SYNAPSE 30:318–328 (1998)

Cellular Electrophysiological Effects of Chronic Fluoxetine and Duloxetine Administration on Serotonergic Responses in the Aging Hippocampus

JANE E. SMITH AND JOAN M. LAKOSKI*
Departments of Pharmacology and Anesthesia, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania

KEY WORDS aging; serotonin; hippocampus; electrophysiology; duloxetine; fluoxetine

ABSTRACT The pharmacological and physiological effects of chronic administration of the selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor (SSRI) fluoxetine and the dual 5-HT/norepinephrine (NE) reuptake inhibitor duloxetine were compared on 5-HT-mediated electrophysiological responses recorded in the hippocampus of young (3–5 months) and old (17–20 months) female Fischer 344 rats. Fluoxetine, duloxetine, or vehicle (saline) was administered once daily for 14 days (10 mg/kg, i.p.) and extracellular recordings of spontaneously firing CA1 and CA3 pyramidal neurons were conducted 24 h following the last injection using microiontophoretic drug application techniques in a chloral hydrate anesthetized preparation. The recovery times (RT_{50} values; sec) following 5-HT application on pyramidal neurons were significantly increased in the young and old chronic fluoxetine (FLX) treated groups (73% and 104%, respectively; $P < 0.05$), but not chronic duloxetine- (DLX) or vehicle- (VEH) treated groups. Following prolonged application of duloxetine (5–10 min), the 5-HT RT_{50} values were significantly increased in the young FLX groups as compared to the age-matched DLX- and VEH-treated groups. In contrast, a significant decline in the time to recovery produced by 5-HT (52%) was observed in the old vs. young FLX-treated group following the second co-application of 5-HT with duloxetine. Within each drug treatment and age group, co-application of duloxetine and 5-HT did not alter the inhibitory responses (IT_{50} values; nC) produced by the application of 5-HT alone. These results demonstrate cellular adaptive changes in serotonergic neuronal function occur following repeated exposure to 5-HT reuptake inhibitors in an age-dependent manner. **Synapse 30:318–328, 1998.**

INTRODUCTION

The serotonergic system of the CNS is organized with serotonin (5-hydroxytryptamine, 5-HT) -containing cell bodies in the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) innervating widespread regions of the forebrain, including the hippocampus (Azmitia, 1991; Kohler and Steinbusch, 1982). Multiple 5-HT receptors, including the 5-HT_{1A} subtype (Hall et al., 1985; Hoyer et al., 1994; Vergé et al., 1986), are distributed throughout the hippocampus (Brown et al., 1993; Chalmers and Watson, 1991; Grossman et al., 1993). Direct physiological effects of 5-HT application in this region include 5-HT_{1A} receptor-mediated inhibition of pyramidal cell firing (Andrade and Nicoll, 1987; Chaput et al., 1988; Dugar and Lakoski, 1997; Smith and Lakoski, 1997a,b). The 5-HT transporter (5-HTT) is also uniformly distributed throughout the hippocampus (Hensler et al., 1994; Sur et al., 1996) and serves as a target of inhibitors selective for 5-HT reuptake (SSRIs). As deficits in central serotonergic transmis-

Contract grant sponsor: U.S.P.H.S.; Contract grant number: P01 AG10514 awarded by the National Institute of Aging; Contract grant sponsors: Pennsylvania State University Gerontology Center Faculty Development Award, Pennsylvania State University College of Medicine, Department of Pharmacology; Contract grant sponsor: National Institute of Aging U.S.P.H.S.; Contract grant number: T32 AG00048.

*Correspondence to: Joan M. Lakoski, Ph.D., Departments of Pharmacology and Anesthesia, The Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033–0850. E-mail: jml19@psu.edu

Present address for Jane Smith: Departments of Environmental Health and Pharmacology, Box 357234, Room F553, University of Washington, Seattle, WA 98195.

Received 8 July 1997; Accepted 3 February 1998

sion have been implicated in the etiology of depression (Blier et al., 1987; Coppen, 1967; Meltzer and Lowy, 1987; Murphy et al., 1978), blockade of 5-HT reuptake processes by SSRIs serve to enhance the synaptic actions of this indoleamine.

Depression is a prominent psychiatric disorder in the elderly community, specifically in the female population (Blazer, 1980; Casey, 1994; Khan et al., 1993; Koenig and Blazer, 1992; Weissman and Olfson, 1995). In the elderly patient, the time required for the improvement of depressive symptoms with antidepressant therapy has been reported to be as long as 6–12 weeks (Slotkin et al., 1989). However, a delay of only 2–3 weeks after initiation of antidepressant treatment is typically required for the onset of clinical efficacy in younger adult patients (Baldessarini, 1990; Briley and Moret, 1993; Richelson, 1991). This pattern of therapeutic delay is characteristic of SSRIs and involves adaptive changes in the serotonergic system (Blier and de Montigny, 1994; Siever et al., 1986; Watchel, 1989). For example, pretreatment with the SSRIs citalopram or paroxetine for several weeks decreased the physiological sensitivity of somatodendritic 5-HT_{1A} autoreceptors in the DRN, independent of changes in hippocampal responses (Chaput et al., 1986; de Montigny et al., 1990; Piñeyro et al., 1994). Investigations of the cellular physiological effects of chronic exposure to SSRIs on serotonergic function have not yet been examined across the lifespan.

The delayed and/or reduced effectiveness of antidepressant therapy in the elderly may involve age-related declines in the serotonergic neuronal system. Age-associated alterations have been characterized with respect to the synthesis, release, and turnover of serotonin (Gozlan et al., 1990; Moretti et al., 1987; Schlicker et al., 1989; Tanila et al., 1994; Venero et al., 1993), 5-HT receptor density (Arranz et al., 1993; Burnet et al., 1994; Marcusson et al., 1984), density of the 5-HT transporter (Arora et al., 1993), as well as physiological sensitivity to this indoleamine (Arentsen and Lakoski, 1993; Smith and Lakoski, 1997b). Declines in recovery following 5-HT-induced inhibition in the aging hippocampus have also been identified following microiontophoretic application of reuptake inhibitors (Smith and Lakoski, 1997b) or neurochemical denervation of serotonergic afferent input to the hippocampus (Dugar and Lakoski, 1997). However, the adaptive responses which occur in this neuronal system following the chronic administration of 5-HT reuptake inhibitors have not yet been characterized with respect to aging.

The present study investigated the in vivo electrophysiological profile of 5-HT, duloxetine, a dual 5-HT and norepinephrine (NE) reuptake inhibitor, and fluoxetine, an SSRI, in the hippocampus of the aging female Fischer 344 rat. A previous study noted that duloxetine and fluoxetine exhibit similar, but not identical, pharmacological and physiological profiles in the hippocampus (Smith and Lakoski, 1997a). Following chronic pretreatment with duloxetine (DLX), fluoxetine FLX), or vehicle (VEH), 5-HT-mediated responses were recorded in a chloral hydrate anesthetized preparation and the inhibitory (IT_{50} values) and recovery responses (RT_{50} values) to 5-HT application alone and with concomitant DLX application were determined in young and old treatment groups.

MATERIALS AND METHODS

Animals

Virgin female Fischer 344 rats (NIA; Bethesda, MD) were used at ages 3–5 months and 17–20 months. These ages represent different stages of the reproductive lifespan of the female rat, including the sexually mature young adult and reproductively senescent groups, respectively (Finch et al., 1984; Lakoski, 1994; Wise, 1983). Ongoing studies in our laboratory are utilizing this model to examine the effects of pre- and postmenopausal hormone environments on the physiological profile of central serotonergic systems. For studies with chronic FLX administration, an older age group (24–27 months) was included for comparison to the younger age groups. Animals were maintained under standard housing conditions with a 12-h light/dark cycle (lights on 0700). Food and water were provided ad libitum.

