Effects of 5-HT₃ Receptor Antagonism on Hippocampal Theta Rhythm, Memory, and LTP Induction in the Freely Moving Rat

Ursula Stäubli and Fang Bo Xu

Center for Neural Science, New York University, New York, New York 10003

Serotonergic brainstem projections to hippocampus are thought to preferentially target and increase, via 5-HT₃ receptors, the excitability of a distinct subpopulation of interneurons that primarily regulate GABA_B-mediated inhibition in the dendritic region of pyramidal cells. Hippocampal slice work suggests that the between-burst hyperpolarization caused by slow (GABA_B) IPSPs plays a significant role in controlling the strength of LTP induced with theta burst stimulation. According to the above observations it was assumed that blockade of hippocampal 5-HT₃ receptors should reduce the hyperpolarization and thereby enhance both the frequency of the naturally occurring theta rhythm and the induction of LTP; moreover, if LTP-like mechanisms provide the substrate for certain forms of memory, such treatment was expected to facilitate learning. Each of the above predictions was tested and confirmed in the present set of experiments. The effects of ondansetron, a potent and selective antagonist of the 5-HT₃ receptor, were examined on (1) frequency of the hippocampal theta rhythm, (2) induction of LTP in field CA1 of freely moving rats, and (3) retention of olfactory and spatial memory in tasks known to depend on an intact hippocampus. When injected intraperitoneally into freely moving rats, the drug reliably and significantly increased the frequency of the hippocampal theta rhythm in a dose-dependent manner. Second, at concentrations that facilitate theta frequency (100 µg/kg and 500 µg/kg), an injection of the drug 30 min prior to delivering electrical stimulation bursts significantly increased the magnitude and duration of LTP compared to that obtained in the same animals after vehicle injections. Third, when tested at a dose of 100 µg/kg, ondansetron was also found to improve retention in an odor matching problem and in a spatial task.

[Key words: hippocampus, 5-HT, 5-HT₃ receptors, interneurons, GABA_B, slow IPSP, hyperpolarization, theta rhythm, long-term potentiation, olfactory memory, spatial memory]

Brainstem projections to forebrain have long been implicated in the regulation of behavioral states such as attention, arousal, sleep, etc., and there is substantial evidence that they exert a

powerful influence on the encoding of memory (e.g., Landfield, 1976; Vertes, 1986; Winson, 1986; Vanderwolf et al., 1990; Baumgarten, 1993). The present study focussed on 5-HT, one of the major transmitters of the diffuse ascending brainstem systems, to examine if its influence on learning is mediated via an action on the cellular machinery responsible for the encoding of memory. This broad question was analyzed in the context of a working hypothesis concerning the link between brain rhythms and the long-term potentiation (LTP) effect, a form of synaptic plasticity thought to underlie certain variants of memory. According to this idea, *global behavioral states* (e.g., alert, moderate arousal) and activities (e.g., exploration) appropriate for learning could be correlated with *local network states* that promote the occurrence of long-lasting synaptic changes (e.g., LTP) and the formation of memory. The impetus for this argument comes from the following observations. (1) LTP in field CA1 of hippocampus is optimally elicited with very short stimulation bursts (30 msec) separated by the period of the theta rhythm (Larson and Lynch, 1986; Larson et al., 1986; Pavlides et al., 1988; Huerta and Lisman, 1993; and others) that mimic pyramidal cell firing patterns reported to occur during learning (Otto et al., 1991). (2) The occurrence and characteristics of the above patterns of brain activity are in large part controlled by populations of interneurons acting via GABA_A and GABA_B receptors, respectively. Moreover, the degree of LTP is known to be tied to both within and between characteristics of the theta burst responses used to induce it (Arai and Lynch, 1992). Thus, blockade of the feedforward inhibition generated by GABA_A receptor-mediated fast IPSPs increases the magnitude and duration of depolarization within a short burst (Larson and Lynch, 1986; Mott and Lewis, 1991) and thereby facilitates the formation of LTP (Wigstrom and Gustafsson, 1983) while reduction of hyperpolarizing currents that occur between bursts, which are due, in part, to GABA_B receptor-mediated slow IPSPs, increases the ceiling of LTP (Arai and Lynch 1992). (3) The class of interneurons modulated by serotonergic brainstem afferents are distinct in their chemical composition (calbindin positive), their anatomical location and possibly the type of inhibition they exert (Freund et al., 1990); that is, they appear to be primarily located in stratum (s.) radiatum/s.lacunosum-moleculare, are activated via 5-HT₃ receptors and primarily associated with feed-forward, GABA_B-mediated inhibition in the dendritic region of pyramidal cells (Freund et al., 1990; Buhl et al., 1994).

