Modulation by Serotonin 5-HT4 Receptors of Long-term Potentiation and Depotentiation in the Dentate Gyrus of Freely Moving Rats

Tetanization-induced long-term potentiation (LTP) in the hippocampus can be depotentiated by low-frequency stimulation. 5-HT4 receptors are expressed in the hippocampus and are suggested to be involved in hippocampus-dependent cognitive processes. Since the role of these receptors in the dentate gyrus has yet not been characterized, this study investigated the effects of 5-HT4 receptors on basal synaptic transmission, LTP and depotentiation in the dentate gyrus of freely moving rats. Male Wistar rats were chronically implanted with a recording electrode in the dentate gyrus granule cell layer, a stimulation electrode in the medial perforant path and a cannula for drug administration in the ipsilateral ventricle. The 5-HT4 agonist methoxtryptamine dose-dependently inhibited basal synaptic transmission and LTP. Priming of receptors by a dose of this agonist which elicited no significant change of basal synaptic transmission inhibited depotentiation. These effects could be prevented by the 5-HT4 antagonist RS 39864, which did not produce independent effects on synaptic transmission, LTP or depotentiation. The effects of methoxtryptamine were confirmed with the highly selective 5-HT4 agonist, RS 67333. These results strongly support a role for 5-HT4 receptors in hippocampal synaptic plasticity and provide an important link to findings with regard to the involvement of 5-HT in processes related to learning and memory.

Introduction

Cellular mechanisms believed to contribute to the storage and processing of information in the mammalian brain (Bear, 1996) comprise, for example, long-term potentiation (LTP) (Bliss and Lomo, 1973), long-term depression (LTD) (Dudek and Bear, 1992) and depotentiation (Barrionuevo et al., 1980; Staubli and Lynch, 1990). LTP comprises a use-dependent persistent enhancement of synaptic efficacy which occurs as a result of patterned stimulation of afferent fibres (Bliss and Lomo, 1973). Depotentiation, on the other hand, comprises a reversal of tetanization-induced LTP, which occurs following low-frequency stimulation (LFS) of afferent fibres (Staubli and Lynch, 1990; Staubli and Chun, 1996; Kulla et al., 1999). This phenomenon can be distinguished from LTD in vivo, in that a prior potentiation of synaptic strength is required, whereas in the case of LTD synaptic depression can be induced in naive synapses (Manahan-Vaughan, 1997; Kulla et al., 1999). Furthermore, depotentiation can only be induced when LFS is applied within 5 min of LTP induction (Staubli and Lynch, 1990; Staubli and Chun, 1996; Kulla et al., 1999), whereas LTD can be induced at any time-point in previously potentiated synapses (Manahan-Vaughan, 1997).

LTP typically requires a critical involvement of kinase-dependent phosphorylation in order for robust expression to occur (Cheng et al., 1994; Lisman, 1994; Petit et al., 1994; Masaaki and Hatase, 1998). On the other hand, there is growing evidence that a persistent reduction of synaptic weight, which occurs as a consequence of LTD or depotentiation, predominantly depends upon dephosphorylation as a result of phosphatase modulation (Mulkey et al., 1993; O’Dell and Kandel, 1994; Masaaki and Hatase, 1998). Furthermore, the inhibition of protein phosphatase-1 is a key step in this dephosphorylation pathway (Mulkey et al., 1993; Wagner and Alger, 1996). The role of adenylyl cyclase, acting via cAMP-dependent protein kinase A, in the inhibition of protein phosphatase-1 has been well documented (Mulkey et al., 1993). Recent work by this group has shown that depotentiation in the dentate gyrus of freely moving rats is modulated by group II metabotropic glutamate receptors (mGluRs) which are negatively coupled to adenylyl cyclase and dopamine D1/D5 receptors which are positively coupled to adenylyl cyclase (Kulla et al., 1999; Kulla and Manahan-Vaughan, 2000). Thus, the involvement of cAMP-coupled metabotropic receptors in depotentiation in vivo has been demonstrated, consistent with a crucial role for cAMP in this phenomenon.

The dentate gyrus is a gateway for neuronal transmission entering the hippocampal formation and plays, therefore, a strategic part in hippocampus-dependent information storage. One of the major neurotransmitters influencing hippocampal neurotransmission is serotonin (5-hydroxytryptamine, 5-HT). Innervation originates from the dorsal and median raphe nuclei (Conrad et al., 1974). 5-HT receptors are classified into seven distinct receptor classes — 5-HT1–7 (Hoyer and Martin, 1996). The 5-HT4 receptors, which are expressed in hippocampus (Waechter et al., 1996; Markstein et al., 1999), are G-protein-coupled and increase intracellular levels of cAMP by activation of adenylyl cyclase (Fagni et al., 1992; Ansamay et al., 1995; Eglen et al., 1995a; Torres et al., 1995). In recent years, growing evidence has emerged that the 5-HT4 receptor is involved in various forms of cognitive processes in the mammalian brain, particularly different learning tasks (Fontana et al., 1997; Kennett et al., 1997; Letty et al., 1997; Marchetti-Gauthier et al., 1997; Meneses and Hong, 1997; Meneses, 1998; Terry et al., 1998). On the other hand, there is little knowledge of the contribution of the 5-HT4 receptor to different forms of synaptic plasticity. Given the demonstrated role of the 5-HT4 receptor in learning processes and our previous findings with regard to the involvement of cAMP-coupled receptors in synaptic plasticity, this study set about to elucidate the role of 5-HT4 receptors in basal synaptic transmission, LTP and depotentiation in the dentate gyrus of freely moving rats.

