Fluoxetine Selectively Alters 5-Hydroxytryptamine$_{1A}$ and $\gamma$-Aminobutyric Acid$_B$ Receptor-Mediated Hyperpolarization in Area CA1, but not Area CA3, Hippocampal Pyramidal Cells$^1$

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ABSTRACT
Fluoxetine is a 5-hydroxytryptamine (5-HT, serotonin)-selective reuptake inhibitor (SSRI) and is one of the main drugs used for the treatment of depression. Because it takes 2 to 3 weeks of treatment before clinical efficacy is manifest, the acute actions of fluoxetine cannot account for the clinical actions of the drug. The chronic effects of fluoxetine have not been completely delineated. The experiments detailed here investigate the chronic effects of fluoxetine on 5-HT and $\gamma$-aminobutyric acid (GABA) receptor-mediated actions using intracellular recording techniques in hippocampal brain slices. Rats were treated with fluoxetine for 3 weeks via osmotic minipumps implanted s.c. Fluoxetine and norfluoxetine plasma levels were determined. The hippocampal pyramidal cell characteristics and the 5-HT$_{1A}$ and GABA$_B$ receptor-mediated hyperpolarization were measured in the CA1 and the CA3 subfields. The 5-HT$_4$ receptor-mediated decrease in the slow afterhyperpolarization amplitude was also recorded in area CA1. The time constant, magnitude of the change in resistance during 300-ms hyperpolarizing current pulses and half-decay time of the sAHP were altered by chronic fluoxetine treatment in area CA1 pyramidal cells. No changes were seen in any of the active or passive membrane properties of the CA3 hippocampal pyramidal cells. Fluoxetine treatment increased the potency of 5-HT for the 5-HT$_{1A}$ receptor-mediated hyperpolarization in area CA1, but not area CA3, and decreased the potency of baclofen for the GABA$_B$ receptor-mediated hyperpolarization in area CA1, but not area CA3. The characteristics of the concentration-response curve for the 5-HT-mediated decrease in sAHP amplitude in area CA1 were not altered by fluoxetine treatment. Chronic fluoxetine selectively and differentially altered the cell characteristics and the 5-HT$_{1A}$ and GABA$_B$ receptor-mediated responses in area CA1 of the hippocampus, which forms the final common output of the hippocampus.

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; DMSO, dimethylsulfoxide; fAHP, fast afterhyperpolarization; GABA, $\gamma$-amino-butyric acid; sAHP, slow afterhyperpolarization; SSRI, serotonin-selective reuptake inhibitor; MAO, monoamine oxidase.
tivity in hippocampus (Newman et al., 1992). G protein α subunit levels are not altered in the hippocampus by chronic fluoxetine treatment (Lesch et al., 1992).

Previous electrophysiological studies have measured the effects of chronic antidepressant drug treatment, including tricyclic antidepressants, MAO inhibitors and SSRIs such as fluoxetine, by measuring changes in extracellularly recorded dorsal raphe and CA3 hippocampal pyramidal cell firing rate elicited by 5-HT (for review, see Chaput et al., 1991; Blier et al., 1987). These studies have implicated an increased sensitivity of the 5-HT neurotransmitter system. Specifically, it has been suggested that fluoxetine decreases the release of 5-HT from presynaptic terminals in CA3 after chronic fluoxetine treatment; no change was found in the postsynaptic 5-HT-elicited responses in either the dorsal raphe or the CA3 subfield of the hippocampus (Blier et al., 1988).

Intracellular recording techniques are useful for determining whether chronic drug treatment alters basic cell characteristics and for determining the mechanism of action underlying neurotransmitter-elicited changes recorded by extracellular recording techniques. Only one intracellular recording study has been conducted to determine the long-term modulatory action of the tricyclic antidepressant imipramine on 5-HT receptor function in area CA1 of the hippocampus (Beck and Halloran, 1989). The long-term effects of chronic fluoxetine treatment on the physiological response of hippocampal pyramidal cells has not been determined using intracellular recording techniques.

The passive and active cell characteristics of CA1 and CA3 hippocampal pyramidal cells are different (Beck et al., 1992; Brown et al., 1981; Spruston and Johnston, 1992), i.e., the input resistance and time constant of the CA3 cells are greater than those of the CA1 cells. This distinction can be attributed to differences in the types, densities and distribution of ion channels between the CA1 and CA3 pyramidal neurons. Also, the concentration-response curves characteristics of the 5-HT1A and GABA_B Receptor-mediated responses are different in CA3 than in CA1. These differences are attributed to differences in receptor-effector number, receptor-effector coupling or recognition site of the receptors (Beck et al., 1992; Beck et al., 1995). Our hypothesis is that the effects of chronic treatment with fluoxetine on hippocampal pyramidal cell characteristics and/or on receptor-mediated responses will not be the same in the CA1 and CA3 subfields of the hippocampus because the ion channels and receptor-effector coupling are not the same in the two subfields. The purpose of the experiments reported here was to determine the modulatory effects of chronic fluoxetine treatment on pyramidal cell characteristics and on 5-HT receptor-mediated responses in areas CA1 and CA3 of the hippocampus. Because 5-HT and GABA receptor systems have been found to share receptor-effector components (Andrade et al., 1986; Okuhara and Beck, 1994), GABA_B receptor-elicited responses and Beck were measured for comparison.