In agreement with the above, recent studies demonstrated that serotonin blocks LTP in field CA1 of hippocampus *in vitro* in a dose-dependent manner and that the 5-HT-induced suppression of potentiation is, at least in part, due to the facilitatory action

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Correspondence should be addressed to Dr. Ursula Stäubli, New York University, Center for Neural Science, 4 Washington Place, New York, NY 10003. Copyright © 1995 Society for Neuroscience 0270-6474/95/152445-08\$05.00/0

of 5-HT on GABA_B-mediated long hyperpolarizations that occur between successive bursts of theta stimulation (Stäubli and Otaky, 1994); others have shown that the suppression of LTP by 5-HT can be prevented by antagonists of the 5-HT₃ receptor (Corradetti et al., 1992). Moreover, there are several reports of memory enhancing effects induced by selective 5-HT₃ receptor antagonists in rodents, primates, and humans (e.g., Costall et al., 1989; Jones et al., 1990; Crook and Lakin, 1991; Domeney et al., 1991; Carey et al., 1992), while agonists of 5-HT receptors were found to cause amnesia (Carli et al., 1992, 1993).

The questions addressed in the present experiments concern the extent to which the modulatory effects of 5-HT on memory encoding are mediated via its action on the cellular machinery underlying LTP. To this purpose, a potent and selective 5-HT₃ receptor antagonist, ondansetron, was used to manipulate the ascending serotonergic system. It was expected that the drug would promote the occurrence and shorten the period between successive theta waves (via action on the GABA_B-mediated suppression of between-burst hyperpolarization) and thus create a pattern of input activity that promotes LTP induction. The final set of studies tested if the changes induced by the drug on theta rhythm and LTP have the predicted facilitatory effects on forms of memory shown recently to be enhanced by other classes of drugs that promote LTP (Stäubli et al., 1994a,b).

Materials and Methods

Subjects

Male Long-Evans rats, 3 months of age, were used. The animals were housed individually and kept in a 12 hr:12 hr light-dark cycle. Food and water were available ad libitum for subjects involved in electrophysiological studies, while animals tested in behavioral tasks had restricted access to food to maintain their weight at 90% of same age controls. All experiments were conducted after the animals were well used to being handled. The animal care and use protocol for the present project was approved by the New York University Animal Welfare Committee.

Preparation for chronic hippocampal physiology

Preparation of animals with chronically implanted electrodes followed procedures essentially as described in previous work (Stäubli and Lynch, 1987, 1990; Stäubli et al., 1988). Subjects were deeply anesthetized with a mixture of ketamine and xylazine and pretreated with atropine (to prevent excessive salivation), following which a platinum/iridium recording electrode (75 μ m) was lowered under stereotaxic guidance into stratum radiatum of field CA1 and a stainless-steel monopolar stimulating electrode (125 μ m) positioned into field CA3 ipsilateral to the recording electrode to activate the Schaffer collateral and commissural projections to the recording site. An indifferent electrode (stainless-steel, 125 μ m) with 1 mm insulation removed from the tip was lowered lateral to the stimulating electrode, but outside the hippocampus, and a skull screw over the cerebellum served as ground. Physiological recording was used to adjust the position of the electrodes so as to maximize the amplitude of the negative-going dendritic field EPSP elicited by single stimulation pulses. Following these steps, the leads of the electrodes were connected to a headstage that was permanently affixed to the rats' skull. Ten days later the animals were acclimated to a chronic recording cage (30 $\times$ 30 $\times$ 58 cm) and to the attachment of a recording lead to the headstage. A total of 12 animals that had stable chronic recording and stimulation arrangements were involved in the various electrophysiological tests described below.