Materials and Methods

Electrode Implantation
Male Wistar rats (7–8 weeks old) underwent electrode implantation into the dentate gyrus as described previously (Manahan-Vaughan et al., 1998; Kulla and Manahan-Vaughan, 2000). Briefly, under sodium pentobarbitone anaesthesia (‘Nembutal’, 40 mg/kg, i.p., Serva, Germany), animals underwent implantation of a monopolar recording and a bipolar stimulating electrode (made from 0.1 mm diameter teflon coated stainless steel) into the dentate gyrus as described previously (Manahan-Vaughan et al., 1998; Kulla and Manahan-Vaughan, 2000).
steel hole. A drill hole was made (1.0 mm diameter) for the recording electrode (2.8 mm posterior to bregma and 1.8 mm lateral to the midline) and a second drill hole (1.5 mm diameter, 6.9 mm posterior to bregma and 4.1 mm lateral to the midline) for the stimulating electrode. The dura was pierced through both holes and the recording and stimulating electrodes lowered into the dentate gyrus granule cell layer and the medial perforant path, respectively. Recordings of evoked field potentials via the implanted electrodes were taken throughout surgery. A cannula was also implanted into the residual cerebral ventricle, through which drug application was made. Once verification of the location of the electrodes was complete, the entire assembly was sealed and fixed to the skull with dental acrylic (Paladur, Heraeus Kulzer GmbH, Germany). The animals were allowed 7–10 days to recover from surgery before experiments were conducted. Throughout the experiments the animals could move freely. Experiments were consistently conducted at the same time of day (commencing at 09:00 h). Baseline experiments to confirm stability of evoked responses were routinely carried out at least 24 h before LTP or depotentiation experiments were conducted. Where possible, the animals served as their own controls. Thus, basal synaptic transmission (in the absence of injection) was monitored over a 24 h period in all animals in order to confirm stability of evoked responses. Subsequently, a control experiment (e.g. depotentiation or basal synaptic transmission) was carried out in the presence of vehicle injection and –1 week later the same experiment was carried out in the same animal in the presence of a drug injection.

Measurement of Evoked Potentials

Responses were evoked by stimulating at low frequency (0.025 Hz, 0.2 ms stimulus duration, 10 000 Hz sample rate). For each time-point, five evoked potentials were averaged. Both field excitatory post-synaptic potential (fEPSP) slope and population spike (PS) amplitude were monitored. The amplitude of PS was measured from the peak of the first positive deflection of the evoked potential to the peak of the following negative potential. fEPSP slope was measured as the maximal slope through the five steeps points obtained on the first positive deflection of the potential. By means of input/output curve determination the maximum PS amplitude was found for each individual animal and all potentials employed as baseline criteria were evoked at a stimulus intensity which produced 40% of this maximum.

LTP was induced by a high-frequency tetanus (HFT) of 200 Hz (10 bursts of 15 stimuli, 0.2 ms stimulus duration, 10 s interburst interval). Depotentiation was generated using low-frequency stimulation (LFS) at 5 Hz (600 pulses). The stimulus amplitude for both protocols was the same as that used for recordings.

The cortical electroencephalogram (EEG) was monitored throughout the course of each experiment; however, no alteration in EEG was seen as a result of HFT, LFS or drug application.

Compounds and Drug Treatment

The 5-HT4 receptor agonist RS 67333 and the 5-HT4 receptor antagonist RS 39604 were obtained from Tocris Cookson Ltd (Bristol, UK). The 5-HT4 receptor agonist 5-methoxytryptamine was obtained from Sigma, Taufkirchen, Germany. For injection, drugs were dissolved in distilled water (or 0.9% NaCl in the case of methoxytryptamine). Compounds or vehicle were injected in a 5 μl volume over a 6 min period via a Hamilton syringe. Agonist injection was carried out 30 min prior to tetanization and, when appropriate, antagonist injection occurred a further 30 min prior to agonist application, to enable diffusion from the lateral cerebral ventricle to the hippocampus to occur (Manahan-Vaughan et al., 1998). Throughout the experiments, injections were administered following measurement of the baseline for 30 min. In LTP experiments, a tetanus was applied 30 min following injection, with measurements then taken at t = 2, 5, 10, 15 and then 15 min intervals up to 4 h, with additional measurements taken after 24 h. LFS to induce depotentiation was given 5 min after tetanization had occurred and the experimental protocol for measuring evoked responses was then followed as above.

Data Analysis

The baseline fEPSP or PS data were obtained by averaging the response to stimulating the perforant path, to obtain five sweeps at 0.025 Hz, every 5 or 15 min as described above. The data were then expressed as mean ± pre-injection baseline reading ± SEM. Statistical significance was estimated using analysis of variance (ANOVA) with repeated measures, followed by post-hoc Student’s t-tests. The probability level interpreted as statistically significant was P < 0.05.