**Materials and Methods**

**Antidepressant treatment.** Male Sprague-Dawley rats (75–125 g) were anesthetized with ether, and an osmotic minipump (Alzet, Pala Alto, CA) implanted s.c. in the back. Fluoxetine hydrochloride was dissolved in 50% DMSO at a concentration of 11.7 g/l to produce an average dose of 6.68 mg/kg at the final weight of approximately 250 g for the rat after 3 weeks of treatment. Therefore, at the beginning of the treatment, the dose of fluoxetine would be higher because the rat weighed less. The control group of rats were implanted with minipumps containing 50% DMSO or physiological saline.

**Hippocampal slice preparation.** The rats were anesthetized with ether and decapitated. Trunk blood was collected in centrifuge tubes containing 0.3 ml of a solution of 0.3 M EDTA (pH = 7.4) and 1000 KIU of trasolol. The blood was centrifuged at 8600 rpm for 20 min at 4°C, and the plasma was removed and frozen. Plasma fluoxetine and norfluoxetine levels were determined by a solid-phase extraction procedure developed by the Toxicology/Psychopharmacology laboratory at the Hines VA hospital. Separation, identification and quantification of the two drugs were accomplished by HPLC.

The brain was rapidly removed and rinsed in ice-cold ACSF containing (mM): NaCl (124), KCl (3), NaHPO_4 (1.25), MgSO_4 (2), CaCl_2 (2.5), dextrose (10), NaHCO_3 (28). The hippocampus was dissected free, and, starting at the dorsal/ septal tip, 500 to 600-μs sections were cut on a vibratome. Slices were placed in a holding vial containing room-temperature ACSF bubbled with 95% O_2/5% CO_2, pH = 7.4. After at least 1 h, a slice was transferred to the recording chamber, where it was perfused continuously with ACSF at 32°C ± 1°C and bubbled with 95% O_2/5% CO_2 at a flow rate of 2 to 3 ml/min. Fluoxetine was not included in the ACSF.

**Intracellular recording techniques.** Intracellular recordings were made as previously described (Beck et al., 1992). Electrodes were pulled from borosilicate capillary tubing (1.2 mm O.D., 0.69 mm I.D., Sutter Instruments, Novato, CA) on a Brown and Flaming electrode puller (Sutter Instruments, Novato, CA) to obtain resistances of 40 to 140 MΩ (2 M KCl or 2 M KCH_3COO/10 mM KCl). Pyramidal cells in area CA1 or CA3 were impaled by briefly (10–50 ms) increasing the capacitance compensation or by briefly increasing positive current ejection through the recording electrode. The pyramidal cells were hyperpolarized after impalement to facilitate sealing, which usually took from 15 to 30 min. Electrical signals were amplified using an Axoclamp2A amplifier (Axon Instruments, Foster City, CA), stored on disk for later analysis using pClamp software (Axon Instruments, Foster City, CA) and recorded on a Gould chart recorder, Series 2200 or 3200 (Gould Incorporated, Cleveland, OH).

The resting membrane potential (in millivolts) was the potential of the cell with no holding current applied through the electrode. This value was read directly from a display on the amplifier and checked by withdrawing the electrode at the end of the experiment. The time constant (τ) was calculated from a single exponential fit of the falling phase of the potential response to a 100- or 200-pA, 300-ms hyperpolarizing current pulse. The input resistance (in megohms) was obtained from the slope of the linear portion of an I-V plot generated from hyperpolarizing current pulses 300 ms in duration obtained under current clamp. Input resistance for CA1 cells was measured at two time-points: approximately 100 ms after the start of the current pulse and at the end of the current pulse at 300 ms. For CA3 cells, the input resistance was measured at the end of the current pulse at 300 ms.

Action potential characteristics were measured from a single action potential generated by a 2 to 4-ms current pulse of sufficient intensity. Action potential threshold (in millivolts) was measured at the point just before the rapid rise of the action potential. Action potential height (in millivolts) was measured from threshold to the peak of the spike, and action potential duration was measured at a point that was 50% of the maximum height (in milliseconds). The amplitude (in millivolts) of the fAHP was measured from threshold to the peak of the hyperpolarized potential that followed a single action potential.