Drug solutions

For chronic drug administration studies, DLX HCl (courtesy of Eli Lilly; Indianapolis, IN) and FLX HCl (Sigma Chemical, St. Louis, MO) were dissolved in 0.9% NaCl and stored at 4°C until use. A 0.9% NaCl solution was prepared for vehicle injections to a control treatment group.

For microiontophoretic studies, DLX HCl (0.01 M in 0.1 M NaCl), FLX HCl (0.01 M in 0.1 M NaCl) and serotonin creatinine sulfate (5-HT; 0.04 M; Research Biochemicals International; Natick, MA) were used at pH 5.5. Drug solutions were aliquoted and stored at -80°C until use.

Chronic drug administration protocol

Animals were handled for 1 week prior to initiation of injections and randomly divided into three treatment groups, including FLX, DLX, and control (VEH). DLX or FLX was administered (10 mg/kg, i.p., 0.1% body weight) once daily for 14 days between 0800–1000 h. Control animals were treated with VEH (0.9% NaCl, i.p.) in a similar manner. Throughout the injection period, body weight was monitored daily and vaginal smears performed to determine the pattern of estrous cyclicity.

Vaginal smears indicated that the estrous cycle was disrupted in the young treatment groups with animals in metestrous or diestrous stages of the cycle (character-

ized by low circulating estrogen levels). The old treatment groups were in diestrous before and throughout the injection period.

Electrophysiological recordings

Extracellular recordings in the hippocampus were performed 24 h following the last drug injection; recording sessions were initiated between 0900 and 1100 h to control for possible circadian variations in the endocrine environment. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.; Research Biochemicals International) and placed in a Kopf small animal stereotaxic instrument. This anesthetic has been reported to have no significant effect on spontaneously active hippocampal pyramidal cell firing rates in aging male and female rats (Olpe and Steinmann, 1982; Dugar and Lakoski, 1997; Smith and Lakoski, 1997a,b). A constant level of anesthesia was maintained throughout the experiment with supplemental doses of chloral hydrate administered via a cannulated lateral tail vein. Body temperature was maintained at approximately 36°C using a thermostatically regulated heating pad.

Extracellular recordings from pyramidal neurons of the CA1 and CA3 regions of the hippocampus were conducted according to the procedures of Smith and Lakoski (1997b). Briefly, a small burr hole was drilled 3.8 mm lateral to the midline and 4.0 mm posterior to bregma (Paxinos and Watson, 1986) for electrode placement. Five-barrel micropipettes (Model P-5, ASI Instruments, Warren, MI) were pulled (Narishige vertical electrode puller) and beveled under a microscope to a tip diameter of 6–10 µm. The side barrels were filled with 5-HT, DLX, FLX, or 2 M NaCl for channel balancing (Salmoiraghi and Weight, 1967). The recording barrel was filled with a 2% Pontamine Sky Blue solution (impedance 1–4 MΩ). Neurons of the CA1 and CA3 regions were found between 2.0 and 2.5 mm and 2.6 and 3.2 mm vertical to the surface of the rat brain, respectively. Conventional amplification methods were used as described in Dugar and Lakoski (1997) and Smith and Lakoski (1997b).

Platinum leads connected to an electrophoresis unit (Model E104 B/4, Fintronics Inc., Orange, CT, USA) were used for current (5–40 nA) ejection of drugs from the electrode barrels. The duration of drug ejection ranged from 1 to 10 min followed by at least a 2-min recovery interval between successive drug applications. The currents used for the microiontophoretic application of 5-HT were applied in a random order (e.g., 5, 40, 20, and 10 nA) to control for current-dependent drug effects. To avoid variability that might be introduced in the analysis of co-application data, the majority of cells (95%) were tested under conditions in which DLX was co-applied at 10 nA. This current has been previously demonstrated to have minimal to no direct effects on spontaneous baseline firing (Smith and Lakoski, 1997a,b). Typically, a current of -10 nA was used to retain cationic drugs in the electrode barrels.

Recording sites were verified histologically by dye ejection at the close of each experiment. Current (-20 µA) was passed through the recording barrel for 40 min using a bipolar constant current source (VL-1200d, Fintronics). The brain was removed and stored in 10% buffered formalin. Brain sections (50 µm) were prepared, placed on subbed slides (0.5% gelatin), and counterstained with cresyl violet. Electrode placement was confirmed microscopically. Only data obtained from CA1 and CA3 hippocampal pyramidal subfields were included for further analysis.

Microiontophoretic data analysis

Spontaneous baseline firing rates were calculated using the mean rate of cell firing for 1 min prior to the initial drug application and reported as mean ± SEM. Inhibition of pyramidal cell firing produced by drug application was assessed using the IT_{50} value. As defined by de Montigny and Aghajanian (1977), the IT_{50} value (nC) is the current in nA × time in seconds required to obtain a 50% decrease in firing rate.

The recovery phase following inhibition of cell firing was assessed using the RT_{50} value. The RT_{50} value is defined as the time in seconds required for the cell to recover to 50% of the mean baseline firing rate (de Montigny et al., 1980) and was calculated from the minute following the interval of drug application. The RT_{50} value is an index of the ability of a presynaptically located transporter to remove a neurotransmitter from the synaptic cleft (de Montigny et al., 1980).

Statistical analysis

Results are reported as mean ± SEM with Sigma Stat (Version 1.0, Jandel Scientific, San Rafael, CA) used for statistical comparisons. Spontaneous cell firing rates, IT_{50}, and RT_{50} values were compared using a two-way ANOVA across age and drug treatment groups followed by Student-Newman-Keuls' method for pairwise comparisons, with $P < 0.05$ chosen as the level of significance. Data recorded from neurons located in CA1 and CA3 subfields were combined due to a lack of significant differences in physiological and pharmacological parameters at each age. Only 1–2 cells per animal were included for analysis in order to avoid bias of the results with respect to drug treatment or age. Integrated frequency histograms were scanned for reproduction (Logitech ScanMan Model 256; Logitech, Fremont, CA) into Logitech FotoTouch (Version 2.1; Logitech).

RESULTS

Effects of chronic administration of DLX, FLX, or VEH on hippocampal pyramidal cell firing rates

The spontaneous firing rates of hippocampal pyramidal neurons were not significantly altered in age-

TABLE I. Spontaneous cell firing rate of hippocampal pyramidal neurons following chronic fluoxetine, duloxetine, or vehicle administration in the chloral hydrate/anesthetized female Fischer 344 rat

	Hippocampal cell firing rates (spikes/10 sec)		
Age	Fluoxetine	Duloxetine	Vehicle
3–5 Months	102.5 ± 13.8 n = 11	93.3 ± 12.9 n = 18	101.0 ± 15.5 n = 12
17–20 Months	106.3 ± 6.5 n = 7	55.3 ± 5.7 n = 6	131.3 ± 30.6 n = 8

Data are expressed as mean ± SEM.

matched FLX, DLX, or VEH treatment groups (Table I; $F_{[2, 56]} = 1.932$, $P < 0.154$); no significant differences in cell firing rates were observed between ages.

Sensitivity of hippocampal cells to application of 5-HT alone following chronic FLX, DLX, or VEH

The inhibitory response (IT_{50} values) produced by 5-HT application in hippocampal neurons was not significantly altered following chronic FLX or DLX administration as compared to chronic VEH groups in both ages (Fig. 3; $F_{[2, 5]} = 0.337$, $P < 0.715$). As illustrated in recordings of hippocampal neurons from 3 and 5 month FLX, DLX, and VEH animals (Fig. 1), microiontophoretic application of 5-HT (10, 20, and 40 nA) consistently inhibited cell firing with a 50% reduction in baseline firing observed in the FLX, DLX, and VEH treated animals (IT_{50} = 600, 800, and 1,200 nC, respectively). Similarly, recordings of hippocampal cells from 18–20 month animals illustrate the inhibitory effects of 5-HT in FLX, DLX, and VEH treated groups (Fig. 2). The inhibitory response produced by 5-HT application (10 nA) in an 18 month VEH animal (IT_{50} = 400 nC; Fig. 2C) was not altered or slightly increased following FLX or DLX in 20 and 19 month animals (IT_{50} = 300 and 400 nC, Fig. 2A and B, respectively).

