0 μg/g. Control fish received an equivalent amount of saline.

Results

Results are expressed in Figure 4 as the mean number of bites ± SEM. A significant difference between the groups during the pre-treatment observations ($P < 0.05$) indicated that the two groups were not drawn from the same population, and so the post-treatment number of bites could not be compared directly. The "change" scores (Table 4) were compared instead.

The "change" in number of bites was significantly lower for DOI-treated fish as compared to saline-injected controls ($P < 0.05$).

A comparison of the number of pre- and post-treatment bites for the control group shows no significant difference ($P = 0.760$), while the same comparison for the DOI group shows a significantly lower number of bites post-treatment ($P < 0.005$).

One fish was sedated by DOI-treatment (Table 4).

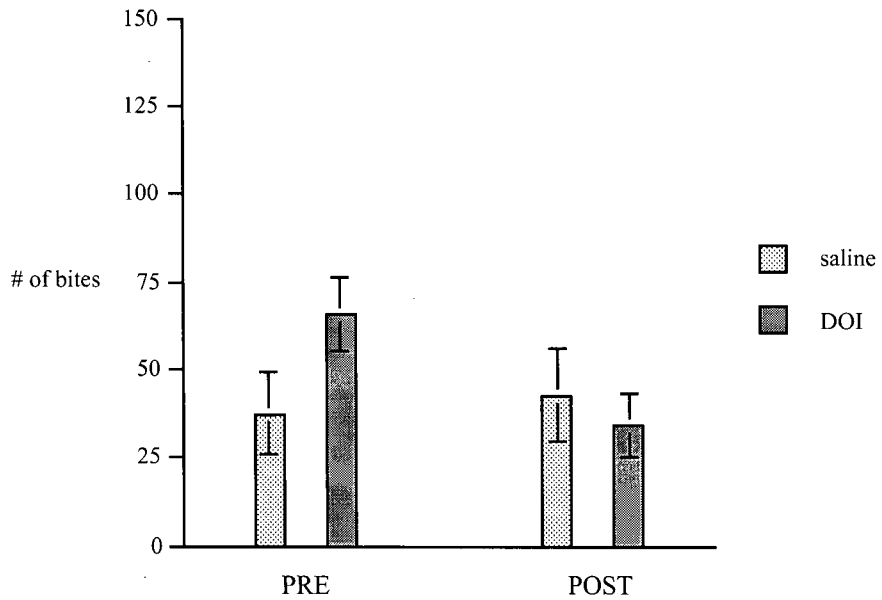

Figure 4. Mean number of mirror-directed bites ± SEM for saline (N = 18) and DOI (N = 18) treated firemouths, pre- and post-treatment.

Table 4. Number of mirror-directed bites for saline- and DOI-treated firemouths.

PRE	TREATMENT	POST	CHANGE
6	saline	10	+ 4
2	saline	30	+ 28
55	saline	14	- 41
8	saline	3	- 5
NA†	saline	21	+ 21
1	saline	0	- 1
187	saline	120	- 67
83	saline	62	- 21
104	saline	159	+ 55
16	saline	6	- 10
9	saline	10	+ 1
NA†	saline	184	+ 184
57	saline	41	- 16
46	saline	15	- 31
52	saline	68	+ 16
37	saline	3	- 34
9	saline	5	- 4
NA*	saline	18	+ 18
45	DOI	NA	- 45
149	DOI	62	- 87
68	DOI	52	- 16
87	DOI	13	- 74
24	DOI	NA*	- 24
NA†	DOI	0	0
28	DOI	10	- 18
88	DOI	24	- 64
101	DOI	22	- 79
57	DOI	33	- 24
5	DOI	10	+ 5
48	DOI	107	+ 59
13	DOI	7	- 6
154	DOI	144	- 10
100	DOI	4	- 96
79	DOI	47	- 32
64	DOI	29	- 35
71	DOI	52	- 19

NA = no aggression
0 = displayed toward the mirror but did not bite
* = did not move greater than one body length
† = under the shelter for the entire observation

DISCUSSION

Experiment 1 is the first study to assess the effects of depleting brain 5-HT on the aggressive behavior of any Teleost species. The finding that PCPA increases mirror-directed biting is also the first report of a serotonergic manipulation eliciting an aggressive behavior in fish. This finding provides strong evidence that brain 5-HT inhibits mirror-directed biting in male firemouths.

Experiments 2, 3 and 4 are the first studies to assess which 5-HT receptor subtypes mediate serotonergic inhibition of aggressive behavior in any Teleost species. The results of experiment 4 are the first clear report of a selective anti-aggressive effect of the 5-HT_2 agonist DOI in any vertebrate species.

Although I will compare my results with similar work in other fish species, I would also like to compare my results with the mammalian literature. To facilitate such a discussion, I will outline several reasons why I think that mirror-directed biting is a behavior that is comparable to offensive behaviors in rats. I will also review evidence that information obtained through studies on mirror-directed biting is comparable with data on more "natural" aggressive interactions. While most of this may appear to be a literature review, I will present this idea at this point because I think it is an intellectual "result" of my research, and because the results of experiment 1 are an important point in support of this hypothesis.

Evidence that mirror-directed biting is an offensive behavior

Brain areas associated with offensive aggression in male rats

As pointed out by Adams (1979), several forebrain structures are involved in offensive behavior in rats. Septal lesions encompassing the medial septal nucleus, nucleus of the diagonal band of Broca, and to a lesser extent, the lateral septal nuclei, reduce the duration of piloerection, lateral attack and "on top" (Blanchard *et al*, 1977).

Lesions of the medial amygdala have no effect on the offensive behavior of naive resident rats. On the other hand, similar lesions in "experienced" rats (i.e., individuals who

have had at least one prior "victory" experience in the resident-intruder test) results in a significant reduction in offensive aggression. This suggests that the medial amygdala modulates aggressive behavior on the basis of past social experiences (Vochteloo and Koolhaas, 1987).