The sAHP was elicited by a 900-pA depolarizing current pulse at a membrane potential of −62 to −65 mV, and the amplitude was measured 100 ms after the offset of the current pulse. The half-decay time (in milliseconds) is the amount of time it takes for the sAHP to decay to half its peak amplitude.

**Hippocampal slice preparation.** The rats were anesthetized with ether and decapitated. Trunk blood was collected in centrifuge tubes containing 0.3 ml of a solution of 0.3 M EDTA (pH = 7.4) and 1000 KIU of trasolol. The blood was centrifuged at 8600 rpm for 20 min at 4°C, and the plasma was removed and frozen. Plasma fluoxetine and norfluoxetine levels were determined by a solid-phase extraction procedure developed by the Toxicology/Psychopharmacology laboratory at the Hines VA hospital. Separation, identification and quantification of the two drugs were accomplished by HPLC.

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Concentration-response curves. Drugs were tested by the addition of concentrated stock drug solution into a reservoir of 50 to 60 ml of ACSF. For concentration-response curves, four to seven concentrations of drug were tested in increments of at least one-half log unit.

Concentration-response curves were analyzed according to the formula for a hyperbolic/logistic function:

$$E = E_{\text{max}} / [1 + (EC_{50}/A)^n]$$

where $E$ is the response produced by A, the concentration of drug, $E_{\text{max}}$ is the maximal response to drug, $EC_{50}$ is the concentration of drug eliciting a half-maximal response and $N$ is the slope index of the concentration-response curve. From this analysis, estimates of $E_{\text{max}}$, $EC_{50}$ and $N$ were obtained.

Statistical analyses. Our hypothesis was that fluoxetine would alter the cell characteristics, and/or the characteristics of the 5-HT or baclofen concentration-response curves for 5-HT$_{1A}$, GABA$_B$ and/or 5-HT$_4$ receptor activation. Previously, we and others have demonstrated that there were significant differences between the two hippocampal subfields in cell characteristics (Beck et al., 1994; Brown et al., 1981; Spruston and Johnston, 1992) and concentration-response characteristics for 5-HT (Beck et al., 1992) and baclofen (Beck et al., 1995). To confirm these previous findings, an ANOVA was conducted comparing the control data from CA1 with the control data from CA3. To test for modulatory actions of fluoxetine, an ANOVA was conducted on data within a particular subfield. Data are reported as mean ± S.E.M. The mean geometric means of the EC$_{50}$ values were used for statistical comparisons. A $P < .05$ was considered significant.

Chemicals and drugs. The chemicals for making the ACSF and 5-HT hydrochloride were purchased from Sigma (St. Louis, MO). Fluoxetine was generously donated by Eli Lilly (Indianapolis, IN). Baclofen was generously donated by Ciba-Geigy (Suffern, NY). DMSO was obtained from Fisher Scientific (Pittsburgh, PA).

Results

Data were obtained from 12 rats treated with fluoxetine and 16 sham control rats; 68 total cells, 34 from fluoxetine treated rats and 34 from sham-treated control rats. Each individual cell was tested as an independent sample, even though more than one cell may have been obtained from each rat.

Fluoxetine plasma levels. On the day of the experiment, trunk blood was collected, and plasma fluoxetine and norfluoxetine levels were determined. The data recorded from cells from rats that had detectable plasma fluoxetine and norfluoxetine levels were used for analysis as data from treated rats; data from two rats with no detectable plasma levels of norfluoxetine or fluoxetine were discarded. Control animals had fluoxetine levels and norfluoxetine levels that were not detectable ($n = 3$). For the treated rats, the fluoxetine levels were $107 ± 15$ ng/ml (range 65–200) and norfluoxetine levels were $118 ± 22$ ng/ml (range 19–200 ng/ml), $n = 12$.

Cell properties. The passive and active characteristics of the CA1 and CA3 hippocampal pyramidal cells from control and fluoxetine-treated rats are summarized in table 1. Previous studies have reported that the passive cell characteristics are not the same in CA1 and CA3 pyramidal cells (Beck et al., 1994; Brown et al., 1981; Spruston and Johnston, 1992), i.e., the input resistance and the time constant of the CA3 cells are greater than those of the CA1 cells. The results of the experiments reported here confirmed those studies, i.e., the input resistance measured at 300 ms and the time constant of the CA3 cells were significantly greater than those of the CA1 cells (table 1).