Results

The 5-HT4 Receptor Agonist Methoxytryptamine Dose-dependently Inhibits Basal Synaptic Transmission in the Dentate Gyrus of Freely Moving Rats

The involvement of 5-HT4 receptors in basal synaptic transmission was initially investigated using the serotonergic agent methoxytryptamine, which is an agonist at 5-HT4 receptors (Monferini et al., 1993). Basal synaptic transmission in the presence of the vehicle was stable with regard to both PS amplitude and fEPSP slope over the 24.5 h period monitored (Fig. 1A,B,D). Administration of methoxytryptamine dose-dependently reduced basal synaptic transmission, however.

Whereas 2.7 μg methoxytryptamine (n = 8) had no effect on either PS amplitude or fEPSP compared to controls (n = 8), basal transmission was increasingly inhibited by raising the concentration of the compound in the range of 5.6–22 μg (Fig. 1C). The dose–response curves (Fig. 1C) for PS amplitude and fEPSP slope demonstrated a marked decrease of the 24 h post-injection values with increasing drug concentration. Whereas 3.6 μg reduced PS values to 77 ± 9% (not significant) and fEPSP values to 87 ± 4% (n = 8) (not significant), 4.5 μg reduced PS values to 72 ± 9% and fEPSP values to 83 ± 4% (n = 8). Increasing the concentration to 9 μg produced a reduction of PS values to 48 ± 16% and fEPSP values to 76 ± 3% (n = 8) at 24 h post-injection. Compared to controls (n = 8), t-test analysis showed significant differences for the 24 h post-injection values (4.5 μg; PS P < 0.05, fEPSP P < 0.05; 9 μg; PS P < 0.05, fEPSP P < 0.001).

When the concentration of methoxytryptamine was raised to 22 μg (n = 8), a substantial reduction in basal synaptic transmission became evident 45 min post-injection, as was the case for RS 67333 (Fig. 6A,B) (P < 0.001 for PS; P < 0.05 for fEPSP). Five minutes post-injection, PS and fEPSP values were 100 ± 1 and 98 ± 6%, respectively. At 24 h post-injection, PS and fEPSP values were 41 ± 5 and 64 ± 8%, respectively (t-test, P < 0.01 for PS, P < 0.01 for fEPSP compared to controls, n = 8). ANOVA confirmed the statistical significance between the control and the 22 μg drug groups (Tables 1 and 2).

The 5-HT4 Receptor Antagonist RS 39604 Prevents the Inhibitory Effects of the 5-HT4 Receptor Agonist, Methoxytryptamine on Basal Synaptic Transmission

The depressant effects on basal synaptic transmission produced by 22 μg methoxytryptamine were inhibited by application of the 5-HT4 antagonist RS 39604 prior to agonist administration (25 μg, n = 8; Fig. 2). Vehicle injection prior to methoxytryptamine administration (n = 8) produced a depression in basal synaptic transmission which was similar to that seen when 22 μg methoxytryptamine was applied alone. Basal synaptic transmission decreased in the presence of methoxytryptamine, to 40 ± 5% for PS and 75 ± 3% for fEPSP values. This depression persisted for 24 h where values were 39 ± 5% for PS and 71 ± 5% for fEPSP. Prior injection of 25 μg RS 39604 prevented this inhibition, resulting in basal synaptic transmission which was stable for 24 h. This response showed no statistical difference to vehicle controls (for ANOVA, see Table 1).

As shown in Figure 1, application of 2.7 μg methoxytryptamine had no effect on basal synaptic transmission. Application
of 25 µg RS 39604 prior to the agonist (n = 8) did not elicit alterations in basal synaptic transmission. The absence of statistical difference compared to vehicle-injected controls was confirmed by ANOVA for both PS and fEPSP (Tables 1 and 2).

**The 5-HT4 Receptor Agonist Methoxytryptamine has no Effect on Long-term Potentiation in the Dentate Gyrus of Freely Moving Rats**

In the dentate gyrus, robust LTP was induced by delivering 200 Hz high-frequency tetanization (HFT, 10 bursts of 15 stimuli, 0.2 ms stimulus duration) to the medial perforant path (Fig. 3A,B). The effect of agonist priming of 5-HT4 receptors with a concentration of methoxytryptamine which had no independent effects on basal synaptic transmission was tested. Injection of 2.7 µg methoxytryptamine 30 min prior to HFT (n = 6) resulted in no detectable effect on the magnitude or time-course of LTP with regard to either PS amplitude or fEPSP slope compared to vehicle injected controls (n = 7). ANOVA supported an absence of statistical significance between the control and the 2.7 µg drug groups (Tables 1 and 2).

**Depotentiation is Inhibited by Agonist Priming of the 5-HT4 Receptor with Methoxytryptamine**

It was shown that the 5-HT4 receptor agonist methoxytrypt-
The 5-HT4 Receptor Agonist RS 67333 Dose-dependently Inhibits Basal Synaptic Transmission in the Dentate Gyrus of Freely Moving Rats

To confirm that the modulatory effects on synaptic transmission seen with methoxytryptamine were, in fact, mediated by the 5-HT4 receptor, the experiments were repeated in the presence of the highly selective 5-HT4 receptor agonist RS 67333.