The bed nucleus of the stria terminalis has connections to the medial amygdala and the medial hypothalamus. Lesions of medial accumbens and the bed nucleus of the stria terminalis reduce the frequency and duration of lateral attack, the number of bites and the duration of "on top" (Albert *et al*, 1989).

The hypothalamus has been investigated more than any other brain structure with respect to offensive aggression. Lesions of the medial hypothalamus reduce the frequency and duration of lateral attack, the number of bites and the duration of "on top" (Albert *et al*, 1987). Lesions of the lateral hypothalamus completely abolish offensive behavior (Adams, 1971), while electrical stimulation of the same area results in teeth-chattering, piloerection, lateral attack and biting (Mos *et al*, 1982). Conversely, lesions of the mammillary bodies result in an increase in offensive behavior (Olivier *et al*, 1983). More specifically, lesions of the nucleus premammillaris ventralis, which lies posterior to the ventromedial hypothalamus, result in an increase in the duration of lateral attack and "on top" (van den Berg *et al*, 1983).

The hypothalamus has connections with the midbrain central gray. Lesions of the midbrain central gray reduce offensive behaviors (Mos *et al*, 1983), while electrical stimulation of this area results in approach, piloerection, lateral attack and biting (Mos *et al*, 1982).

Finally, lesions of the ventromedial tegmentum of the brain stem completely abolish offensive behaviors (Adams, 1986).

Brain areas associated with mirror-directed biting in male Teleosts

While a number of studies have assessed the effects of relatively large forebrain lesions on aggressive behavior in Teleost fish (reviewed in de Bruin, 1980), little work has been done on the role of specific brain nuclei in such behavior.

Electrical stimulation in the area surrounding the lateral recess of the third ventricle

causes a significant increase in the frequency of mirror-directed biting in both *Lepomis macrochirus* (Demski and Knigge, 1971) and the cichlid *Tilapia heudelotii macrocephala* (Demski, 1973). These authors later reported that the stimulation sites which elicited this behavior were located in the nucleus ventricularis (Demski *et al*, 1975).

Direct effects of brain 5-HT on offensive circuitry

The influence of serotonergic neurons on the offensive circuit in rats has been assessed with 5,7-dihydroxytryptamine (5,7-DHT), a neurotoxin that destroys 5-HT neurons (Baumgarten and Lachenmayer, 1972). Vergnes *et al* (1988) injected 5,7-DHT into the lateral hypothalamus of individually-housed male Wistar rats. Six days later a weight matched male was introduced. The result was that PCPA-treated residents showed a significantly higher frequency and duration of lateral attack as compared to vehicle-injected controls. As these injections were administered in an ascending pathway, this finding suggests that 5-HT neurons inhibit offensive behaviors at the level of the hypothalamus, amygdala and septum.

Although no study has directly assessed the influence of serotonergic neurons on a specific brain area involved in mirror-directed biting in fish, it is worthwhile to note that Winberg and Nilsson (1993) reported an inverse correlation between telencephalic and hypothalamic 5-HT turnover and dominance aggression in juvenile *Salvelinus alpinus*.

Neuroendocrine evidence that mirror-directed biting is an offensive behavior

Offensive behaviors such as piloerection and lateral attack are hormone-dependent (reviewed in Albert *et al*, 1992). Francis *et al* (1992) have reported that castration reduces mirror-directed biting in the African cichlid *Haplochromis burtoni*, suggesting that mirror-directed biting is a hormone-dependent behavior.

As mentioned above, the effects of PCPA are restricted to offensive aggression in rats (Vergnes *et al*, 1986). Thus, my finding that PCPA increase mirror-directed biting in male firemouths suggests that this behavior is an offensive one.

I should note that a possible neuroendocrine link between these two lines of evidence

has been reported by Bonson and Winter (1992) and Bonson *et al* (1994). These authors found that testosterone-induced dominance could be reversed with various 5-HT receptor agonists.

Summary

Several points have emerged in the last few pages. First, offensive behaviors in male rats are subserved by several hypothalamic nuclei (Adams, 1971; Mos *et al*, 1982; Albert *et al*, 1987), while at least one hypothalamic nuclei subserves mirror-directed biting in Teleost fish (Demski and Knigge, 1971; Demski, 1973). Second, offensive behaviors in male rats are hormone-dependent (Albert *et al*, 1992), as is mirror-directed biting in cichlids (Francis *et al*, 1992). Third, the effects of PCPA in rats are restricted to offensive behavior (Vergnes *et al*, 1986), so my finding that PCPA increase mirror-directed biting suggests that this behavior is an offensive one. These three lines of evidence suggest that it is reasonable to assume that mirror-directed biting is comparable to offensive behavior in rats and other mammals.

I also want to note how studies on mirror-directed biting relate to more "natural" aggressive interactions. Blanchard and Blanchard (1977) addressed a similar issue with respect to rats, and they reported that the behavior of rats in the resident-intruder test is identical to interactions between wild rats.

Two points emerge when examining the fish literature. First, the findings of Demski and Knigge (1971) and Demski (1973) were not limited to mirror-directed biting. Stimulation of the nucleus ventricularis in *Lepomis macrochirus* also resulted in a significant increase in the number of bites directed toward another fish (Demski and Knigge, 1971). Similarly, stimulation of the nucleus ventricularis in *Tilapia heudelotii macrocephala* resulted in a significant increase in the number of bites toward a fish confined to a glass tube, as well as toward a freely-swimming fish (Demski, 1973). These findings demonstrate that bites directed toward mirrors and freely-swimming fish are in fact subserved by the same brain area.

Second, the inverse correlation between telencephalic and hypothalamic 5-HT turnover and dominance aggression in pairs of juvenile *Salvelinus alpinus* following the removal of an

opaque partition (Winberg and Nilsson, 1993) is comparable to an earlier report. Winberg *et al* (1991) reported an inverse correlation between telencephalic and "brain stem" (i.e., the remainder of the brain) 5-HT turnover and dominance aggression in group-housed juvenile *Salvelinus alpinus*. These findings demonstrate similar roles for brain 5-HT in both "artificial" test situations and in more "natural" group interactions.