Comparing the hippocampal pyramidal cell properties recorded from control and treated rats can give an indication of whether chronic fluoxetine treatment altered any of the active or passive ionic conductances. Chronic fluoxetine treatment altered some of the cell characteristics from CA1 hippocampal pyramidal cells; no changes were seen in any of the active or passive membrane properties of the CA3 hippocampal pyramidal cells. Fluoxetine treatment for 3 weeks significantly increased the time constant and the half-decay time of the sAHP in CA1 pyramidal cells. The input resistance of CA1 hippocampal pyramidal cells decreases during long-duration hyperpolarizing current pulses, i.e., the voltage response “sags.” This “sag” can be measured by comparing the resistance of the cell at approximately 100 ms and at 300 ms. The magnitude of this change in resistance was significantly greater in the cells recorded from fluoxetine-treated rats ($12.94 ± 1.63$ MΩ) as compared with control rats ($6.69 ± 0.75$ MΩ).

5-HT$_{1A}$ receptor-mediated hyperpolarization. As previously reported, 5-HT elicited a hyperpolarization in both CA1 and CA3 hippocampal pyramidal cells (fig. 1). Also in confirmation of previous findings (Beck et al., 1992), the magnitude of the hyperpolarization was greater in area CA3 than in area CA1, and 5-HT was more potent in area CA1 than in area CA3 (table 2; fig. 2).

### TABLE 1

<table>
<thead>
<tr>
<th>Cell Property</th>
<th>CA1-Control</th>
<th>CA1-Fluoxetine</th>
<th>CA3-Control</th>
<th>CA3-Fluoxetine</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP (mV)</td>
<td>$-62.6 ± 1.8$ (12)</td>
<td>$-60.4 ± 0.8$ (15)</td>
<td>$-59.5 ± 1.5$ (11)</td>
<td>$-61.0 ± 1.8$ (11)</td>
</tr>
<tr>
<td>Resistance$^a$ 100 ms (MΩ)</td>
<td>$45.8 ± 2.9$ (10)</td>
<td>$54.8 ± 3.8$ (9)</td>
<td>$63.7 ± 6.1$ (10)</td>
<td>$69.5 ± 7.1$ (11)</td>
</tr>
<tr>
<td>Resistance$^a$ 300 ms (MΩ)</td>
<td>$41.9 ± 3.0$ (10)</td>
<td>$34.2 ± 2.7$ (13)</td>
<td>$32.4 ± 4.4$ (10)</td>
<td>$32.5 ± 5.9$ (9)</td>
</tr>
<tr>
<td>Tau$^d$ (ms)</td>
<td>$18.0 ± 1.2$ (10)</td>
<td>$27.8 ± 2.7$ (13)</td>
<td>$32.4 ± 4.4$ (10)</td>
<td>$32.5 ± 5.9$ (9)</td>
</tr>
<tr>
<td>AP thresh (mV)</td>
<td>$-51.2 ± 1.2$ (11)</td>
<td>$-49.5 ± 1.2$ (12)</td>
<td>$-48.1 ± 1.1$ (5)</td>
<td>$-45.6 ± 2.1$ (9)</td>
</tr>
<tr>
<td>AP duration (ms)</td>
<td>$1.4 ± 0.05$ (11)</td>
<td>$1.4 ± 0.05$ (12)</td>
<td>$1.3 ± 0.04$ (5)</td>
<td>$1.2 ± 0.03$ (8)</td>
</tr>
<tr>
<td>AP amp (mV)</td>
<td>$87.8 ± 1.5$ (11)</td>
<td>$88.5 ± 2.6$ (12)</td>
<td>$72.9 ± 2.8$ (5)</td>
<td>$79.0 ± 1.5$ (9)</td>
</tr>
<tr>
<td>IAH$_B$ amp (mV)</td>
<td>$4.4 ± 0.7$ (10)</td>
<td>$4.2 ± 0.7$ (12)</td>
<td>$8.5 ± 0.5$ (9)</td>
<td>$9.7 ± 0.5$ (9)</td>
</tr>
<tr>
<td>sAHP amp (mV)</td>
<td>$7.4 ± 0.6$ (16)</td>
<td>$8.9 ± 0.6$ (13)</td>
<td>$10.7 ± 2.6$ (8)</td>
<td>$10.3 ± 1.8$ (9)</td>
</tr>
<tr>
<td>sAHP decay time$^e$ (ms)</td>
<td>$986 ± 67$ (16)</td>
<td>$1175 ± 76$ (13)</td>
<td>$808 ± 114$ (8)</td>
<td>$807 ± 122$ (9)</td>
</tr>
</tbody>
</table>

$^a$ Characteristics include resting membrane potential (RMP), input resistance measured at 100 ms (Res 100) and at 300 ms (Res 300), tau, threshold to fire an action potential (AP thresh), height of the action potential (AP height), duration of the action potential (AP duration), amplitude of the sAHP (sAHP amp) and sAHP half-decay time. Values are mean ± S.E.M., and the numbers of neurons tested are given in parentheses.