EEG analysis

The effect of ondansetron on hippocampal EEG activity was examined at six dosages (μ g/kg): 10, 50, 100, 500, 1000, and 5000. Each concentration was tested with at least 3 d between consecutive drug injections. Recordings began after the rat had been attached to the cable for 10–20 min in the cage. Ondansetron was given via intraperitoneal (i.p.) route of administration, at a dilution of 0.1–1.0 mg/ml and always pre-

ceded by a vehicle injection (saline). Samples of EEG activity were taken for 1–2 min before (baseline) and after saline injection, and following ondansetron administration at five different time points, that is, at 5, 15, 30, 60, and 120 minutes, respectively. All EEG recordings were monitored on-line on thermo paper by means of a polygraph while the animal was moving about the cage, a behavior that reliably causes theta pattern activity to appear in the hippocampal EEG. The sequence at which different concentrations were administered was randomized across four animals, all of which were also part of the group of six animals used for the first LTP study (100 μ g/kg) described below.

LTP induction in vivo

Recording sessions began by adjusting current intensity (25–100 μ A) to produce a response that was 50–60% of the maximum amplitude of the population spike-free response (usually 3–6 mV). Single biphasic stimulation pulses (150 μ sec pulse width) were delivered once every 25 sec, and responses were amplified via a FET operational amplifier (10 $\times$) inside the headstage and a second stage amplifier (bandpass 1–3000 Hz) and digitized at 10 kHz for analysis on line and storage on disk. Baseline evoked EPSPs were recorded daily during a 30–45 min period for at least 3 d or until the responses (initial slope and amplitude) were stable across days. Usable baseline responses were obtained in approximately 75% of the animals implanted. The drug dosages to be tested for their effects on LTP induction were selected on the basis of the results from the preceding experiment that examined interactions between ondansetron and EEG activity (i.e., 100 and 500 μ g/kg).

LTP was induced using a stimulation paradigm involving pairs of short (30 msec), high-frequency (100 Hz) bursts of pulses delivered to the Schaffer collateral/commissural projections to hippocampal region CA1. A train of 10 such bursts produces a robust and extremely stable LTP effect in rats with chronically implanted electrodes, when the bursts are separated by 200 msec (i.e., "theta burst stimulation" or "TBS") (Stäubli and Lynch, 1987, 1990). Recent studies indicate that five pairs of theta bursts delivered at the frequency of one pair every 30 sec induce a small and transient (3–24 hr) potentiation effect (Stäubli et al., 1994b). Accordingly, this stimulation pattern was used to test if intraperitoneal injections of ondansetron would facilitate the induction of LTP. Six animals each received theta burst stimulation both in presence and absence of the drug to allow for within subject comparisons of the amount and duration of LTP produced. In all cases, baseline recordings (1 stimulation pulse per 25 sec) were collected for at least 60 min before attempts were made to induce LTP. Vehicle (saline) or drug was injected 30 min before theta burst stimulation, and recording was continued for 180 min after induction of LTP. Twenty-four hours later, the rats were returned to the recording apparatus and field EPSPs recorded during 60 min. A minimal period of 5 d was interposed between two successive episodes of LTP induction; the stimulation current was kept constant for a given subject throughout the entire experiment. The first experiment involved ondansetron at 100 μ g/kg and six animals; in five of these, LTP was tested in presence of the drug first, followed 5 d later by a second LTP episode in presence of saline. A second experiment was conducted "blindly" thereafter and involved six newly implanted rats and two consecutive LTP tests with the sequence of drug (500 μ g/kg) and saline injections counterbalanced across animals.

Behavioral methods

Behavioral studies were carried out with injections of ondansetron at a dosage found to be effective in modifying hippocampal theta rhythm and the induction of LTP *in vivo* (i.e., 100 μ g/kg).

Olfactory match-to-sample task. An elevated eight-arm radial maze made from painted wood was modified to accommodate olfactory delayed match-to-sample performance. Eight arms, 9 cm wide and 54 cm long, were radiating from the center platform (40 cm diameter); each of them was enclosed by a 1 cm thick wall extending 11 cm above the floor of the arm and covered by a removable Plexiglas top. Located across each arm at the entrance were 2 cm high wooden hurdles which the rat had to climb over to get access from the center platform. A hole drilled in the floor at the distal end of each arm served as recessed water well. Odors were prepared by enclosing a small amount (~3 ml) of a given odor concentrate (International Flavors and Fragrances, Inc.) into a 50 ml Erlenmeyer flask and gassing it with air monitored by a flow-meter at a rate of 0.1–1.0 liters/min (depending on intensity of the odor concentrate used). The odor-saturated air leaving the flask then joined with a clean air stream regulated by a second flow-meter at 4.5–