Brain 5-HT and aggression in Teleosts, mammals and birds

The results of experiment 1 demonstrate that treatment with PCPA results in an increase in mirror-directed biting. This finding is directly comparable to at least one other report on the pharmacological manipulation of the brain 5-HT system and its effects on aggression in Central American cichlids. Munro (1986) reported that intracranial injections of 5-HT significantly reduced mirror-directed biting in female *Aequidens pulcher*.

My results for experiment 1 may also be comparable to the effects of pharmacological manipulation of the brain 5-HT system on other aggressive behaviors in fish. As mentioned above, Avis and Peeke (1979) reported that increasing available 5-HT reduced the display frequency in male convict cichlids, while ventricular injections of 5-HT reduced the frequency of aggressive EOD "chirping" in male *Apteronotus leptorhynchus* (Maler and Ellis, 1987).

Based on the assumptions made above, the finding that treatment with PCPA increases mirror-directed biting in firemouth cichlids is comparable to the effects of this drug on offensive aggression in rats. Vergnes *et al* (1986) found that PCPA increased the frequency and duration of lateral attack, as well as the frequency of offensive upright in male rats.

The results of experiment 1 may also be comparable to the effects of this drug in wild mice and group-housed monkeys. Matte and Tornow (1978) reported that PCPA increased the latency to attack and the total duration of fighting time in male mice. Similarly, Raleigh *et al* (1980) reported that chronic PCPA-treatment resulted in an increase in the frequency of obvious attack behaviors such as hitting, chasing and biting in male vervet monkeys.

Finally, the results of experiment 1 may also be comparable to the effects of PCPA on birds. Buchanan *et al* (1994) reported that PCPA-treatment increases the frequency of

aggressive "pecking" in group-housed chicks.

5-HT_{1A} receptors and offensive aggression in male rodents and firemouths

Based on the assumptions made above concerning offensive aggression in rats and mirror-directed biting in fish, the results of experiment 2 do not agree with the rodent literature. The finding that 8-OH-DPAT does not inhibit mirror-directed biting in male firemouths is in contrast with the effects of this drug on offensive behavior in rodents (McMillen *et al*, 1987; Olivier *et al*, 1989; White *et al*, 1991; Mos *et al*, 1992). This finding is also in conflict with the results for experiment 3, which showed that another 5-HT_{1A} agonist, buspirone, does inhibit mirror-directed biting. The results for experiment 3 do agree with similar work in rodents (Olivier *et al*, 1984; McMillen *et al*, 1988; Olivier *et al*, 1989; White *et al*, 1991; Mos *et al*, 1992). There are three possible explanations for the conflicting results of experiments 2 and 3.

First, it could be said that buspirone binds to fish brain 5-HT_{1A} receptors, while 8-OH-DPAT does not. This argument does not seem tenable, as Palacios and Dietl (1988) have reported that [^{3}H]8-OH-DPAT labels 5-HT_{1A} sites in fish brain.

Second, the dose of 0.2 μg/g of 8-OH-DPAT may not have been high enough to inhibit aggression. The reason I did not use any higher doses was that they produced the "rotting" effects I described above. The fact that I might have used a higher dose became clear to me some time after the end of the experiment, when the fish that I did the dose determination on, as well as all their untreated tank mates, became sick. One of the symptoms of these sick fish was a "rotting" appearance. When I learned that the cause of this symptom was poor water conditions, it was clear to me that the fish may have been in a weakened state when I did the dose determination. In other words, "rotting" may not have been a side effect of 8-OH-DPAT, and so the doses of 0.4, 0.8 and 1.6 μg/g might have been used after all.

Third, the species-specific effects of buspirone suggests that a dose of 1.0 μg/g may have been too high. As I pointed out above, buspirone exerts species-specific effects in rodents: only a low dose of buspirone (2.0 mg/kg) selectively reduces offensive behavior in rats (Mos *et al*, 1992), while aggression is dose-dependently reduced in mice at doses up to 10

mg/kg, without a concomitant increase in inactivity (Olivier *et al*, 1989). The idea that buspirone may exert species-specific effects is supported by the fact that the lowest dose (2.0 mg/kg) used in rodents produced atypical behavior in the firemouths. As I described above, all of the fish in the preliminary observations (2.0 μg/g) swam at the surface of water during the observations. No firemouth ever did this in an observation tank before, and the fact that 2 of the 10 fish in experiment 3 (Table 3) engaged in this behavior suggests that a dose of 1.0 μg/g may still have been too high.

Needless to say, until these issues are investigated in further detail, conclusions regarding the role of 5-HT_{1A} receptors in firemouth aggression must remain tentative.

5-HT_2 receptors and offensive aggression in male rodents and firemouths

The results of experiment 4 demonstrate that treatment with DOI reduces mirror-directed biting in male firemouths. As only one treated fish did not move more than one body length during its observation (Table 4), it is unlikely that this behavioral change was caused by an overall behavioral sedation. This suggests that serotonergic inhibition of aggression in male firemouths is mediated by 5-HT_2 receptors.

Two interesting possibilities are suggested by comparing the results of experiment 4 with the mammalian literature. The first possibility is that DOI exerts species-specific effects. DOI produces an overall behavioral sedation in rats (Mos *et al*, 1992), while it inhibits aggression in mice at "high doses" (Sánchez *et al*, 1993). Unfortunately it is unclear from this latter report whether the anti-aggressive effect in mice is due to an overall behavioral sedation. What is clear, however, is that DOI reduces mirror-directed biting without sedating firemouths, even though the fish in my experiment received a dose (2.0 μg/g) that was 8 times higher than the lowest dose (0.25 mg/kg) which caused a behavioral sedation in rats (Mos *et al*, 1992).