$^b$ CA1 subfield, one-within, one-between factor ANOVA, interaction of treatment by resistance measured at 100 and 300 ms, $F = 13.03$, $P = .0022$.

$^c$ CA1 different from CA3, $F = 24.1$, $P = .0001$.

$^d$ CA1-control different from CA1-fluoxetine, $F = .891$, $P = .0071$. CA1 different from CA3, $F = 10.06$, $P = .0053$.

$^e$ CA1-control different from CA1-fluoxetine, $F = 7.46$, $P = .01$. 

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After chronic treatment with fluoxetine for 3 weeks, the EC50 and slope of the concentration-response curve for 5-HT acting at the 5-HT1A receptor in area CA1 were altered (table 2; fig. 2). The EC50 for 5-HT was shifted to the left, i.e., more potent, after fluoxetine treatment. The slope of the concentration-response curves for the CA1 pyramidal cells taken from treated rats was more shallow than the slope of the curves for the CA1 pyramidal cells from control rats (table 2). In contrast, the Emax, EC50, and slope of the concentration-response curves for the 5-HT1A-elicited hyperpolarization in area CA3 hippocampal pyramidal cells were not altered after fluoxetine treatment (table 2; fig. 2).

**GABA	extsubscript{B} receptor-mediated hyperpolarization.** Baclofen elicits a hyperpolarization in both area CA1 and area CA3, and the GABA	extsubscript{B} receptor and the 5-HT1	extsubscript{A} receptor appear to share some component of the receptor-effector pathway (Okuhara and Beck, 1994; Andrade et al., 1986). The data from the CA1 and CA3 cells from the sham-treated rats were reported in a previous study (Beck et al., 1995), which describes the finding that baclofen is less potent at eliciting a hyperpolarization through activation of the GABA	extsubscript{B} receptor in area CA1 than in CA3 (table 3). Like the 5-HT1A-elicited hyperpolarization, the characteristics of the baclofen concentration-response curve for the GABA	extsubscript{B} receptor-mediated hyperpolarization differ in area CA1 and area CA3 hippocampal pyramidal cells (Beck et al., 1995). However, unlike the 5-HT1A response, the GABA	extsubscript{B} response is less potent in CA1 than in CA3. Like the 5-HT1A response, the maximal response elicited by baclofen is greater in CA3 than in CA1 (Beck et al., 1995).

After chronic treatment with fluoxetine for 3 weeks, the characteristics of the concentration-response curve for baclofen were altered in area CA1, but not in area CA3. As with the 5-HT1A receptor-mediated hyperpolarization, the effect of fluoxetine was selective for the CA1 subfield. Fluoxetine altered the EC50 for baclofen acting at the GABA	extsubscript{B} receptor in area CA1 (table 3, fig. 3). In contrast to the increased potency of 5-HT for the 5-HT1A-elicited hyperpolarization in area CA1, the EC50 for baclofen was less potent after fluoxetine treatment. There were no changes in the Emax or the slope of the baclofen concentration-response curve in area CA3, and there were no significant effects of fluoxetine treatment on the Emax, EC50 or slope of the baclofen concentration-response curve in area CA3 (table 3; fig. 3).

**5-HT4 receptor-mediated decrease in sAHP amplitude in area CA1.** Five sAHPs were generated by a 900-pA, 300-ms current pulse both before and during the perfusion of the slice with 5-HT. The five sAHPs were averaged, and the amplitude and the half-decay time of the sAHP were measured. During the hyperpolarization elicited by 5-HT, the resting membrane potential was brought back to base-line levels by injecting direct current so that the sAHP could be generated (fig. 1). In the CA3 subfield, the magnitude of the hyperpolarization was very large; because of the large increase in potassium conductance, even with direct current injection to bring the membrane potential back to base-line levels, it was not possible to generate a train of action potentials to elicit the sAHP in every cell. Therefore, data on the chronic effects of fluoxetine treatment on the 5-HT-elicited decrease in sAHP amplitude were not obtained from area CA3 pyramidal cells.

Fluoxetine treatment did not produce any statistically significant changes in the concentration-response curve characteristics for the 5-HT4 receptor-mediated decrease in sAHP amplitude or in the decrease in sAHP half-decay time (fig. 4, table 4). These data were analyzed both as absolute change in the magnitude of the sAHP amplitude and as the percent change in sAHP amplitude compared with the base-line sAHP amplitude value. The Emax, EC50 and slope values for the percent change in sAHP amplitude by 5-HT for the control and fluoxetine-treated groups are presented in table 4.