In contrast to the IT_{50} values, the rate of recovery (RT_{50} values) following 5-HT application was significantly increased in both the young and old FLX as compared to DLX and VEH treated groups (Fig. 3; $F_{[2, 56]} = 15.344$, $P < 0.0001$). As shown in frequency histograms of pyramidal neurons from young DLX and VEH animals, cell firing recovers rapidly following 5-HT application (RT_{50} = 10 and 10 sec; Fig. 1B and C, respectively). This recovery response was increased in the young FLX animal (RT_{50} = 70 sec; Fig. 1A). Similar to young animals, cell firing in old DLX and VEH animals rapidly recovers following 5-HT microiontophoresis (RT_{50} = 10 and 30 sec; Fig. 2B and C, respectively). Additionally, the time to recovery was increased in the 19 month FLX-treated animal (RT_{50} = 70 sec; Fig. 2A).

Responses of hippocampal cells to co-application of 5-HT and DLX following chronic of FLX, DLX, or VEH

As observed with the application of 5-HT alone, the IT_{50} values produced by 5-HT co-applied with DLX were not altered following pretreatment with FLX, DLX, or VEH in either age group (Figs. 1, 2; summary data not shown). However, the RT_{50} values were significantly altered in an age- and treatment-related manner with co-application of 5-HT and DLX (Figs. 2, 3, 4).

As illustrated in a Figure 1A and B, the first co-application of DLX increased the recovery response following 5-HT application in young FLX and DLX animals (RT_{50} = 70, 80, and 80 sec and 10, 50, and 30 sec; 5-HT alone, first and second co-applications; FLX and DLX animals, respectively). However, in the VEH animal co-application of DLX with 5-HT did not increase the time to recovery produced by 5-HT alone until the second co-application (RT_{50} = 10, 10, and 30 sec; 5-HT alone, first and second co-applications, respectively; Fig. 1C).

The RT_{50} value following 5-HT application increased with the second co-application of DLX and 5-HT in the old DLX animal (RT_{50} = 10, 10, and 20 sec; 5-HT alone, first and second co-applications, respectively; Fig. 2B). However, the recovery response to 5-HT was increased with the first co-application of DLX in the old FLX animal (RT_{50} = 70 and 110 sec; 5-HT alone and first co-application, respectively; Fig. 2A). In the same cell, the recovery response was decreased with the second co-application of 5-HT with DLX (RT_{50} = 50 sec; Fig. 2A). Similar to the young VEH and old DLX animals, the time to recovery produced by 5-HT application only increased following prolonged DLX application in the old VEH animal (RT_{50} = 30, 30, and 50 sec; 5-HT alone, first and second co-applications, respectively; Fig. 2C).

Statistical analysis of DLX co-application with 5-HT was carried out for RT_{50} values obtained across ages and treatment groups (N's, see Fig. 4; first co-application: $F_{[2, 42]} = 9.716$, $P < 0.0003$; second co-application; $F_{[2, 31]} = 7.180$, $P < 0.0027$). The RT_{50} values for 5-HT alone and co-applied with DLX in the young FLX group were significantly increased as compared to the recovery responses for the same applications in both 3–5 month and 17–20 month DLX and VEH animals. Likewise, the time to recovery following 5-HT application alone was significantly enhanced ($P < 0.05$) in the old FLX group as compared to 3–5 month and 17–20 month DLX and VEH animals. However, with the co-application of 5-HT and DLX the pattern of recovery was different in the old FLX group than in the young FLX group, such that the RT_{50} value for 5-HT was not altered with the first DLX co-application. Furthermore, the time to recovery for the second co-application of 5-HT with DLX was significantly reduced (52%) as compared to the same application in the young FLX

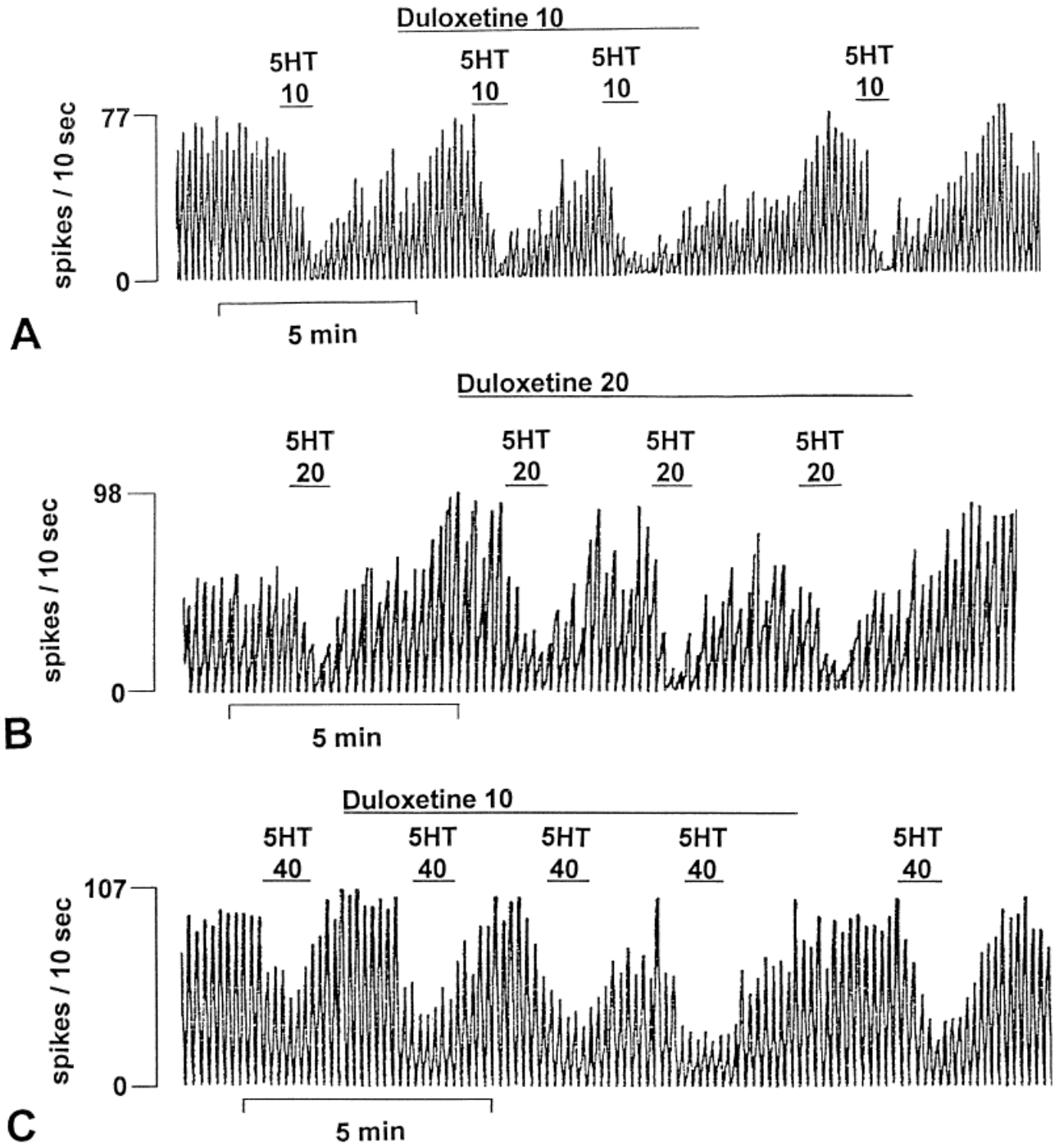

Fig. 1. Effects of microiontophoretic application of 5-HT and DLX on spontaneously active hippocampal pyramidal neurons recorded in 3 and 5 month old chloral hydrate-anesthetized female Fischer 344 rats following chronic FLX, DLX, or VEH administration. Representative integrated firing rate histograms show the inhibition of cell firing produced by 5-HT alone and co-applied with DLX. The inhibition produced by 5-HT application alone and co-applied with DLX was similar in the FLX (**A**), DLX (**B**), and VEH (**C**) animals. In contrast, the recovery phase following 5-HT application was increased in the FLX, but not the DLX, animal as compared to the VEH control. The RT_{50} values for 5-HT alone were increased with DLX co-application in the DLX animal. In contrast, DLX co-application did not significantly alter the RT_{50} values produced by 5-HT alone in the FLX or VEH animals. Bars indicate the duration of drug application (minutes) with current applied in nA indicated by numbers.