The second possibility is that there are species differences in the presence and/or density of central 5-HT_2 receptor subtypes. This idea is supported by several points. First, 5-HT_{2A} and 5-HT_{2C} receptors are apparently absent in fish brain (Palacios and Dietl, 1988). Second, 5-HT_{2B} receptors are apparently absent in rat brain (Kursar *et al*, 1992; Helton *et al*, 1994;

Pompeiano *et al*, 1994), while 5-HT_{2B} mRNA has been found in the brain of mouse (Loric *et al*, 1992), cat and monkey (Helton *et al*, 1994) and human (Schmuck *et al*, 1994). Finally, given the apparent absence of 5-HT_{2A} and 5-HT_{2C} receptors in fish brain, the findings of Hensley and Cohen (1992) and myself can only be explained by the presence of 5-HT_{2B} receptors in fish brain.

Summary and conclusions

Based on a review of the literature and my results for experiment 1, I have proposed that mirror-directed biting is comparable to biting seen in more "natural" interactions between fish, and to offensive behaviors in rats. Based on these assumptions, the following conclusions were drawn.

The finding that PCPA increases mirror-directed biting in male firemouths adds to the small body of pharmacological literature which suggests that 5-HT is a neurotransmitter associated with inhibiting aggression in both male (Avis and Peeke, 1979) and female (Munro, 1986) Central American cichlids, as well as in more distantly related species (Maler and Ellis, 1987). This finding is also comparable to the effects of PCPA on aggression in rodents (Matte and Tornow, 1978; Vergnes *et al*, 1986), vervet monkeys (Raleigh *et al*, 1980) and birds (Buchanan *et al*, 1994).

PCPA selectively depletes brain 5-HT in fish (Johnston *et al*, 1993; Winberg *et al*, 1993; Adams, Drew and Ebbesson, unpublished data), so it is likely that the increase in mirror-directed biting seen in experiment 1 was caused by a blockade of the brain 5-HT system. Although PCPA reduces brain 5-HT to a level that would not be seen in normal animals, there is evidence that brain 5-HT turnover is inversely correlated with aggression in fish (Winberg *et al*, 1991; Winberg and Nilsson, 1993). Nevertheless, a clear demonstration that brain 5-HT inhibits mirror-directed biting remains to be done with application of 5-HT to brain areas such as the nucleus ventricularis.

As this thesis is the first work to report the role of 5-HT receptor subtypes on aggressive behavior in fish, the results of experiments 2, 3 and 4 were only compared with the

mammalian literature. The finding that 8-OH-DPAT has no effect on mirror-directed biting does not agree with previous work in rodents (McMillen *et al*, 1987; Olivier *et al*, 1989; White *et al*, 1991; Mos *et al*, 1992). This finding also does not agree with the results for experiment 3, which showed that another 5-HT_{1A} agonist, buspirone, does inhibit mirror-directed biting in male firemouths. The results for experiment 3 do agree with previous work in rodents (Olivier *et al*, 1984; McMillen *et al*, 1988; Olivier *et al*, 1989; White *et al*, 1991; Mos *et al*, 1992). Possible explanations for these conflicting results were discussed.

The finding that DOI reduces mirror-directed biting in male firemouths is the first clear report of a selective anti-aggressive effect for this drug in any species. Based on this finding, as well as previous autoradiographic work (Palacios and Dietl, 1988), it is proposed that serotonergic inhibition of mirror-directed biting in male firemouths is mediated by 5-HT_{2B} receptors. Nevertheless, this suggestion remains to be confirmed with the treatment of a 5-HT_{2B} antagonist + DOI.

Side effects of drugs used in experiments 2, 3 and 4 were monitored by noting lack of movement, and, in experiment 3, the appearance of unusual behavior.

In conclusion, the clear effects of PCPA and DOI on mirror-directed biting in male firemouths strongly suggests that brain 5-HT neurons tonically inhibit such behavior, and supports the hypothesis that brain 5-HT activity is associated with inhibiting offensive aggression throughout vertebrate taxa.

REFERENCES

Adams DB, 1971. Defense and territorial behaviour dissociated by hypothalamic lesions in the rat. Nature 232:573-574.

Adams DB, 1979. Brain mechanisms for offense, defense, and submission. Brain and Behavioral Sciences 2:201-241.

Adams DB, 1986. Ventromedial tegmental lesions abolish offense without disturbing predation or defense. Physiology and Behavior 38:165-168.

Ahlenius S, Larsson K, 1989. New aspects on the serotonergic modulation of male rat sexual behaviour. In: Behavioural Pharmacology of 5-HT (Bevan P, Cools AR, Archer T, eds). Hillsdale, New Jersey: Lawrence Erlbaum Associates; 35-53.

Albert DJ, Dyson EM, Walsh ML, Gorzalka BB, 1987. Intermale social aggression in rats: suppression by medial hypothalamic lesions independently of enhanced defensiveness or decreased testicular testosterone. Physiology and Behavior 39:693-698.

Albert DJ, Jonik RH, Walsh ML, 1992. Hormone-dependent aggression in male and female rats: experiential, hormonal, and neural foundations. Neuroscience and Biobehavioral Reviews 16:177-192.

Albert DJ, Petrovic DM, Walsh ML, Jonik RH, 1989. Medial accumbens lesions attenuate testosterone-dependent aggression in male rats. Physiology and Behavior 46:625-631.

Albert PR, Zhou QY, Van Tol HHM, Bunzow JR, Civelli O, 1990. Cloning, functional expression, and mRNA tissue distribution of the rat 5-hydroxytryptamine$_{1A}$ receptor gene. Journal of Biological Chemistry 265:5825-5832.

Andén NE, Dahlström A, Fuxe K, Larsson K, Olson L, Ungerstedt U, 1966. Ascending monoamine neurons to the telencephalon and diencephalon. Acta Physiologica Scandinavica 67:313-326.