Basal synaptic transmission in the presence of the vehicle was stable with regard to both PS amplitude and fEPSP slope over the 24.5 h period monitored (Fig. 4A,B). As was the case with methoxytryptamine, administration of RS 67333 dose-dependently reduced basal transmission (Fig. 4A,B). Whereas 5 µg RS 67333 (n = 7) had no effect on either PS amplitude or fEPSP compared to controls (n = 6), basal transmission was significantly and increasingly inhibited by raising the concentration of the compound in the range of 7.5–50 µg (Fig. 4C). The dose–response curves (Fig. 4C) for PS amplitude and fEPSP slope showed a marked decrease of the 24 h post-injection values with increasing drug concentration. Whereas 7.5 µg reduced PS values to 83 ± 7% and fEPSP values to 91 ± 3% (n = 6), 10 µg reduced PS values to 86 ± 4% and fEPSP values to 78 ± 8% (n = 6). Increasing the concentration to 25 µg produced at reduction at 24 h of PS values to 51 ± 4% and fEPSP values 70 ± 9% (n = 6). Compared to controls (n = 6), t-test analysis showed significant differences for the 24 h post-injection values (7.5 µg, PS P < 0.05, fEPSP P < 0.05; 10 µg, PS P < 0.01, fEPSP P < 0.05; 25 µg, PS P < 0.0001, fEPSP P < 0.01).

When the concentration of RS 67333 was raised to 50 µg (n = 6), a substantial reduction in basal synaptic transmission became evident as soon as 45 min post-injection (Fig. 4A). Five minutes post-injection, PS and fEPSP values were 90 ± 4 and 102 ± 3% respectively. At 24 h post-injection, PS and fEPSP values were 63 ± 2 and 67 ± 7%, respectively [P < 0.05 (t-test) from 45 min post-injection]. ANOVA confirmed the statistical significance between the control and the 50 µg-drug groups (Tables 1 and 2).

The 5-HT4 Receptor Agonist RS 39604
Dose-dependently Prevents the Inhibitory Effects of the 5-HT4 Receptor Agonist RS 67333 on Basal Synaptic Transmission

For a confirmation that the inhibitory effects on basal synaptic transmission of the agonist RS 67333 were mediated by 5-HT4 receptors, the 5-HT4 receptor antagonist RS 39604 was applied prior to RS 67333 administration (Fig. 5). Vehicle application prior to application of 50 µg RS 67333 produced a significant reduction in basal synaptic transmission (Fig. 5), which was not statistically different from the effects obtained when 50 µg RS 67333 was applied alone (Fig. 4). Applying 10 µg RS 39604 (n = 7) did not result in a complete block of the inhibitory action of the agonist compared to vehicle/50 µg RS 67333 injected controls (n = 6). A complete block of the inhibitory action of the agonist was produced by antagonist concentrations of 25 µg (n = 6; Fig. 5), or 50 µg (n = 6; not shown). Neither PS amplitude values nor fEPSP values in either group showed significant differences to vehicle controls (n = 6). ANOVA confirmed these results (Tables 1 and 2).

The 5-HT4 Receptor Agonist RS 67333 Dose-dependently Inhibits Long-term Potentiation in the Dentate Gyrus of Freely Moving Rats

In the dentate gyrus, robust LTP was induced by delivering 200 Hz high-frequency tetanization (HFT, 10 bursts of 15 stimuli, 0.2 ms stimulus duration) to the medial perforant path (Fig. 6). Initially, the effect of agonist priming of 5-HT4 receptors with a concentration of the agonist RS 67333, which had no independent effects on basal synaptic transmission (Fig. 4), was
tested. Injection of 5 μg RS 67333, 30 min prior to HFT (n = 6) resulted in no detectable effect on the magnitude or time-course of LTP with regard to either PS amplitude or fEPSP slope compared to vehicle-injected controls (n = 6) (Fig. 6).

Increasing the concentration of RS 67333 to 7.5, 10 and 50 μg was associated with an increasing inhibition of LTP however (Fig. 6C). Whereas no significant reduction in the initial magnitude of LTP could be seen, significant changes could be detected in the 24 h post-HFT values. Thus, in comparison to vehicle-injected controls (n = 6) where 24 h post-HFT PS values were 205 ± 27% and fEPSP values were 122 ± 8% (n = 6), a reduction to PS 170 ± 12% and fEPSP 105 ± 6% could be seen when 7.5 μg RS 67333 was injected 30 min prior to HFT. An even more dramatic reduction could be seen when 10 μg RS 67333 was applied (Fig. 6), where 24 h post-HFT PS and fEPSP values were 138 ± 27 and 103 ± 7%, respectively (n = 6). Application of 50 μg RS 67333 caused a reduction of LTP values to 106 ± 11% (PS) and 80 ± 12% (fEPSP) 24 h post-HFT (n = 6). Whereas ANOVA did not confirm the 7.5 μg group as being significantly different in comparison to the controls, significant differences were found for the 10 and 50 μg groups (Tables 1 and 2).

These effects may have been associated with the depressive effects of the higher concentrations of agonists on basal synaptic transmission. For example, 10 μg RS 67333 (n = 6) produced a significant depression in basal synaptic transmission with regard to PS amplitude (n = 6). This effect was more pronounced with regard to fEPSP (Fig. 6). Twenty-four hours after drug injection, PS amplitude and fEPSP slope values were still significantly

### Table 1

<table>
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<tr>
<th>Treatment</th>
<th>n</th>
<th>Within factor</th>
<th>P &lt;</th>
<th>Between factor</th>
<th>P &lt;</th>
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<tr>
<td>Basal transmission</td>
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<tr>
<td>Methoxytryptamine 25 μg</td>
<td>8</td>
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In the case of agonist injection, comparison was made with vehicle-injected controls; in the case of antagonist/agonist injection, comparison was made with vehicle/agonist-injected controls (as described in the Results section).