group. In an additional FLX group of 22–24 month animals, the RT_{50} value for the first co-application of 5-HT and DLX was also significantly increased (89%) as compared to the recovery response produced by the application of 5-HT alone (Table II; $F_{[2, 13]} = 4.380$, $P < 0.0352$). In this oldest age group, the time to recovery

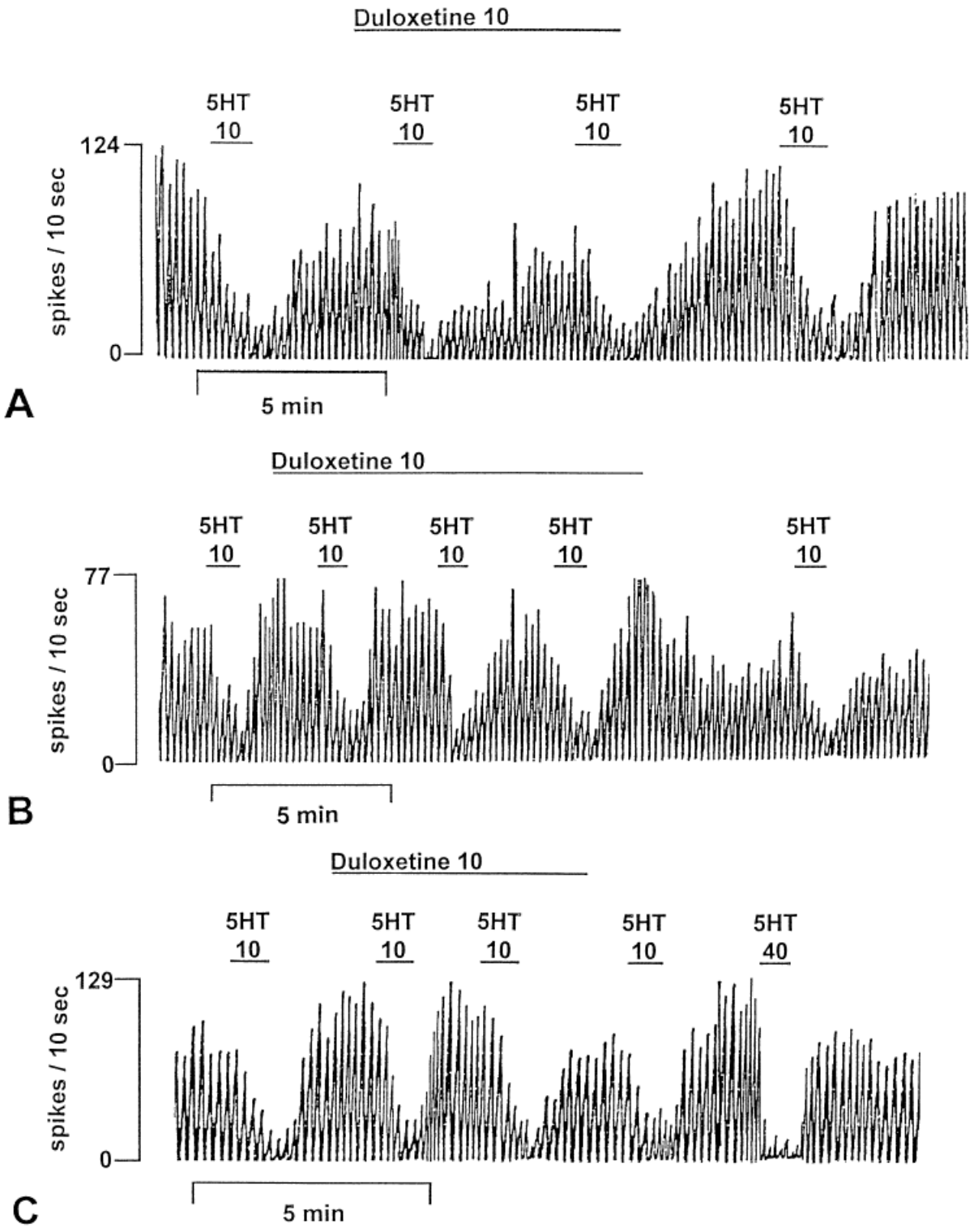

Fig. 2. Effects of microiontophoretic application of 5-HT and DLX on spontaneously active hippocampal pyramidal neurons recorded in 18, 19, and 20-month-old chloral hydrate-anesthetized female Fischer 344 rats following DLX, FLX, or VEH treatment. Representative integrated histograms illustrate the rapid inhibition of cell firing produced by 5-HT application in all treatment groups. The recovery phase following 5-HT application alone was significantly increased in the FLX (**A**) animal as compared to the DLX (**B**) animal and VEH (**C**) control. The IT_{50} values produced by the microiontophoresis of 5-HT were unchanged with DLX co-application. Similarly, DLX co-application did not alter the RT_{50} values produced by 5-HT in the DLX and VEH groups (**B,C**). In the FLX animal (**A**), the RT_{50} was increased with the first co-application of 5-HT; however, an attenuation of the recovery response was seen with the second co-application of 5-HT. Bars indicate the duration of drug application (minutes) with current applied in nA indicated by numbers.