**Discussion**

The major findings of this study were that chronic fluoxetine treatment selectively altered the cell characteristics and the concentration-response curve characteristics of 5-HT and baclofen to elicit a membrane hyperpolarization in area CA1 hippocampal pyramidal cells. In contrast, chronic treatment with fluoxetine did not alter the pyramidal cell characteristics or the concentration-response curve characteristics in area CA3 hippocampal pyramidal cells.

The differences in the passive cell characteristics between CA1 and CA3 pyramidal cells, as previously noted (Brown et al., 1981; Bilkey and Schwartzkroin, 1990; Beck et al., 1994), were confirmed by the results collected in this experiment. Interestingly, the chronic effects of fluoxetine were manifested only on the cell characteristics of the CA1 neurons. The “sag” in membrane potential, measured by the difference in resistance measured at approximately 100 ms and 300 ms, was greater in fluoxetine-treated animals. This “sag” is thought to be due to the activation of a mixed cationic conductance termed Iq or If (Brown et al., 1990; Halliwell and Adams, 1982). Further experiments will be necessary to de-
termine which ionic conductance(s) were changed to account for the measured increase in resistance at 300 ms. Chronic fluoxetine treatment also increased the time constant and the half-decay time of the sAHP of CA1 pyramidal cells. A change in the time constant could be due to an alteration in input resistance and/or capacitance and could account for the increased "sag" recorded in fluoxetine-treated cells. The increased time constant would make the cell more sensitive to synaptic activity and increase the likelihood of synaptic potentiation summing. In contrast, the prolonged time course of the sAHP would decrease the excitability of the cell and decrease the probability that synaptic input would summate. Therefore, the net effect of chronic fluoxetine would make the cell more responsive at low synaptic activity and less responsive to large, prolonged synaptic input.

Chronic fluoxetine treatment altered the EC50 for both the 5-HT and baclofen concentration-response curves for the 5-HT1A and GABAB receptor-mediated hyperpolarization of area CA1 pyramidal cells, but not area CA3 pyramidal cells. Fluoxetine was present in the plasma and brain on the day of the experiment, because the fluoxetine minipumps were not removed before preparation of the brain slices. The acute effect of fluoxetine is to prevent the uptake of 5-HT into nerve terminals; one explanation for the increase in potency of the 5-HT1A receptor-mediated hyperpolarization in areas CA1 and CA3 in control and fluoxetine-treated cells. Note that the y axis is different for the CA1 and CA3 summary graphs. The number of neurons per data point was 9 to 15 for CA1 and 6 to 9 for CA3. Values are mean ± S.E.M.

### TABLE 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC50 (−log)</th>
<th>Emax (mV)</th>
<th>Slope</th>
<th>EC50 (−log)</th>
<th>Emax (mV)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.29 ± 0.04</td>
<td>11.62 ± 0.95</td>
<td>2.21 ± 0.21</td>
<td>4.94 ± 0.09</td>
<td>18.51 ± 1.77</td>
<td>2.04 ± 0.23</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>5.64 ± 0.10</td>
<td>11.24 ± 0.88</td>
<td>1.42 ± 0.12</td>
<td>5.00 ± 0.13</td>
<td>18.79 ± 1.91</td>
<td>1.73 ± 0.28</td>
</tr>
</tbody>
</table>

a Estimates were obtained by fitting concentration-response data from individual cells to the formula for a logistic/hyperbolic function. Values listed are the mean ± S.E.M., and the numbers of cells tested are given in parentheses.

b CA1 control different from CA3 control, F = 14.97, P = .0014; CA1 control different from CA1 fluoxetine, F = 11.05, P = .0034.

c CA1 control different from CA3 control, F = 14.06, P = .0017.

d CA1 control different from CA1 fluoxetine, F = 10.29, P = .0044.

### TABLE 3

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC50 (−log)</th>
<th>Emax (mV)</th>
<th>Slope</th>
<th>EC50 (−log)</th>
<th>Emax (mV)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.63 ± 0.06</td>
<td>9.25 ± 0.54</td>
<td>1.24 ± 0.16</td>
<td>5.88 ± 0.07</td>
<td>18.53 ± 1.64</td>
<td>1.56 ± 0.44</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>5.37 ± 0.09</td>
<td>9.80 ± 1.30</td>
<td>1.51 ± 0.39</td>
<td>5.90 ± 0.15</td>
<td>16.27 ± 5.11</td>
<td>1.35 ± 0.06</td>
</tr>
</tbody>
</table>

a Estimates were obtained by fitting concentration-response data from individual cells to the formula for a logistic/hyperbolic function. Values listed are the mean ± S.E.M., and the numbers of cells tested are given in parentheses.

b CA1 control different from CA1 fluoxetine, F = 6.65, P = .0275; CA1 control different from CA3 control, F = 32.83, P = .0001.