Appel NM, Mitchell WmM, Garlick RK, Glennon RA, Titeler M, De Souza EB, 1990. Autoradiographic characterization of (±)-1-(2,5-dimethoxy-4-[^{125}I]iodophenyl)-2-aminopropane ([^{125}I]DOI) binding to 5-HT$_2$ and 5-HT$_{1C}$ receptors in rat brain. Journal of Pharmacology and Experimental Therapeutics 255:843-857.

Arvidsson LE, Hacksell U, Nilsson JLG, Hjorth S, Carlsson A, Lindberg P, Sanchez D, Wikström H, 1981. 8-hydroxy-2-(di-*n*-propylamino)tetralin, a new centrally active 5-hydroxytryptamine receptor agonist. Journal of Medicinal Chemistry 24:921-923.

Avis HH, Peeke HVS, 1979. The effects of pargyline, scopolamine, and imipramine on territorial aggression in the convict cichlid (*Cichlasoma nigrofasciatum*). Psychopharmacology 66:1-2.

Baerends GP, Baerends-van Roon JM, 1950. An introduction to the study of the ethology of cichlid fishes. Behaviour, Supplement 1: 1-243.

Batten TFC, Berry PA, Maqbool A, Moons L, Vandesande F, 1993. Immunolocalization of catecholamine enzymes, serotonin, dopamine and L-dopa in the brain of *Dicentrarchus labrax* (Teleostei). Brain Research Bulletin 31:233-252.

Baumgarten HG, Lachenmayer L, 1972. 5,7-dihydroxytryptamine: improvement in chemical lesioning of indoleamine neurons in the mammalian brain. Zeitschrift für Zellforschung und Mikroskopische Anatomie 135:399-414.

Blanchard RJ, Blanchard DC, 1977. Aggressive behavior in the rat. Behavioral Biology 21:197-224.

Blanchard DC, Blanchard RJ, Takahashi LK, Takahashi T, 1977. Septal lesions and aggressive behavior. Behavioral Biology 21:157-161.

Bonson KR, Johnson RG, Fiorella D, Rabin RA, Winter JC, 1994. Serotonergic control of androgen-induced dominance. Pharmacology Biochemistry and Behavior 49:313-322.

Bonson KR, Winter JC, 1992. Reversal of testosterone-induced dominance by the serotonergic agonist quipazine. Pharmacology Biochemistry and Behavior 42:809-813.

Broekkamp CL, Jenck F, 1989. The relationship between various animal models of anxiety, fear-related psychiatric symptoms and response to serotonergic drugs. In: Behavioural Pharmacology of 5-HT (Bevan P, Cools AR, Archer T, eds). Hillsdale, New Jersey: Lawrence Erlbaum Associates; 321-335.

Buchanan CP, Shrier EM, Hill WL, 1994. Time-dependent effects of PCPA on social aggression in chicks. Pharmacology Biochemistry and Behavior 49:483-488.

Chambard JC, van Obberghen-Schilling E, Haslam RJ, Vouret V, Pouysségur J, 1990. Chinese hamster serotonin (5-HT) type 2 receptor cDNA sequence. Nucleic Acids Research 18:5282.

Conn PJ, Sanders-Bush E, 1984. Selective 5HT-2 antagonists inhibit serotonin stimulated phosphatidylinositol metabolism in cerebral cortex. Neuropharmacology 23:993-996.

Conn PJ, Sanders-Bush E, Hoffman BJ, Hartig PR, 1986. A unique serotonin receptor in choroid plexus is linked to phosphatidylinositol turnover. Proceedings of the National Academy of Sciences 83:4086-4088.

Dahlström A, Fuxe K, 1964. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiologica Scandinavica 62, Supplementum 232:1-55.

de Bruin JPC, 1980. Telencephalon and behavior in Teleost fish: a neuroethological approach. In: Comparative Neurology of the Telencephalon (Ebbesson SOE, ed). New York: Plenum Press; 175-201.

de Vivo M, Maayani S, 1985. Inhibition of forskolin-stimulated adenylate cyclase activity by 5-HT receptor agonists. European Journal of Pharmacology 119:231-234.

Demski LS, 1973. Feeding and aggressive behavior evoked by hypothalamic stimulation in a cichlid fish. Comparative Biochemistry and Physiology A 44:685-692.

Demski LS, Evan AP, Saland LC, 1975. The structure of the inferior lobe of the Teleost hypothalamus. Journal of Comparative Neurology 161:483-498.

Demski LS, Knigge KM, 1971. The telencephalon and hypothalamus of the bluegill (*Lepomis macrochirus*): evoked feeding, aggressive and reproductive behavior with representative frontal sections. Journal of Comparative Neurology 143:1-16.

Ekström P, Ebbesson SOE, 1989. Distribution of serotonin-immunoreactive neurons in the brain of sockeye salmon fry. Journal of Chemical Neuroanatomy 2:201-213.

Ekström P, van Veen T, 1984. Distribution of 5-hydroxytryptamine (serotonin) in the brain of the Teleost *Gasterosteus aculeatus* L. Journal of Comparative Neurology 226:307-320.

Fargin A, Raymond JR, Lohse MJ, Kobilka BK, Caron MG, Lefkowitz RJ, 1988. The genomic clone G-21 which resembles a b-adrenergic receptor sequence encodes the 5-HT_{1A} receptor. Nature 335:358-360.

Foguet M, Hoyer D, Pardo LA, Parekh A, Kluxen FW, Kalkman HO, Stühmer W, Lübbert H, 1992. Cloning and functional characterization of the rat stomach fundus serotonin receptor. EMBO Journal 11:3481-3487.

Forbes IT, Jones GE, Murphy OE, Holland V, Baxter GS, 1995. *N*-(1-methyl-5-indoyl)-*N'*-(3-methyl-5-isothiazolyl)urea: a novel, high-affinity 5-HT2B receptor antagonist. Journal of Medicinal Chemistry 38:855-857.