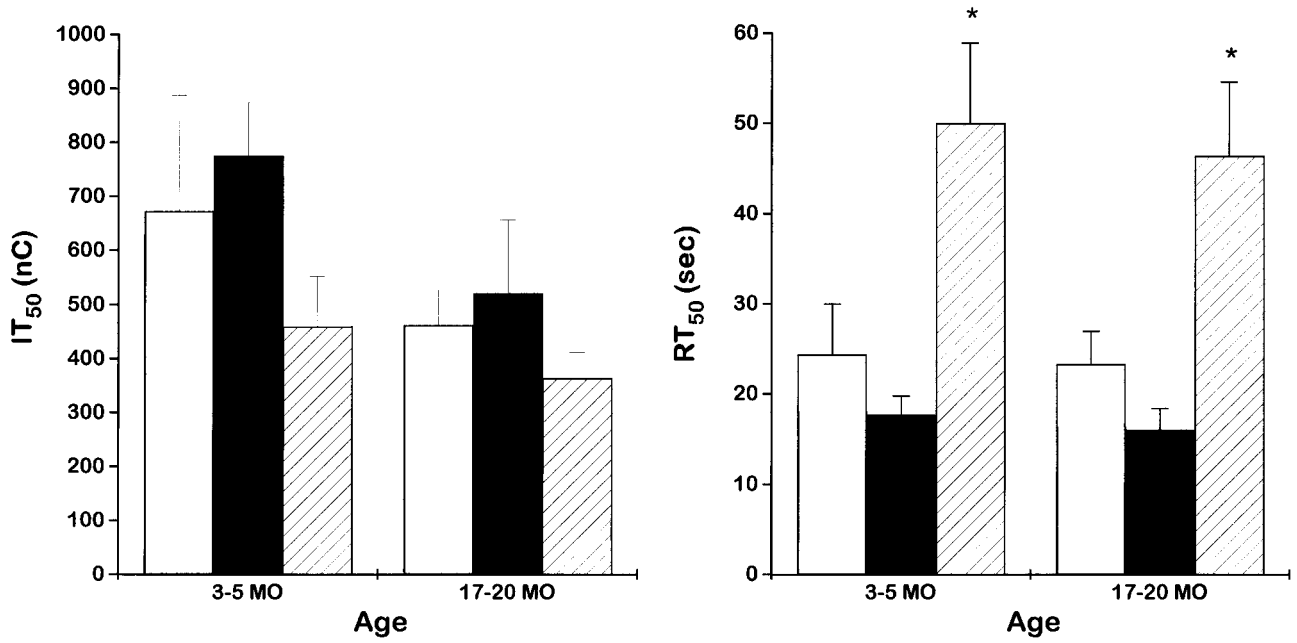

Fig. 3. Inhibitory and recovery responses for the microiontophoretic application of 5-HT in CA1 and CA3 hippocampal neurons recorded from chloral hydrate-anesthetized female Fischer 344 rats following FLX, DLX, or VEH administration. Data are IT_{50} and RT_{50} values using responses obtained from cells recorded from 3–5 month and 17–20 month animals (n [cells/animals] = 11/9, 18/9, 12/7 and 7/6, 6/5, 8/7; FLX, DLX, VEH; young and old, respectively); responses to iontophoretic currents were pooled to obtain mean ± SEM values. White bars indicate values for the VEH group. Hatched and shaded bars indicate values for the DLX and FLX groups, respectively. *Indicates significant difference from VEH and DLX groups ($P < 0.05$).

following 5-HT application returned to baseline values with the continued application of DLX (second co-application).

DISCUSSION

This study addressed the physiology and pharmacology of serotonergic neuronal responses recorded in the aging hippocampus following repeated administration of an SSRI in comparison with a putative antidepressant with high affinity for both the norepinephrine (NE) and 5-HT transporter. The 5-HT recovery response (RT_{50} values) was significantly enhanced in the FLX treatment groups as compared to the DLX and VEH groups across ages. However, with co-application of 5-HT and DLX, the recovery responses produced by 5-HT were significantly decreased in the old FLX animals as compared to the same co-application in the young FLX group. This pattern of recovery was observed in an older FLX group (22–24 month) as well as with co-application of FLX and 5-HT in 17–20 month FLX animals (data not shown). This biphasic recovery response was previously noted in drug-naive 24–27 month female Fischer 344 rats (Smith and Lakoski, 1997b). However, the present study is the first to report this pattern of recovery for 5-HT-mediated neuronal inhibition in old animals following chronic pretreatment with FLX.

The prolonged recovery phase observed following 5-HT application suggests that adaptive changes occur in serotonergic neurotransmission in young and old animals following repeated administration of FLX as compared to age-matched VEH groups. This finding in the young animals is in contrast with previous reports of 5-HT recovery responses following 21 day paroxetine treatment (Piñeyro et al., 1994); however, these differences may be due to the experimental paradigms utilized, including animal models, injection protocols, and chronic drug treatments. Using the RT_{50} value as an index of presynaptic 5-HT transporter (5-HTT) function (de Montigny et al., 1980), alterations in the density or affinity of this reuptake site may account for alterations in the recovery response. For example, an increase in the density of the 5-HT transporter has been observed in the frontal cortex, striate cortex, and CA1 pyramidal cell layer of the hippocampus following long-term fluoxetine administration (32–43%, 55% and 111%, respectively; Hrdina and Vu, 1993). Significant increases in 5-HT transporter mRNA have also been reported in the DRN following chronic administration of the monoamine oxidase inhibitor clorgyline and the tricyclic antidepressant imipramine, and result in an enhanced expression of this protein (Lopez et al., 1994). However, a decrease in transporter mRNA levels has also been observed in the DRN following prolonged pretreatment with FLX (Lesch et al., 1993).

Alternatively to adaptive changes at the transporter site, an increase in 5-HT concentration and/or a desensitization of autoreceptors may also contribute to the

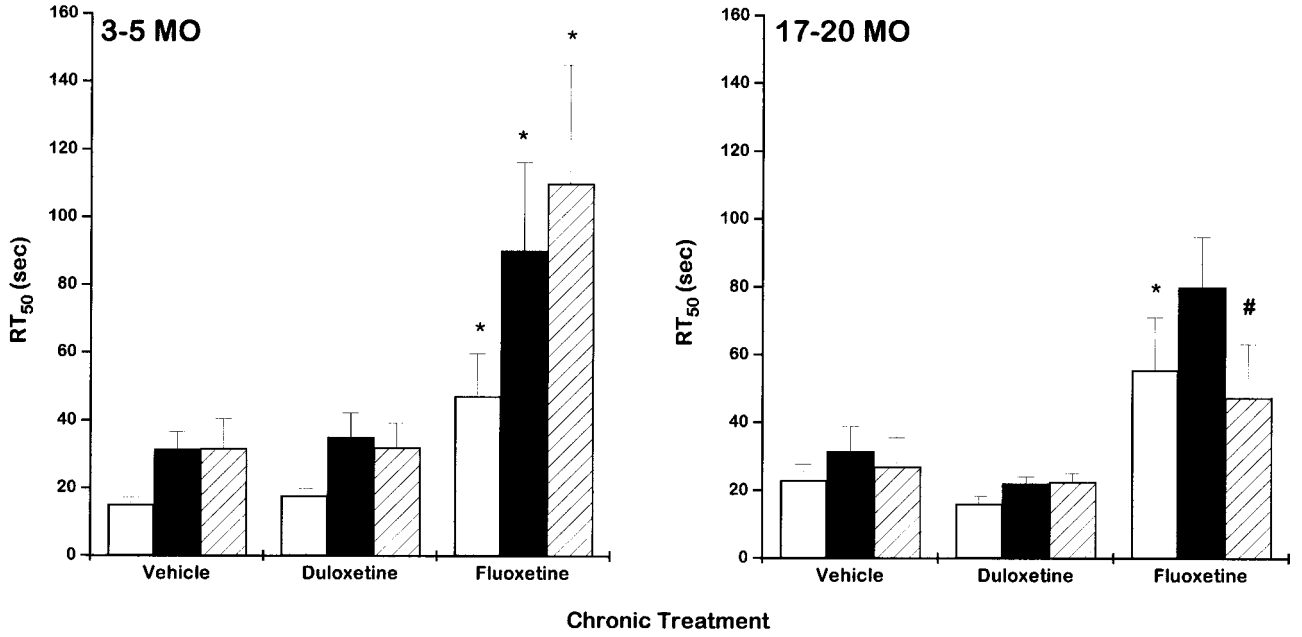

Fig. 4. Recovery rates determined for the microiontophoretic application of 5-HT alone and co-applied with DLX in the CA1 and CA3 hippocampal neurons recorded in chloral hydrate-anesthetized young and old female Fischer 344 rats following chronic FLX, DLX, or VEH administration. Data are RT_{50} values were obtained from responses in cells recorded in 3–5 and 17–20-month-old animals (N [cells/animals] = 7/5, 18/8, 7/7; 3–5 month VEH, DLX, and FLX treatment, respectively; 7/5, 5/5, 4/4; 17–20 month VEH, DLX, and FLX treatment, respectively); responses to iontophoretic currents were pooled to obtain mean ± SEM values and utilized in a two-way ANOVA of all treatment groups. White bars indicate values for the application of 5-HT alone. Hatched and shaded bars indicate values for the first and second co-applications of 5-HT and DLX, respectively. * Indicates significance from the same drug application in the 3–5 month and 17–20 month VEH and DLX groups ($P < 0.05$). # Indicates significant difference from the second co-application of 5-HT and DLX in the 3–5 month FLX group ($P < 0.05$).