c CA1 control different from CA3 control, F = 7.66, P = .0183.
In this study, there was a significant shift to the left in the EC$_{50}$ and a decrease in the slope of the 5-HT concentration-response curves recorded in cells from rats treated with fluoxetine for 3 weeks. Therefore, because the acute effects of fluoxetine did not result in significant changes in the EC$_{50}$ or E$_{\text{max}}$ of the concentration-response curves for the 5-HT$_{1A}$ receptor-mediated hyperpolarization, our results cannot be attributed to an acute effect of residual fluoxetine. Also, the amount of time before a cell is obtained after the preparation of the tissue ranges from 2 h to 10 h. Any residual fluoxetine present in the tissue after decapitation is probably removed in that amount of time. Therefore, we conclude that the changes in EC$_{50}$ and slope of the 5-HT and baclofen concentration-response curves are due to the chronic effects of fluoxetine.

The alteration of the 5-HT and baclofen concentration-response curves by chronic fluoxetine was in the opposite direction, i.e., an increase in potency for the 5-HT$_{1A}$ receptor-mediated response and a decrease in potency of the GABA$_{B}$ response. The GABA$_{B}$ and 5-HT$_{1A}$ receptors share some component(s) of their effector pathways, because no additivity is measured when saturating concentrations of 5-HT and baclofen are administered together (Andrade et al., 1986; Okuhara and Beck, 1994). If fluoxetine was modulating some shared component of the pathway, the changes in the concentration-response curve characteristics for 5-HT and baclofen would be expected to be similar. Chronic fluoxetine treatment could be altering any component of the receptor-effector pathway: receptor number, coupling efficiency between receptor and G protein or G protein and ion channel, number of G proteins, number of ion channels or kinetics of the ion channel. However, because we found that fluoxetine treatment produced differential effects on the 5-HT$_{1A}$ and GABA$_{B}$ receptor-mediated hyperpolarization, we propose that fluoxetine is altering some component of the receptor-effector pathway that is not shared by these two receptor-effector pathways.

**Table 4**

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC$_{50}$ (-log)</th>
<th>E$_{\text{max}}$ % decrease</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.48 ± 0.09 (8)</td>
<td>50.81 ± 4.23 (8)</td>
<td>2.57 ± 0.34 (8)</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>5.78 ± 0.13 (7)</td>
<td>57.69 ± 8.97 (8)</td>
<td>2.19 ± 0.33 (8)</td>
</tr>
</tbody>
</table>

*E$_{\text{max}}$ values are the percent inhibition of the sAHP amplitude as compared with the base-line sAHP amplitude. Estimates were obtained by fitting concentration-response data from individual cells to the formula for a logistic/hyperbolic function. Values listed are the mean ± S.E.M., and the numbers of cells tested are given in parentheses.

**Fig. 3.** Summary graphs of baclofen concentration-response curves for the GABA$_{B}$ receptor-mediated hyperpolarization in areas CA1 and CA3 in control and fluoxetine-treated cells. Note that the y-axis scale is different for the CA1 and CA3 summary graphs. The number of neurons per data point for CA1 was 6 to 10, and 3 to 6 neurons for CA3. Values are mean ± S.E.M.

**Fig. 4.** Summary graphs of 5-HT concentration-response curves for the 5-HT$_{4}$ receptor-mediated decrease in sAHP amplitude (panel A) and half-decay time (panel B). The values are given as percent decrease compared with control. The number of neurons per data point was 7 to 13. Values are mean ± S.E.M.
Differences in receptor number cannot account for the divergence in the $E_{\text{max}}$ values for 5-HT$_{1A}$ or GABA$_B$ receptor-mediated responses recorded in area CA1 and CA3 in sham-treated rats (Beck et al., 1992; Beck et al., 1995). The density of 5-HT$_{1A}$ and GABA$_B$ receptors is higher in area CA1 than in area CA3 of the hippocampus (Chu et al., 1990; Knott et al., 1993; Pazos and Palacios, 1985; Ahlenius and Larsson, 1987; Welner et al., 1988; Goslau et al., 1995). The density of the GABA$_B$ receptors is relatively homogenous in the cell body and dendritic fields of both areas CA1 and CA3 (Chu et al., 1990; Knott et al., 1993). Within the CA1 subfield, the 5-HT$_{1A}$ receptors are in all of the layers; in area CA3 the receptors are located in stratum oriens. The larger input resistance of the CA3 cells can only partially account for the larger magnitude of the response elicited by 5-HT$_{1A}$ and GABA$_B$ receptor activation in area CA3 as compared with area CA1; the mechanism underlying this difference has not been definitively identified.