### Table 2

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<th>Treatment</th>
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<th>Within factor</th>
<th>P &lt;</th>
<th>Between factor</th>
<th>P &lt;</th>
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<td>Basal transmission</td>
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<td>Methoxytryptamine 25 μg</td>
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<td>1.0</td>
<td>F(1,32) = 0.90</td>
<td>0.34</td>
</tr>
<tr>
<td>Depotentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methoxytryptamine 2.7 μg</td>
<td>11</td>
<td>F(1,31) = 2.02</td>
<td>0.05</td>
<td>F(1,31) = 35.18</td>
<td>0.0001</td>
</tr>
<tr>
<td>RS39604 25 μg/methoxytryptamine 2.7 μg</td>
<td>10</td>
<td>F(1,31) = 0.13</td>
<td>1.0</td>
<td>F(1,31) = 1.62</td>
<td>0.20</td>
</tr>
<tr>
<td>RS39604 25 μg</td>
<td>6</td>
<td>F(1,39) = 0.27</td>
<td>1.0</td>
<td>F(1,39) = 0.75</td>
<td>0.387</td>
</tr>
<tr>
<td>RS 67333 5 μg</td>
<td>10</td>
<td>F(1,39) = 0.50</td>
<td>1.0</td>
<td>F(1,39) = 2.89</td>
<td>0.09</td>
</tr>
</tbody>
</table>

In the case of agonist injection, comparison was made with vehicle-injected controls; in the case of antagonist/agonist injection comparison was made with vehicle/agonist-injected controls (as described in the Results section).
depressed compared to vehicle injected-controls (PS, 86 ± 4%, t-test, \( P < 0.05 \); fEPSP, 78 ± 8%, t-test, \( P < 0.01 \)). The effects were confirmed by ANOVA (Table 1).

**Depotentiation is Inhibited by Priming of the 5-HT\(_4\) Receptor with RS67333**

It was shown that the 5-HT\(_4\) receptor agonist RS 67333 in a 5 \( \mu \)g concentration had no significant effects on basal synaptic transmission or LTP (Fig. 6). However, injection of 5 \( \mu \)g of RS 67333 (\( n = 10 \)) significantly inhibited depotentiation (Fig. 7). Whereas no change in the initial phase of depotentiation occurred compared to vehicle controls (\( n = 10 \)), a significant reduction in the magnitude of depotentiation was noted from \( t = 150 \) min post-LFS with regard to both PS and fEPSP values (t-test, \( P < 0.05 \)), which persisted for <24 h. At this point, PS and fEPSP values were 193 ± 17 and 124 ± 5%, respectively, in
RS 67333-treated animals, whereas PS amplitude was 138 ± 9% and fEPSP slope was 102 ± 5% in control animals. The inhibition of depotentiation by 5 µg RS 67333 could be blocked when the 5-HT4 antagonist RS 39604 (25 µg) was injected prior to RS 67333 (n = 6). In contrast, application of 50 µg RS 67333 (n = 6) elicited a marked depression of basal synaptic transmission [P < 0.05 (t-test) from 45 min post-injection]. Line breaks indicate a change in time-scale. (C) Dose–response curve for the inhibitory effect of RS 67333 (5–50 µg applied in a 5 µl injection volume to the lateral cerebral ventricle) on basal synaptic transmission in the dentate gyrus of freely moving rats. The values represent PS (i) and fEPSP (ii) values observed at 24 h post-LFS. Compared to controls (n = 6), t-test analysis showed significant differences for the 24 h post-injection values (7.5 µg, PS P < 0.05, fEPSP P < 0.05; 10 µg, PS P < 0.01, fEPSP P < 0.05; 25 µg, PS P < 0.0001, fEPSP P < 0.01). (D) Original analog traces showing the field potentials evoked from the dentate gyrus pre-injection, 5 min and 24 h following application of (i) 5 µg RS 67333 and (ii) 50 µg RS 67333. Vertical scale bar corresponds to 5 mV, horizontal scale bar corresponds to 4 ms.

Figure 4. The 5-HT4 receptor agonist RS 67333 dose-dependently inhibits basal synaptic transmission in the dentate gyrus of freely moving rats. (A,B) Test-pulse stimulation when given in the presence of the 5-HT4 receptor agonist RS 67333 (5 µg, n = 7) does not affect basal PS amplitude (A) or fEPSP slope (B) compared to vehicle-injected controls (n = 6).

Induction of depotentiation in the presence of 10 µg RS 67333 resulted in no lasting effects on depotentiation (Fig. 8A,B). Twenty-four hours after induction of depotentiation, PS and fEPSP values were 140 ± 13 and 106 ± 5%, respectively, in RS 67333-treated animals (compared to PS values of 138 ± 9% and fEPSP slope of 102 ± 5% in control animals).