TABLE II. Recovery rates determined for the microiontophoretic application of serotonin (5-HT) alone and co-applied with duloxetine in the CA1 and CA3 hippocampal pyramidal neurons recorded in chloral hydrate anesthetized senescent female Fischer 344 rats following chronic fluoxetine administration

	RT_{50} values (sec)		
Age	5-HT	Duloxetine + 5-HT 1	Duloxetine + 5-HT 2
22–24 Months	45.0 ± 12.0	85.0 ± 8.9*	57.5 ± 7.5

Data are RT_{50} values obtained from cells recorded for 24-month-old animals (n [cells/animals] = 6/4). Duloxetine + 5-HT 1 and duloxetine + 5-HT 2 = first and second co-application of 5-HT with duloxetine, respectively.
*Indicates significant difference from the application of 5-HT alone ($P < 0.05$).

increase in the recovery phase following exogenous 5-HT application. For example, increases in 5-HT concentration in the hippocampus have been observed following chronic administration of FLX (10 mg/kg, i.p.; Invernizzi et al., 1995; Kreiss and Lucki, 1995). Additionally, long-term pretreatment with this SSRI attenuated a decrease in 5-HT release produced by (±)-8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT; Kreiss and Lucki, 1995). This loss in sensitivity to 5-HT_{1A} receptor agonists has also been reported following chronic fluoxetine exposure in cellular physiological studies in the DRN and hippocampus (Blier et al., 1987). In examining the cellular mechanisms of these neuronal responses observed following antidepressant treatment, some investigators have reported no changes in 5-HT receptor binding following long-term antidepressant treatment (Goodnough and Baker, 1994; Maggi et al., 1980; Peroutka and Snyder, 1980). However, chronic FLX treatment has been observed to increase the density of 5-HT_2 receptors (Hrdina and Vu, 1993; Klimek et al., 1994). Furthermore, electrophysiological studies have provided evidence for the action of FLX at the 5-HT_2 receptor (Ni and Miledi, 1997). Actions of FLX at multiple 5-HT binding sites may also contribute to a complex series of cellular responses produced by chronic SSRI administration.

The synaptic actions of 5-HT were no longer enhanced with co-application of DLX or FLX in the 17–20 month FLX treatment group. These results suggest that the physiological responses to 5-HT observed following long-term FLX administration may be affected by age-related alterations in the function of the 5-HTT. It is not yet known if these changes are mediated at the 5-HTT sites; however, significant decreases in the density of the 5-HTT have been observed in senescent animals (Arora et al., 1993). Conversely, an increase in 5-HTT mRNA has been noted in the raphe nuclei of senescent male Sprague Dawley rats (Meister et al., 1995), suggestive of post-translational modifications in this transporter with aging. The identification of polymorphisms in the promoter region of the 5-HTT

gene associated with affective disorders (Heils et al., 1995; Lesch et al., 1996 ; Stober et al., 1996) also indicate another regulatory site in gene expression that may occur with aging to effectively decrease 5-HTT function.

In addition to alterations at the 5-HTT site, age-related decreases in the concentration of 5-HT available in the hippocampus may contribute to the decline in the recovery phase observed with aging. Declines in 5-HT levels and the ratio of 5-hydroxyindoleacetic acid to 5-HT were reported in the aging hippocampus, striatum, hypothalamus, and cerebral cortex (Tanila et al., 1994; Gozlan et al., 1990). Alternatively, the concentration of endogenous 5-HT available at the pyramidal cell synapse may not be sufficient to enhance the recovery time with prolonged DLX co-application. However, increases in 5-HT turnover (Moretti et al., 1987; Venero et al., 1993) or no change in 5-HT levels (Ponzio et al., 1982; Wester et al., 1984) have also been reported with advanced age.

Decreases in spontaneous cell firing rates in the DRN (Lakoski, 1989) and hippocampus (Lippa et al., 1981) have been identified with aging, which could contribute to the different pattern of recovery seen in the old FLX treatment group. However, in the present study the spontaneous baseline firing rates were found not to be altered as a function of both drug treatment and age. Therefore, age-related differences in the serotonergic responses following chronic FLX treatment were independent of alterations in spontaneous firing rates.

Similar to previous reports from our laboratory in young female Fischer 344 rats (Smith and Lakoski, 1997b), the recovery times following 5-HT and DLX co-application did not change in the young DLX and VEH groups. Additionally, the time to recovery following 5-HT was not altered with co-application of DLX in the old DLX and VEH animals. These results indicate that the adaptive changes that occur in 5-HT neurotransmission following chronic FLX administration do not occur with long-term DLX treatment. The different patterns of recovery in the DLX vs. the FLX groups may be due to the reuptake blocking profile of DLX at both the NE and 5-HT transporters (Fuller et al., 1995; Kasamo et al., 1996; Wong et al., 1993) or be a function of the dose of drug chronically administered. As previously reported, repeated administration of DLX had no effect on basal levels of 5-HT or NE in the rat frontal cortex and nucleus accumbens (Kihara and Ikeda, 1995). These results are in contrast to the increase in 5-HT concentrations observed with chronic FLX treatment (Inernizzi et al., 1995; Kreiss and Lucki, 1995) and may explain the lack of alteration in the RT_{50} values recorded in the DLX groups. However, Kihara and Ikeda (1995) also demonstrated an augmentation of the increase in 5-HT and NE output induced by an exogenous DLX challenge following chronic DLX pretreatment.

Similar to previous studies in which citalopram or tandospirone were chronically administered (Chaput et al., 1986; de Montigny et al., 1990; Godbout et al., 1991), the inhibitory responses to the microiontophoretic application of 5-HT were unaltered in hippocampal pyramidal neurons in young and old FLX and DLX groups as compared to VEH treated groups. Additionally, the inhibitory response produced by 5-HT application was not altered with co-application of DLX in either 3–5 month or 17–20 month FLX, DLX, and VEH groups. The lack of alteration in IT_{50} values produced by microiontophoresis of 5-HT in the hippocampus following chronic antidepressant treatment also confirm previous observations (Chaput et al., 1986; de Montigny et al., 1990; Godbout et al., 1991). Under conditions where 8-OH-DPAT inhibition of DRN cell firing is reduced by chronic administration of cericlamine, no changes were observed in the hippocampal responses to this agonist (Jolas et al., 1994). Other investigators have reported similar losses in the sensitivity of the somatodendritic, but not postsynaptic, $5\text{-}HT_{1A}$ receptors following chronic pretreatment with antidepressants (Kreiss and Lucki, 1995; Li et al., 1996; Piñeyro et al., 1994). Our laboratory has noted an increased sensitivity with aging in both CA1 and CA3 pyramidal neurons to the application of 5-HT (Smith and Lakoski, 1997b). However, in the present study using a paradigm of repeated daily drug administration, no alteration in the inhibitory effects were seen with aging in any treatment groups. This difference may be attributed to the experimental regimen, which included daily drug injections, or to the ages examined.

As previously observed in adult male Sprague Dawley rats (Smith and Lakoski, 1997a), DLX and FLX exhibited different pharmacological and physiological profiles in female Fischer 344 rats. The adaptive physiological responses in 5-HT neurotransmission in young animals following 14-day administration of FLX were not observed in the old animals. These results support the hypothesis that longer treatment with SSRI antidepressants may be required in elderly patients to achieve therapeutic efficacy. The lack of effect of chronic DLX treatment on hippocampal 5-HT function suggests that dual reuptake inhibitors may require an even longer treatment period to produce clinical benefit in patients. Studies addressing the cellular mechanisms of SSRIs and dual reuptake inhibitors across the lifespan will advance our knowledge of the usefulness of these classes of antidepressants in the treatment of depression in the elderly.

REFERENCES

Andrade, R., and Nicoll, R.A. (1987) Pharmacologically distinct actions of serotonin on single pyramidal neurones of the rat hippocampus recorded in vitro. J. Physiol., 394:99–124.

Arentsen, M.I., and Lakoski, J.M. (1993) Cellular electrophysiology of 5-HT_{1A}-mediated responses in CA1 and CA3 hippocampal subfields with aging. Soc. Neurosci. Abstr., 19:1060.