Previous studies have shown that fluoxetine treatment did not alter the amount of binding or the affinity of 5-HT or of the 5-HT$_{1A}$ agonist 8-hydroxy-2-dipropylamino-tetralin using hippocampal homogenate binding assays (Maggi et al., 1980; Peroutka and Snyder, 1980) or in area CA1 or area CA3 using autoradiography techniques (Klimek et al., 1994; Welner et al., 1989). Therefore, because there is no change in the affinity or density of the 5-HT$_{1A}$ receptors, the shift in the slope and potency of the 5-HT concentration-response curve for the 5-HT$_{1A}$ receptor-mediated hyperpolarization in area CA1 cannot be attributed to changes in receptor number or affinity. To our knowledge, no one has assessed GABA$_B$ receptor density in the hippocampus after chronic fluoxetine treatment. One study reported an up-regulation of GABA$_B$ receptors in the frontal cortex after chronic fluoxetine treatment (Lloyd et al., 1985).

The identity of the G protein(s) that link the 5-HT$_{1A}$ and GABA$_B$ receptors in areas CA1 and CA3 to the potassium channel have not been identified, though it is known that they are pertussis toxin-sensitive (Andrade et al., 1986; Okuhara and Beck, 1994). It is entirely possible that the 5-HT$_{1A}$ and GABA$_B$ receptors are not linked to the same G protein in either or both hippocampal subfields. We have found that the distribution of the pertussis toxin-sensitive G proteins G$_i$ and G$_o$ is different in areas CA1 and CA3 (Okuhara et al., 1996). In area CA1, G$_o$ labeling was found in the apical and distal dendrites, whereas it was very diffuse in the neuropil of area CA3 neurons. In area CA3, G$_i$ labeling was fibrous and was concentrated in patches surrounding the pyramidal cell, but it was primarily in the neuropil in the area CA1 neurons (Okuhara et al., 1996). Fluoxetine treatment had no effect on the density of G protein mRNA in whole hippocampus (Lesch et al., 1992). It is possible that chronic fluoxetine treatment has selective effect(s) on G protein number or distribution within the subfields of the hippocampus.

Previous studies have demonstrated that the 5-HT$_{1A}$ receptor also inhibits forskolin-stimulated adenylcyclase activity through a pertussis toxin-sensitive G protein (De Vivo and Maayani, 1986). Chronic fluoxetine treatment has been reported to decrease the 5-HT$_{1A}$ Receptor-mediated inhibition of adenylcyclase (Newman et al., 1992) or to have no effect (Varrault et al., 1991) in whole hippocampal homogenates. Because the 5-HT$_{1A}$ receptor-mediated hyperpolarization is not mediated through a change in adenylcyclase activity (Andrade et al., 1986) the effects of fluoxetine cannot be attributed to a modification of adenyl cyclase activity.

The 5-HT$_{4}$ receptor-mediated decrease in sAHP amplitude recorded in area CA1 was not altered by fluoxetine treatment. This receptor-mediated response was not recorded in isolation, but concomitantly with the 5-HT$_{1A}$ receptor-mediated hyperpolarization. Approximately a 15% decrease in sAHP amplitude occurs as a consequence of the opening of the inward rectifying potassium channel after 5-HT$_{1A}$ receptor activation (Andrade and Nicoll, 1987). The shift to the left in the concentration-response curve for 5-HT for the decrease in sAHP amplitude could be due primarily to the larger magnitude of the hyperpolarization elicited by lower 5-HT concentrations in fluoxetine-treated animals. It is interesting that fluoxetine selectively altered the 5-HT$_{1A}$ receptor-mediated response and did not alter the 5-HT$_{4}$ response. The effects of fluoxetine cannot be ascribed to a general nonspecific action on all 5-HT receptors.

In conclusion, chronic fluoxetine treatment had selective actions on neurotransmitter receptor-mediated responses across hippocampal subfields and within a subfield. Fluoxetine selectively modulated area CA1 hippocampal pyramidal cell characteristics, as well as 5-HT$_{1A}$ and GABA$_B$ receptor-mediated effects in area CA1, without altering area CA3 hippocampus pyramidal cell characteristics or neurotransmitter actions. Also, fluoxetine selectively altered 5-HT$_{1A}$, but not 5-HT$_{4}$ receptor-mediated responses within area CA1. The actions of fluoxetine on the 5-HT$_{1A}$ and GABA$_B$ receptor-mediated hyperpolarization were in the opposite direction, even though it has been shown that these neurotransmitter receptors share some component(s) of their receptor-effector pathway. We conclude that the effects of fluoxetine are not diffuse but selective and that they appear to be on some component(s) of the receptor-effector pathways that are not shared across neurotransmitter systems or hippocampal subfields.

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References


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