The 5-HT4 Receptor Antagonist RS 39604 has no Effect on Basal Synaptic Transmission, LTP or Depotentiation

To investigate whether the 5-HT4 receptor antagonist RS 39604 has any independent effects on basal synaptic transmission LTP or depotentiation, the concentration of antagonist which was effective in blocking the inhibitory effects of the agonist...
This depotentiation was not altered when 25 μg applied 5 min after HFT, persistent depotentiation occurred vehicle controls (magnitude or time-course of the LTP induced in comparison to injected 30 min prior to HFT did not show any effects on the magnitude or time-course of the LTP). Similar antagonist effects were seen for both PS amplitude (A) and fEPSP slope (B) compared to vehicle-injected controls (n = 5) and controls where vehicle was injected prior to RS 67333 (50 μg). Whereas 10 μg RS 39604 (n = 7) only partially inhibits the depressive effects of RS 67333 (50 μg), application of the antagonist in a concentration of 25 μg (n = 6) completely prevents the inhibitory effects of the agonist on basal synaptic transmission. Neither PS amplitude nor fEPSP values in the RS39604/RS67333 groups showed significant differences to vehicle-injected controls (n = 6). ANOVA analysis confirmed these results. For 25 μg RS9604 versus RS67333, PS values were: within-factor, F(1,39) = 1.40, P < 0.24; fEPSP values were: within-factor, F(1,39) = 0.31, P < 1.0; between-factor, F(1,39) = 13.41, P < 0.3. For 50 μg of the antagonist, PS values were: within-factor, F(1,39) = 0.14, P < 1.0; between-factor, F(1,39) = 0.001, P < 0.99; fEPSP values were: within-factor, F(1,39) = 0.13, P < 1.0; between-factor, F(1,39) = 1.21, P < 0.27. Line breaks indicate a change in time-scale.

RS 67333 was examined. An injection of 25 μg RS9604 (n = 6) had no influence on basal synaptic transmission compared to vehicle-injected controls (n = 6; Fig. 8). ANOVA confirmed the lack of significant differences between drug and baseline vehicle controls (Table 1).

Robust LTP was induced in the dentate gyrus by delivering HFT (Fig. 8). The same concentration of the antagonist (n = 6) injected 30 min prior to HFT did not show any effects on the magnitude or time-course of the LTP induced in comparison to vehicle controls (n = 6; for ANOVA results, see Tables 1 and 2).

When low-frequency stimulation (5 Hz, 600 pulses) was applied 5 min after HFT, persistent depotentiation occurred (Fig. 7). This depotentiation was not altered when 25 μg of the 5-HT4 antagonist (n = 6) was injected prior to induction of depotentiation (Fig. 7). ANOVA verified that no significant differences to depotentiation vehicle controls existed (Tables 1 and 2).

Discussion
The observations of this study demonstrate that basal synaptic transmission, depotentiation and LTP are dose-dependently...
inhibited by 5-HT₄ receptor agonists in the dentate gyrus of freely moving rats. Unlike LTP, depotentiation is inhibited by an agonist concentration which has no significant effect on basal synaptic transmission. Whereas a 5-HT₄ receptor antagonist prevents the agonist-induced inhibition of basal synaptic transmission, depotentiation and LTP, the antagonist has no independent effects on the expression of any of these phenomena. These data suggest that activation of 5-HT₄ receptors results in modulation of LTP and depotentiation in the dentate gyrus in vivo, but also suggest that 5-HT₄ receptor activation is not a critical factor for the expression of either form of synaptic plasticity. On the other hand, the potent effects on depotentiation seen following agonist priming strongly support a role for this receptor in metaplasticity (Abraham and Bear, 1996).

The inhibition of basal synaptic transmission by the serotonergic agent methoxytryptamine, which is an effective agonist

Figure 7. Depotentiation is inhibited by agonist priming of the 5-HT₄ receptor with RS 67333. (A,B) Low frequency stimulation (LFS) at 5 Hz when given 5 min post-HFT in the presence of vehicle injection (n = 10) results in a significant reversal of LTP of both PS (A) and fEPSP (B). Application of RS 67333 (5 µg, n = 10) prior to HFT and LFS results in a significant inhibition of depotentiation. Thus, a significant reduction in the magnitude of depotentiation was noted from t = 150 min post-LFS with regard to both PS and fEPSP values compared to controls (t-test, P < 0.05). Raising the concentration of RS 67333 to 10 µg (n = 8) results in a loss of the inhibitory effects of the agonist with regard to both PS (A) and fEPSP (B). (C,D) Administration of the 5-HT₄ receptor antagonist RS 29604 (25 µg, n = 6) prior to application of RS 67333 (5 µg) results in a significant prevention of the inhibitory effects of the agonist on depotentiation of both the PS (C) and fEPSP (D). RS 39604 (25 µg, n = 6), has no independent effects on the expression of depotentiation. Depotentiation in the combined presence of antagonist and agonist did not show any significant differences to depotentiation in the presence of vehicle only [ANOVA — PS: within-factor, F(1,39) = 0.55, P < 0.99; between-factor, F(1,39) = 1.55, P < 0.213; fEPSP: within-factor, F(1,39) = 0.50, P < 1.0; between-factor, F(1,39) = 2.89, P < 0.09]. Line breaks indicate a change in time-scale. (E) Original analog traces showing the field potentials evoked from the dentate gyrus before HFT, 2 min post-HFT, 5 min post-LFS and 24 h post-LFS in the presence of (i) vehicle or (ii) RS 67333 (5 µg). Vertical scale bar corresponds to 5 mV, horizontal scale bar corresponds to 4 ms.
on basal synaptic transmission, LTP and depotentiation were produced by activation of 5-HT$_4$ receptors was provided by repetition of the key experiments in the presence of the agonist RS 67333 (Eglen et al., 1995a). The actions of RS 67333 mirrored those of methoxytryptamine very closely. Thus, a dose-dependent inhibition of basal synaptic transmission in the range of 5–50 µg was seen, as well as an inhibition of depotentiation using an agonist concentration which did not influence basal synaptic transmission.