Francis RC, Jacobson B, Wingfield JC, Fernald RD (1992): Castration lowers aggression but not social dominance in male *Haplochromis burtoni* (Cichlidae). Ethology 90:247-255.

Frankenhuis-van den Heuvel THM, Nieuwenhuys R, 1984. Distribution of serotonin-immunoreactivity in the diencephalon and mesencephalon of the trout, *Salmo gairdneri.* Anatomy and Embryology 169:193-204.

Fuxe K, 1965. Evidence for the existence of monoamine-containing neurons in the central nervous system. IV. The distribution of monoamine terminals in the central nervous system. Acta Physiologica Scandinavica 64, Supplementum 247:37-84.

Gozlan H, El Mestikawy S, Pichat L, Glowinski J, Hamon M, 1987. Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. Nature 305:140-142.

Hasse JJ, 1981. Characters, synonymy and distribution of the middle American cichlid fish *Cichlasoma meeki.* Copeia 1981:210-212.

Helton LA, Thor KB, Baez M, 1994. 5-hydroxytryptamine$_{2A}$, 5-hydroxytryptamine$_{2B}$, and 5-hydroxytryptamine$_{2C}$ receptor mRNA expression in the spinal cord of rat, cat, monkey and human. Neuroreport 5:2617-2620.

Hensley SH, Cohen JL, 1992. Effects of serotonergic agonists and antagonists on ganglion cells in the goldfish retina. Visual Neuroscience 9:353-364.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PPA, 1994. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacological Reviews 46:157-203.

Hoyer D, Pazos A, Probst A, Palacios JM, 1986a. Serotonin receptors in the human brain. I. Characterization and autoradiographic localization of 5-HT_{1A} recognition sites. Apparent absence of 5-HT_{1B} recognition sites. Brain Research 376:85-96.

Hoyer D, Pazos A, Probst A, Palacios JM, 1986b. Serotonin receptors in the human brain. II. Characterization and autoradiographic localization of 5-HT_{1C} and 5-HT_2 recognition sites. Brain Research 376:97-107.

Jéquier E, Lovenberg W, Sjoerdsma A, 1967. Tryptophan hydroxylase inhibition: the

mechanism by which *p*-chlorophenylalanine depletes rat brain serotonin. Molecular Pharmacology 3:274-278.

Jacobs BL, 1987. Central monoaminergic neurons: single-unit studies in behaving animals. In: Psychopharmacology: The Third Generation of Progress (Meltzer HY, ed). New York: Raven Press; 159-169.

Johnston WL, Atkinson JL, Glanville NT, 1992. Effect of PCPA or tryptophan on brain serotonin and on consumption of a high protein or high carbohydrate diet by rainbow trout, *Oncorhynchus mykiss*. Journal of Nutritional Biochemistry 3:421-428

Julius D, Huang KN, Livelli TJ, Axel R, Jessell TM, 1990. The 5-HT2 receptor defines a family of structurally distinct but functionally conserved serotonin receptors. Proceedings of the National Academy of Sciences 87:928-932.

Kah O, Chambolle P, 1983. Serotonin in the brain of the goldfish, *Carassius auratus*. Cell and Tissue Research 234:319-333.

Khan IA, Thomas P, 1993. Immunocytochemical localization of serotonin and gonadotropin-releasing hormone in the brain and pituitary gland of the Atlantic croaker *Micropogonias undulatus*. General and Comparative Endocrinology 91:167-180.

Koe BK, Weissman A, 1966. *p*-chlorophenylalanine: a specific depletor of brain serotonin. Journal of Pharmacology and Experimental Therapeutics 154:499-516.

Kursar JD, Nelson DL, Wainscott DB, Cohen ML, Baez M, 1992. Molecular cloning, functional expression, and pharmacological characterization of a novel serotonin receptor (5-hydroxytryptamine$_{2F}$) from rat stomach fundus. Molecular Pharmacology 42:549-557.

Lagerspetz KYH, Tirri R, Lagerspetz KMJ, 1968. Neurochemical and endocrinological studies of mice selectively bred for aggressiveness. Scandinavian Journal of Psychology 9:157-160.

Langer SZ, Moret C, Raisman R, Dubocovich ML, Briley M, 1980. High-affinity [^{3}H]imipramine binding in rat hypothalamus: association with uptake of serotonin but not of norepinephrine. Science 210:1133-1135.

Leysen JE, Niemegeers CJE, Van Neuten JM, Laduron PM, 1982. [^{3}H]ketanserin (R 41 468), a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. Molecular Pharmacology 21:301-314.

Loric S, Launay JM, Colas JF, Maroteaux L, 1992. New mouse 5-HT2-like receptor. Expression in brain, heart and intestine. FEBS Letters 312:203-207.

Lovenberg W, Jéquier E, Sjoerdsma A, 1967. Tryptophan hydroxylation: measurement in pineal gland, brainstem, and carcinoid tumor. Science 155:217-219.

Maler L, Ellis WG, 1987. Inter-male aggressive signals in weakly electric fish are modulated by monoamines. Behavioural Brain Research 25:75-81.

Marcinkiewicz M, Vergé D, Gozlan H, Pichat L, Hamon M, 1984. Autoradiographic evidence for the heterogeneity of 5-HT$_1$ sites in the rat brain. Brain Research 291:159-163.

Margolis-Kazan H, Halpern-Sebold LR, Schreibman MP, 1985. Immunocytochemical

localization of serotonin in the brain and pituitary gland of the platyfish, *Xiphophorus maculatus*. Cell and Tissue Research 240:311-314.

Matte AC, Tornow H, 1978. Parachlorophenylalanine produces dissociated effects on aggression "emotionality" and motor activity. Neuropharmacology 17:555-558.