Arora, R.C., Gulati, A., and Crayton, J.W. (1993) Aging and ^{3}H-paroxetine binding in the rat brain: Effect of imipramine and tetrahydroacridine. Life Sci., 52:1767–1775.

Arranz, B., Eriksson, A., Mellerup, E., Plenge, P., and Marcusson, J. (1993) Effect of aging in human cortical pre- and postsynaptic serotonin binding sites. Brain Res., 620:163–166.

Azmitia, E.C., and Whitaker-Azmitia, P.M. (1991) Awakening the sleeping giant: Anatomy and plasticity of the brain serotonergic system. J. Clin. Psychiatry, 52:4–16.

Baldessarini, R.J. (1990) Drugs and the treatment of psychiatric disorders. In: The Pharmacological Basis of Therapeutics, Vol. 8. A.G. Gilman, T.W. Rall, A.S. Nies, and P. Taylor, eds. Pergamon Press, New York, pp. 383–435.

Blazer, D., and Williams, C.D. (1980) Epidemiology of dysphoria and depression in the elderly population. Am. J. Psychiatry, 137:439–444.

Blier, P., and de Montigny, C. (1994) Current advances and trends in the treatment of depression. Trends Pharmacol. Sci., 15:220–226.

Blier, P., de Montigny, C., and Chaput, Y. (1987) Modifications of the serotonin system by antidepressant treatments: Implications for the therapeutic response in major depression. J. Clin. Psychopharmacol., 7:24S–35S.

Briley, M., and Moret, C. (1993) 5-HT and antidepressants: In vitro and in vivo release studies. Trends Pharmacol. Sci., 14:396–397.

Brown, A.M., Young, T.J., Patch, T.L., Cheung, C.W., Kaumann, A., Gaster, L., and King, F.D. (1993) [^{125}I]SB 207710, a potent, selective radioligand for 5-HT_4 receptors. Br. J. Pharmacol. 110:10P.

Burnet, P.W.J., Eastwood, S.L., and Harrison, P.J. (1994) Detection and quantitation of 5-HT_{1A} and 5-HT_{2A} receptor mRNAs in human hippocampus using a reverse transcriptase-polymerase chain reaction (RT-PCR) technique and their correlation with binding site densities and age. Neurosci. Lett., 178:85–89.

Casey, D.A. (1994) Depression in the elderly. South. Med. J., 87:559–563.

Chalmers, D.T., and Watson, S.J. (1991) Comparative anatomical distribution of 5-HT_{1A} receptor mRNA and 5-HT_{1A} binding in rat brain — A combined in situ hybridisation/in vitro receptor autoradiographic study. Brain Res., 561:51–60.

Chaput, Y., de Montigny, C., and Blier, P. (1986) Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: Electrophysiological studies in the rat brain. Naunyn Schmiedebergs Arch. Pharmacol., 333:342–348.

Coppen, A. (1967) The biochemistry of affective disorders. Br. J. Psychiatry, 113:1237–1264.

de Montigny, C., and Aghajanian, G.K. (1977) Preferential action of 5-methoxydimethyltryptamine on presynaptic serotonin receptors: A comparative iontophoretic study with LSD and 5-HT. Neuropharmacology, 16:811–818.

de Montigny, C., Wang, R.Y., Reader, T.A., and Aghajanian, G.K. (1980) Monoaminergic denervation of the rat hippocampus: Microiontophoretic studies on pre- and postsynaptic supersensitivity to norepinephrine and serotonin. Brain Res., 200:363–376.

de Montigny, C., Chaput, Y., and Blier, P. (1990) Modification of serotonergic neuron properties by long-term treatment with serotonin reuptake blockers. J. Clin. Psychiatry, 51:4–8.

Dugar, A., and Lakoski, J.M. (1997) Serotonergic function of aging hippocampal CA3 pyramidal neurons: Electrophysiological assessment following administration of 5,7-dihydroxytryptamine in the fimbria-fornix and cingulum bundle. J. Neurosci. Res., 47:58–67.

Finch, C.E., Felicio, L.S., Mobbs, C.V., and Nelson, J.F. (1984) Ovarian and steroidal influences on neuroendocrine aging processes in female rodents. Endocr. Rev., 5:467–497.

Fuller, R.W., Hemrick-Luecke, S.K., and Snoddy, H.D. (1995) Effects of duloxetine, an antidepressant drug candidate, on concentrations of monoamines and their metabolites in rats and mice. J. Pharmacol. Exp. Ther., 269:132–136.

Godbout, R., Chaput, Y., Blier, P., and de Montigny, C. (1991) Tandospirone and its metabolite, 1-(2-pyrimidinyl)-piperazine-I. Effects of acute and long-term administration of tandospirone of serotonin neurotransmission. Neuropharmacology, 30:676–690.

Goodnough, D.B., and Baker, G.B. (1994) 5-Hydroxytryptamine$_2$ and β-adrenergic receptor regulation in rat brain following chronic treatment with desipramine and fluoxetine alone and in combination. J. Neurochem., 62:2262–2268.

Gozlan, H., Daval, G., Vergé, D., Spampinato, U., Fattaccini, C. M., Gallissot, M.C., El Mestikawy, S., and Hamon, M. (1990) Aging associated changes in serotoninergic and dopaminergic pre- and postsynaptic neurochemical markers in the rat brain. Neurobiol. Aging, 11:437–449.

Grossman, C.J., Kilpatrick, G.J., and Bunce, K.T. (1993) Development of a radioligand binding assay for 5-HT_4 receptors in guinea-pig and rat brain. J. Pharmacol., 109:618–624.

Hall, M.D., El Mestikawy, S., Emerit, M.B., Pichat, L., Hamon, M., and Gozlan, H. (1985) [^{3}H]8-Hydroxy-2-(di-n-propylamino)tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain. J. Neurochem., 44:1685–1696.

Heils, A., Teufel, A., Petri, S., Seemann, M., Bengel, D., Balling, U., Riederer, P., and Lesch, K.P. (1995) Functional promoter and polyadenylation site mapping of the human serotonin (5-HT) transporter gene. J. Neural Transm., 102:247–254.

Hensler, J.G., Ferry, R.C., Labow, D.M., Kovachich, G.B., and Frazer, A. (1994) Quantitative autoradiography of the serotonin transporter to assess the distribution of serotonergic projections from the dorsal raphe nucleus. Synapse, 17:1–15.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., and Humphrey, P.P.A. (1994) International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev., 46:157–203.

Hrdina, P.D., and Vu, T.B. (1993) Chronic fluoxetine treatment upregulates 5-HT uptake sites and 5-HT_2 receptors in rat brain: An autoradiographic study. Synapse, 14:324–331.

Invernizzi, R., Bramante, M., and Samanin, R. (1995) Extracellular concentrations of serotonin in the dorsal hippocampus after acute and chronic treatment with citalopram. Brain Res., 696:62–66.

Jolas, T., Haj-Dahmane, S., Kidd, E.J., Langlois, X., Lanfumey, L., Fattaccini, C.M., Vantalon, V., Laporte, A.M., Adrien, J., Gozlan, H., and Hamon, M. (1994) Central pre- and postsynaptic 5-HT_{1A} receptors in rats treated chronically with a novel antidepressant, cericlamine. J. Pharmacol. Exp. Ther., 268:1432–1443.

Kasamo, K., Blier, P., and de Montigny, C. (1996) Blockade of the serotonin and norepinephrine uptake processes by duloxetine: In vitro and in vivo studies in the rat brain. J. Pharmacol. Exp. Ther., 277:278–286.

Khan, A., Mirolo, H., Mirolo, M.H., and Dobie, D.J. (1993) Depression in the elderly: A treatable disorder. Geriatrics, 48:14–17.

Kihara, T., and Ikeda, M. (1995) Effects of duloxetine, a new serotonin and norepinephrine uptake inhibitor, on extracellular monoamine levels in rat frontal cortex. J. Pharmacol. Exp. Ther., 272:177–183.