RS 67333 has a reported pK$_A$ of 9.1 at 5-HT$_4$ receptors and an affinity of pK$_A$ > 6 to 5-HT$_4$ receptors (Eglen et al., 1995b). The possibility that some of the inhibitory effects of the agonist were mediated by 5-HT$_4$ receptors cannot, therefore, be excluded. Evidence exists of inhibitory postsynaptic 5-HT$_4$ receptors on dentate gyrus granule cells (Piguet and Galvan, 1994). Indeed, reductions in hippocampal basal synaptic transmission as a consequence of 5-HT$_4$ receptor activation have previously been reported (Manahan-Vaughan et al., 1994a,b, 1995). These effects are likely to have been mediated by hyperpolarization of dentate gyrus granule cells via activation of a K$^+$ conductance (Biak and Misgeld, 1997). However, the fact that the 5-HT$_4$ antagonist RS 39604 completely prevented the inhibitory effects of the agonist suggests that its effects were predominantly mediated via 5-HT$_4$ receptors.

5-HT$_4$ receptor stimulation results in the activation of protein kinase A and cAMP in rat hippocampus, which subsequently closes K$^+$ channels (Bockaert et al., 1998). Even short-term agonist exposure results in a transient (~2 h) K$^+$ current inhibition. This results in a reduction of after-hyperpolarization and an increase in neuronal excitability (Andrade and Chaput, 1991; Fagni et al., 1992; Ansanay et al., 1995; Torres et al., 1995). Although these 5-HT$_4$-receptor-mediated events could explain the inhibition of depotentiation elicited by application of the 5-HT$_4$ receptor agonist, it is difficult to see how a 5-HT$_4$ receptor-mediated increase in neuronal excitability could lead to the inhibition of basal synaptic transmission and LTP observed in the current study. However, it has been shown that 5-HT$_4$ receptor stimulation increases intrahippocampal 5-HT levels (Ge and Barnes, 1996), which could indirectly result in neuronal hyperpolarization via activation of 5-HT$_4$ receptors and subsequent inhibition of basal synaptic transmission and LTP. On the other hand, depolarization by 5-HT$_4$ receptor activation of GABAergic interneurons in the guinea pig dentate gyrus hilar region has been demonstrated (Biak and Misgeld, 1997). Furthermore, 5-HT$_4$ receptors increase the frequency of GABA(A) and GABA(B) receptor-mediated inhibitory post-synaptic potentials in dentate gyrus granule cells (Biak and Misgeld, 1997). Thus, the inhibition of basal synaptic transmission, LTP and depotentiation seen in the current study following RS 67333 application could also have occurred as a result of 5-HT$_4$ receptor modulation of GABA transmission in the dentate gyrus.

The late onset of the inhibitory effects of the agonist on basal synaptic transmission and LTP may also be an indication of extrahippocampal effects resulting from 5-HT$_4$ receptor activation. The 5-HT$_4$ receptor is distributed widely throughout the hippocampus (Claeysen et al., 1998) and slow diffusion of the compound from the lateral ventricle to other brain regions, such as the dorsal raphe, could result in altered 5-HT neurotransmission in the hippocampus. In another study, it was demonstrated that application of pharmacological agents to the lateral cerebral ventricle results in an initial specific localization of the injected compound to the lateral ventricle and neighbouring hippocampus (Manahan-Vaughan et al., 1998). This specificity endures for a period of ~60 min post-injection. 

![Figure 8. The 5-HT$_4$ receptor antagonist RS 39604 has no effect on basal synaptic transmission or LTP (A,B) 200 Hz HFT in the presence of vehicle (n = 6) results in a robust long-term potentiation of both PS (A) and fEPSP (B). Prior application of the 5-HT$_4$ receptor antagonist RS39604 (25 µg, n = 6) has no effect on the expression of LTP. Similarly, basal synaptic transmission is unaffected by application of RS39604 (25 µg, n = 6), compared to vehicle-injected controls, (n = 6). (C) Original analog traces showing the field potentials evoked from the dentate gyrus pre-injection, 5 min and 24 h following HFT in the presence of (i) vehicle or (ii) RS 39604 (25 µg). Vertical scale bar corresponds to 5 mV, horizontal scale bar corresponds to 4 ms.](image-url)
Thus, one can assume that at the time-points where LTP and depotentiation were induced, primarily the hippocampal 5-HT₄ receptors were activated.

The dose-dependent inhibition of LTP by the agonist RS 67333 paralleled the inhibition of basal synaptic transmission seen with this compound. The initial magnitude of LTP was unaffected by application of RS 67333, suggesting that pharmacological activation of 5-HT₄ receptors has no effect on the induction of LTP. As was the case with basal synaptic transmission, a reduction in evoked potentials became evident ~3 h after application of the agonist. Thus, a reduction of the later phases of LTP could reflect an equivalent reduction of basal synaptic transmission.