McMillen BA, Scott SM, Williams HL, Sanghera MK, 1987. Effects of gepirone, an aryl-piperazine anxiolytic drug, on aggressive behavior and brain monoaminergic neurotransmission. Naunyn-Schmiedeberg's Archives of Pharmacology 335:454-464.

McMillen BA, DaVanzo EA, Scott SM, Song AH, 1988. N-alkyl-substituted aryl-piperazine drugs: relationship between affinity for serotonin receptors and inhibition of aggression. Drug Development Research 12:53-62.

Mendelson SD, Gorzalka BB, 1986. 5-HT_{1A} receptors: differential involvement in female and male sexual behavior in the rat. Physiology and Behavior 37:345-351

Mendelson SD, Gorzalka BB, 1989. Differential roles of 5-HT receptor subtypes in female sexual behaviour. In: Behavioural Pharmacology of 5-HT (Bevan P, Cools AR, Archer T, eds). Hillsdale, New Jersey: Lawrence Erlbaum Associates; 55-71.

Middlemiss DN, Fozard JR, 1983. 8-hydroxy-2-(di-*n*-propylamino)-tetralin discriminates between subtypes of the 5-HT_1 recognition site. European Journal of Pharmacology 90:151-153.

Mos J, Kruk MR, van der Poel AM, Meelis W, 1982. Aggressive behavior induced by electrical stimulation in the midbrain central gray of male rats. Aggressive Behavior 8:261-284.

Mos J, Lammers JHCM, van der Poel AM, Bermond B, Meelis W, Kruk MR, 1983. Effects of midbrain central gray lesions on spontaneous and electrically induced aggression in the rat. Aggressive Behavior 9:133-135.

Mos J, Olivier B, Tulp MThM, 1992. Ethopharmacological studies differentiate the effects of various serotonergic compounds on aggression in rats. Drug Development Research 26: 343-360.

Munro AD, 1986. Effects of melatonin, serotonin, and naloxone on aggression in isolated cichlid fish (*Aequidens pulcher*). Journal of Pineal Research 3:257-262.

Neil SJ, 1984. Field studies of the behavioral ecology and agonistic behavior of *Cichlasoma meeki* (Pisces: Cichlidae). Environmental Biology of Fishes 10:59-68.

Olivier B, Olivier-Aardema R, Wiepkema PR, 1983. Effect of anterior hypothalamic and mammillary area lesions on territorial aggressive behaviour in male rats. Behavioural Brain Research 9:59-81.

Olivier B, van Aken H, Jaarsma I, van Oorschot R, Zethof T, Bradford D, 1984. Behavioural effects of psychoactive drugs on agonistic behaviour of male territorial rats (resident-intruder model). In: Ethopharmacological Aggression Research (Miczek KA, Kruk MR, Olivier B, eds). New York: Alan R Liss Inc.; 137-156.

Olivier B, Mos J, van der Heyden J, Hartog J, 1989. Serotonergic modulation of social interactions in isolated male mice. Psychopharmacology 97:154-156.

Palacios JM, Dietl MM, 1988. Autoradiographic studies of serotonin receptors. In: The Serotonin Receptors (Sanders-Bush E, ed). Clifton, New Jersey: Humana Press; 89-138.

Parent A, 1983. The monoamine-containing neuronal systems in the Teleostean brain. In: Fish Neurobiology, volume 2 (Davis RE, Northcutt RG, eds). Ann Arbor: University of Michigan Press; 285-315.

Parent A, Dubé L, Braford MR Jr, Northcutt RG, 1978. The organization of monoamine-containing neurons in the brain of the sunfish (*Lepomis gibbosus*) as revealed by fluorescence microscopy. Journal of Comparative Neurology 182:495-516.

Pazos A, Cortés R, Palacios JM, 1985. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Research 346:231-249.

Pazos A, Hoyer D, Palacios JM, 1984. The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site. European Journal of Pharmacology 106:539-546.

Pazos A, Palacios JM, 1985. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. Brain Research 346:205-230.

Peroutka SJ, 1985. Selective interaction of novel anxiolytics with 5-hydroxytryptamine$_{1A}$ receptors. Biological Psychiatry 20:971-979.

Peroutka SJ, Snyder SH, 1979. Multiple serotonin receptors: differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol. Molecular Pharmacology 16:687-699.

Pompeiano M, Palacios JM, Mengod G, 1994. Distribution of the serotonin 5-HT$_2$ receptor family mRNAs: comparison between 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. Molecular Brain Research 23:163-178.

Pritchett DB, Bach AWJ, Wozny M, Taleb O, Dal Toso R, Shih JC, Seeburg PH, 1988. Structure and functional expression of cloned rat serotonin 5-HT$_2$ receptor. EMBO Journal 7:4135-4140.

Raleigh MJ, Brammer GL, Yuwiler A, Flannery JW, McGuire MT, Geller E, 1980. Serotonergic influences on the social behavior of vervet monkeys (*Cercopithecus aethiops sabaeus*). Experimental Neurology 68:322-334.

Rolinski Z, 1975. Pharmacological studies on isolation-induced aggressiveness in mice in relation to biogenic amines. Polish Journal of Pharmacology and Pharmacy 27:37-44.

Saltzman AG, Morse B, Whitman MM, Ivanshchenko Y, Jaye M, Felder S, 1991. Cloning of the human serotonin 5-HT2 and 5-HT1C receptor subtypes. Biochemical and Biophysical Research Communications 181:1469-1478.

Samanin R, 1989. Serotonin and feeding. In: Behavioural Pharmacology of 5-HT (Bevan P, Cools AR, Archer T, eds). Hillsdale, New Jersey: Lawrence Erlbaum Associates; 259-281.

Sánchez C, Arnt J, Hyttel J, Moltzen EK, 1993. The role of serotonergic mechanisms in inhibition of isolation-induced aggression in male mice. Psychopharmacology 110:53-59.

Schmidt AW, Peroutka SJ, 1989. 5-hydroxytryptamine receptor "families." FASEB Journal 3:2242-2249.