Klimek, V., Zak-Knapik, J., and Mackowiak, M. (1994) Effects of repeated treatment with fluoxetine and citalopram, 5-HT reuptake inhibitors, on 5-HT_{1A} and 5-HT_2 receptors in the rat brain. J. Psychiatry Neurosci., 19:63–67.

Koenig, H., and Blazer, D. (1992) Epidemiology of geriatric affective disorders. Clin. Geriatr. Med., 8:235–251.

Kohler, K.C., and Steinbusch, H. (1982) Identification of serotonin and non-serotonin-containing neurons in the midbrain raphe projecting to the entorhinal area and the hippocampal formation. A combined immunohistochemical and fluorescent retrograde tracing study in the rat brain. Neuroscience, 7:951–975.

Kreiss, D.S., and Lucki, I. (1995) Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured in vivo. J. Pharmacol. Exp. Ther., 274:866–876.

Lakoski, J.M. (1989) Cellular electrophysiological approaches to the central regulation of female reproductive aging. In: Neural Control of Reproductive Function. J.M. Lakoski, J.R. Perez-Polo, and D.A. Rassin, eds. Alan R. Liss, New York, pp. 209–220.

Lakoski, J.M. (1994) Neuroendocrinology of aging at the cellular level: Membranes to neural circuits. Neurobiol. Aging, 15:519–520.

Lesch, K.P., Aulakh, C.S., Wolozin, B.L., Tolliver, T.J., Hill, J.L., and Murphy, D.L. (1993) Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. Mol. Brain Res., 17:31–35.

Lesch, K.-P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H., and Murphy, D.L. (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science, 274:1527–1531.

Lippa, A.S., Critchett, D.J., Ehlert, F., Yamamura, H.I., Enna, S.J., and Bartus, R.T. (1981) Age-related alterations in neurotransmitter receptors: An electrophysiological and biochemical analysis. Neurobiol. Aging, 2:3–8.

Lopez, J.F., Chalmers, D.T., Vazquez, D.M., Watson, S.J., and Akil, H. (1994) Serotonin transporter mRNA in rat brain is regulated by classical antidepressants. Biol. Psychiatry, 35:287–290.

Maggi, A., U'Prichard, D.C., and Enna, S.J. (1980) Differential effects of antidepressant treatment on brain monoaminergic receptors. Eur. J. Pharmacol., 61:91–98.

Marcusson, J., Oreland, L., and Winblad, B. (1984) Effect of age on human brain serotonin (S-1) binding sites. J. Neurochem., 43:1699–1705.

Meister, B., Johnson, H., and Ulfhake, B. (1995) Increased expression of serotonin transporter messenger RNA in raphe neurons of the aged rat. Mol. Brain Res., 33:87–96.

Meltzer, H.Y., and Lowy, M.T. (1987) The serotonin hypothesis of depression. In: Psychopharmacology: The Third Generation of Progress. H.Y. Meltzer, ed. Raven Press, New York, pp. 513–526.

Moretti, A., Carfagna, N., and Trunzo, F. (1987) Effect of aging on monoamines and their metabolites in the rat brain. Neurochem. Res., 12:1035–1039.

Murphy, D.L., Cambell, I., and Costa, J.L. (1978) Current status of the indoleamine hypothesis of the affective disorders. In: Psychopharmacology: A Generation of Progress. M.A. Lipton, A. DiMascio, and K.F. Killam, eds. Raven Press, New York, pp. 1235–1247.

Ni, Y.G., and Miledi, R. (1997) Blockage of $5HT_{2C}$ serotonin receptors by fluoxetine (Prozac). Proc. Natl. Acad. Sci. USA, 94:2036–2040.

Olpe, H.R., and Steinmann, M.W. (1982) The effect of vincamine, hydergine and piracetam on the firing rate of locus coeruleus neurons. J. Neural Trans., 55:101–109.

Paxinos, G., and Watson, C. (1986) The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Peroutka, S.J., and Snyder, S.H. (1980) Long-term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. Science, 210:88–90.

Piñeyro, G., Blier, P., Dennis, T., and de Montigny, C. (1994) Desensitization of the neuronal 5-HT carrier following its long-term blockade. J. Neurosci., 14:3036–3047.

Ponzio, F., Calderini, G., Lomuscio, G., Vantini, G., Toffano, G., and Algeri, S. (1982) Changes in monoamines and their metabolite levels in some brain regions of aged rats. Neurobiol. Aging, 3:23–29.

Richelson, E. (1991) Biological basis of depression and therapeutic relevance. J. Clin. Psychiatry, 52:4–10.

Salmoiraghi, G.C., and Weight, F. (1967) Micromethods in neuropharmacology: An approach to the study of anesthetics. Anesthesiology, 28:54–64.

Schlicker, E., Betz, T., and Gothert, M. (1989) Investigation into the age-dependence of release of serotonin and noradrenaline in the rat brain cortex and or autoreceptor-mediated modulation of release. Neuropharmacology, 28:811–815.

Siever, L.J., Coccaro, E.F., Benjamin, E., Rubinstein, K., and Davis, K.L. (1986) Adrenergic and serotonergic receptor responsiveness in depression. In: Antidepressants and Receptor Function. R. Porter, G. Bock, and S. Clark, eds. John Wiley & Sons, New York, pp. 148–169.

Slotkin, T.A., Whitmore, W.L., Barnes, G.A., Ranga, K., Krishnan, R., Blazer, D.G., Knight, D.L., and Nemeroff, C.B. (1989) Reduced inhibitory effect of imipramine on radiolabeled serotonin uptake into platelets in geriatric depression. Biol. Psychiatry, 25:687–691.

Smith, J.E., and Lakoski, J.M. (1997a) Electrophysiological effects of fluoxetine and duloxetine in the dorsal raphe nucleus and hippocampus. Eur. J. Pharmacol. 323:69–73.

Smith, J.E., and Lakoski, J.M. (1997b) Electrophysiological study of the effects of the reuptake inhibitor duloxetine on serotonergic responses in the aging hippocampus. Pharmacology, 55:66–77.

Stober, G., Heils, A., and Lesch, K.P. (1996) Serotonin transporter gene polymorphism and affective disorder. Lancet, 347:1340–1341.

Sur, C., Betz, H., and Schloss, P. (1996) Immunocytochemical detection of the serotonin transporter in rat brain. Neuroscience, 73:217–231.

Tanila, H., Taira, T., Piepponen, T.P., and Honkanen, A. (1994) Effect of sex and age on brain monoamines and spatial learning in rats. Neurobiol. Aging, 15:733–741.

Venero, J.L., de la Roza, C., Machado, A., and Cano, J. (1993) Age-related changes on monoamine turnover in hippocampus of rats. Brain Res., 631:89–96.

Vergé, D., Daval, G., Marcinkiewicz, M., Patey, A., El Mestikawy, S., Gozlan, H., and Hamon, M. (1986) Quantitative autoradiography of multiple $5\text{-}HT_1$ receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. J. Neurosci., 6:3473–3482.

Watchel, H. (1989) Dysbalance of neuronal second messenger function in the etiology of affective disorders: A pathophysiological concept hypothesizing defects beyond first messenger receptors. J. Neural Transm., 75:21–29.

Weissman, M.M., and Olfson, M. (1995) Depression in women: Implications for health care research. Science, 269:799–801.

Wester, P., Hardy, J.A., Marcusson, J., Nyberg, P., and Winblad, B. (1984) Serotonin concentrations in normal aging human brains: Relation to serotonin receptors. Neurobiol. Aging, 5:199–203.

Wise, P.M. (1983) Aging of the female reproductive system. Rev. Biol. Res. Aging, 1:195–222.

Wong, D.T., Bymaster, F.P., Mayle, D.A., Reid, L.R., Krushinski, J.H., and Robertson, D.W. (1993) LY248686, a new inhibitor of serotonin and norepinephrine uptake. Neuropsychopharmacology, 8:23–33.