On the other hand, the maintenance of LTP itself may have been disturbed. For example, 50 µg of the agonist produced a reduction to ~60% of pre-injection PS values with regard to basal synaptic transmission, whereas the same concentration of the agonist reduced PS values to ~90% of pre-injection values (which comprised a reduction of 25% of LTP values).

Interestingly, cAMP-coupled receptors appear to modulate synaptic plasticity in quite distinct ways in the dentate gyrus in vivo. Whereas agonist activation of dopamine D₁/D₅ receptors (which are positively coupled to adenylyl cyclase) has no effect on LTP, depotentiation is inhibited by these receptors. However, antagonism of these receptors has no effect on either phenomenon (Kulla and Manahan-Vaughan, 2000). Furthermore, pharmacological antagonism of group 2 mGluRs (which are negatively coupled to adenylyl cyclase) has no effect on LTP in the dentate gyrus (Manahan-Vaughan et al., 1998).

Interestingly, cAMP-coupled receptors appear to modulate synaptic plasticity in quite distinct ways in the dentate gyrus in vivo. Whereas agonist activation of dopamine D₁/D₅ receptors (which are positively coupled to adenylyl cyclase) has no effect on LTP, depotentiation is inhibited by these receptors. However, antagonism of these receptors has no effect on either phenomenon (Kulla et al., 1999). In addition, agonist activation of group 2 mGluRs inhibits maintenance of LTP, enhances depotentiation (Kulla et al., 1999) and facilitates the expression of long-term depression in the dentate gyrus (Manahan-Vaughan, 1998).

Furthermore, in the present study it was shown that agonist activation of 5-HT₄ receptors dose-dependently inhibits LTP and depotentiation, whereas antagonism of these receptors has no effect. These observations could perhaps be explained by the relative neuronal distribution and expression of the respective receptor types (Smiley et al., 1994; Bergson et al., 1995; Waerber et al., 1996; Lujan et al., 1997), pre-versus post-synaptic localization (Jaber et al., 1996; Vilà et al., 1998; Shigemoto et al., 1997), or the differing effector mechanisms employed by these receptors to elicit alterations in neuronal function (Liu et al., 1992; Chavis et al., 1995; Bijak and Misgeld, 1997; Aosaki et al., 1998; Bockaert et al., 1998).

The observation that the 5-HT₄ antagonist did not elicit any effects on basal synaptic transmission, LTP or depotentiation suggests that activation of this receptor is neither critically involved in tonic regulation of synaptic transmission, nor essential for the expression of synaptic plasticity. Rather, it appears to be the case that the 5-HT₄ receptor is capable of modulation of these phenomena. On the other hand, the potent effects of agonist priming on depotentiation strongly support a role for 5-HT₄ receptors in metaplasticity (Abraham and Bear, 1996).

Application of 10 µg RS 67333 resulted in a loss of the inhibitory effects on depotentiation seen when this agonist was applied in a 5 µg concentration. The lack of effect of the higher agonist concentration may be associated with the depressive effects seen on LTP and basal synaptic transmission when this agonist concentration was used. Intriguingly, both 5-HT₄ receptor agonists inhibited depotentiation at concentrations which had no effects on basal synaptic transmission or LTP. This suggests that, whereas the depressive effects of higher agonist concentrations on basal synaptic transmission and LTP may be related (and explained by altered 5HT neurotransmission in the hippocampus), the agonist inhibition of depotentiation may have been mediated by an entirely different mechanism. In other words, 5-HT₄ receptor activation produced an alteration in synaptic efficacy which did not initially manifest itself as a change in synaptic weight, but which was sufficient to alter the profile of a subsequent depotentiation: a phenomenon which is commonly known as metaplasticity (Abraham and Bear, 1996).

This finding suggests that the 5-HT₄ receptor is capable of differential metaplasticity, in that depotentiation but not LTP is affected by agonist priming. Depotentiation of LTP may function to return potentiated synapses to their previous level of activation (following transduction of an LTP signal) or to shut-down erroneous LTP induction, thereby freeing a synaptic population to undergo renewed LTP. The current data argue against the likelihood that depotentiation reverses LTP by a simple disruption of LTP consolidation however. Given previous reports of a role for the 5-HT₄ receptor in cognitive processing (Fontana, 1997; Kennett, 1997; Letty et al., 1997; Marchetti-Gauthier et al., 1997; Meneses and Hong, 1997; Meneses, 1998; Terry et al., 1998; Marchetti et al., 2000), these data suggest that, under certain circumstances, release of serotonin could activate 5-HT₄ receptors resulting in prevention of depotentiation and, consequently, reinforcement of LTP. This modulation could have a decisive influence on subsequent information storage or retrieval.

In conclusion, 5-HT₄ receptors appear to play a modulatory role in the expression of LTP and depotentiation in the dentate gyrus in vivo. A critical role for these receptors in these phenomena must be excluded on the basis that agonist application elicited no effects on either LTP or depotentiation. The finding that agonist priming, using a drug concentration which had no independent effects on basal synaptic transmission, inhibited depotentiation but not LTP suggests that 5-HT₄ receptors may contribute to metaplasticity of reductions in synaptic weight in the dentate gyrus. This observation, together with reports that 5-HT₄ receptors contribute to certain forms of hippocampally based learning, suggests that activation of 5-HT₄ receptors may participate in cellular processes which underlie information storage in the brain.

Notes
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References