Schmuck K, Ullmer C, Engels P, Lübbert H, 1994. Cloning and functional characterization of the human 5-HT_{2B} serotonin receptor. FEBS Letters 342:85-90.

Shannon M, Battaglia G, Glennon RA, Titeler M, 1984. 5-HT_1 and 5-HT_2 binding properties of derivatives of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA). European Journal of Pharmacology 102:23-29.

Sheard MH, 1973. Aggressive behavior: modification by amphetamine, p-chlorophenylalanine and lithium in rats. Agressologie 14:327-330.

Twarog BM, Page IH, 1953. Serotonin content of some mammalian tissues and urine and a method for its determination. American Journal of Physiology 175:157-161.

van den Berg MJ, ter Horst GJ, Koolhaas JM, 1983. The nucleus premammillaris ventralis (PMV) and aggressive behavior in the rat. Aggressive Behavior 9:41-47.

Vergnes M, Depaulis A, Boehrer A, 1986. Parachlorophenylalanine-induced serotonin depletion increases offensive but not defensive aggression in male rats. Physiology and Behavior 36:653-658.

Vergnes M, Depaulis A, Boehrer A, Kempf E, 1988. Selective increase of offensive behavior in the rat following intrahypothalamic 5,7-DHT-induced serotonin depletion. Behavioural Brain Research 29:85-91.

Vochteloo JD, Koolhaas JM, 1987. Medial amygdala lesions in male rats reduce aggressive behavior: interference with experience. Physiology and Behavior 41:99-102.

Wainscott DB, Cohen ML, Schenk KW, Audia JE, Nissen JS, Baez M, Kursar JD, Lucaites VL, Nelson DL, 1993. Pharmacological characteristics of the newly cloned rat 5-hydroxytryptamine$_{2F}$ receptor. Molecular Pharmacology 43:419-426.

Watson AHD, 1980. The distribution of aminergic neurones and their projections in the brain of the Teleost, *Myoxocephalus scorpius*. Cell and Tissue Research 208:299-312.

Welch BL, Welch AS, 1969. Aggression and the biogenic amine neurohumors. In: Aggressive Behaviour (Garattini S, Sigg EB, eds). Amsterdam: Excerpta Medica Foundation; 188-202.

White SM, Kucharik RF, Moyer JA, 1991. Effects of serotonergic agents on isolation-induced aggression. Pharmacology Biochemistry and Behavior 39:729-736.

Winberg S, Nilsson GE, 1993. Time course of changes in brain serotonergic activity and brain tryptophan levels in dominant and subordinate juvenile Arctic charr. Journal of Experimental Biology 179:181-195.

Winberg S, Nilsson GE, Olsén KH, 1991. Social rank and brain levels of monoamines and monoamine metabolites in Arctic charr, *Salvelinus alpinus* (L.). Journal of Comparative Physiology A: 168:241-246.

Winberg S, Nilsson GE, Spruijt BM, Höglund U, 1993. Spontaneous locomotor activity in Arctic charr measured by a computerized imaging technique: role of brain serotonergic

activity. Journal of Experimental Biology 179:213-232.

Yu L, Nguyen H, Le H, Bloem LJ, Kozak CA, Hoffman BJ, Snutch TP, Lester HA, Davidson N, Lübbert H, 1991. The mouse 5-HT_{1C} receptor contains eight hydrophobic domains and is X-linked. Molecular Brain Research 11:143-149.

APPENDIX

I spent several months trying out various methods of testing aggression in firemouths. The advantages and disadvantages of each are listed below

Two males interacting following the removal of an opaque partition. While this obviously provided the most "realistic" setting, there was the problem of one of the fish being "beaten up" and how this experience might affect future interactions. Another theoretical problem with this procedure is whether it actually measures aggression or just fighting ability. There was also a practical problem related to the speed at which two firemouths circle each other while fighting, which required that the fish would have to be marked, or that the interaction would have to be videotaped. Marking the fish in a way that would not change its behavior, or that of its opponent, proved difficult. On the other hand, videotapes of such interactions would have to be analyzed frame-by-frame, which would be very time-consuming.

Two males interacting through a transparent partition following the removal of an opaque partition. This procedure was an attempt to eliminate both the theoretical and practical problems of the previous arrangement. In this case a new problem emerged: oftentimes one of the fish would feed off the bottom or swim against the glass (as if trying to get through it), rather than interacting with the other fish.

Two males interacting through a grid following the removal of an opaque partition. I had hoped that, because the fish could "smell" each other, the fish would interact rather than engaging in other behaviors. Nevertheless, feeding off the bottom and swimming against the glass persisted.

One male interacting with a mirror following the removal of an opaque partition. The problem that arose with this arrangement was a large number of fish (approximately 67%) would not come out of their shelters and interact with the mirror. I then tried having another fish in the tank, separated from the focal fish by a grid. This was necessary to prevent one of the fish from being repeatedly attacked and eventually killed. The presence of either a male or female did not produce an increase in the number of reactive fish. Here I should mention that,

in all 3 scenarios (isolated, with male, with female), every individual fish was presented with the mirror on 5 consecutive days. Friedman analysis of this data showed no difference in the number of bites between any of the days.

I then tried videotaping an isolated fish, rather than direct observation. Almost every fish reacted to the mirror when I did this, and it was the procedure I adopted.

During subsequent pilot studies it was found that fish who spent the duration of the observation in the shadows at the rear of their shelter could not see their reflection. To eliminate this problem, some minimum criteria was needed to establish that the fish had seen its reflection. As the front of the shelter was open (to be able to observe the fish at all times), it was easy to see when the fish moved to the edge of the shelter facing the mirror, even if it did not move outside it. I then found that the fish could clearly see their reflection while at the mouth of the shelter. Thus, fish were assumed to have seen their reflection if their mouth touched the mirror-facing-edge of the shelter at least once during the observation. Fish that did not do this were not used.