5.5 liters/min, such that the combined volume of clean and odor-saturated air delivered per minute was identical for each odor. The odorized air was carried via tygon tubing to the maze and ejected towards the center platform through an opening in the back wall of the arms. A fan mounted 25 cm above the center platform served to pull the odors down the enclosed arms into the center area of the maze. Onset and release of odorized air at randomly assigned ports was controlled by the experimenter. The animals were given two trials per session, always with the same four odors (anise, opoponax alvina oliffac, sage clary oliffac, cis-3-hexenol). A different member of the group of four odors was used as target odor on different days. In the first trial, the rat was trained to move from the distal end of the start arm down to the center of the maze and enter the target arm (all other arms were blocked) to sample the odor released from the port and collect a drop of water from the recessed well underneath the odor outlet. After spending a variable intertrial interval in the home cage, rats were given a choice of four odors, one of which was the target odor. While the start arm remained the same, the four alleys releasing an odor did not include the arm that contained the target odor in the sample trial. Three daily sessions were run with 5 min delays between sample and test trial, until the animals reached a criterion of seven error-free runs in three consecutive days. Intertrial intervals were increased stepwise up to 6 hr, with interim delays of 15, 30, 60, 120, and 240 min. To minimize proactive interference only one daily session was run at delays of 1 hr or longer, and each delay was maintained for at least 7 d. Experienced rats show excellent retention at intertrial intervals up to 4 hr, but with 6 hr delays there is an evident decay in memory as they typically perform around chance level. Accordingly, 6 hr delays were used to repeatedly test the effects of the drug on learning. Specifically, the animals received a vehicle injection (saline) on four of five weekly trials 30 min before the first session and on one day an injection of ondansetron (100 μ g/kg). To allow for within animal comparisons of the drug effect and to control for changes in performance unrelated to the drug, only half of the animals were tested with ondansetron at one time and the other half received a saline injection; reversed group assignments were carried out the following day. All drug trials were conducted on the third and fourth weekly testing day. This protocol was used with 16 animals during a period of 3 weeks. Data analysis involved all drug and saline trials except the first trial of the week.

Spatial maze. An elevated eight-arm radial maze made of painted wood was used. It was kept in a well-lit room containing numerous distinct extramaze cues such as posters, bookshelves, cabinets, lamps etc. The center platform was 38 cm in diameter with eight arms (12 cm wide, 56 cm long, 3.5 cm high enclosing walls) separated from the central platform by Plexiglas guillotine-type doors that could be raised and lowered by the experimenter via a system of pulleys and strings. During training, a chocolate chip was placed inside a recessed food well located at the end of every alley. Rats were first given one trial per day with all eight arms open and were trained to a criterion of three consecutive trials with no errors (i.e., no repeated entries into a given alley). Subsequent training involved two episodes, in the first of which four arms were blocked. The animals were allowed to enter the open arms to retrieve the food reward. After a delay they were returned to the maze, which now had all eight arms open but contained rewards only in the previously blocked arms (blocked arms were randomized across days). The number of incorrect entries (i.e., arms entered more than once) and the number of correct choices made before a reentry occurred were recorded. The interval between the two episodes was 5 min at first and increased stepwise to 8 hr (interim delays were 15, 30, 60, 120, 240, 360 min, each of which was instated for at least 7 d). Experienced rats show excellent retention at intertrial intervals up to 6 hr in that they make very few (zero or one) reentry errors before obtaining the four remaining rewards, but with 8 hr delays there is an evident decay in memory as they typically commit one to two such errors, even after extensive training. Accordingly, 8 hr delays were used to repeatedly test the effects of the drug on learning. Specifically, the animals received a vehicle injection (saline) on four of five weekly trials 30 min before the first session, and on one day they were injected with ondansetron (100 μ g/kg). To allow for within animal comparisons of the drug effect and to control for changes in performance unrelated to the drug, only half of the animals were tested with ondansetron at one time and the other half received a saline injection; reversed group assignments were carried out the following day. All drug trials were conducted on the third and fourth weekly testing day. This protocol was used with 16

animals during a period of 3 weeks. Data analysis involved all drug and saline trials except the first trial of the week.

Results

EEG analysis. At none of the concentrations tested were any obvious changes in behavioral activity observed. Four 3 sec periods of uninterrupted theta activity were selected for each pre- and post-ondansetron injection time point. Figure 1 displays an example of the effects as they are typically observed following administration of ondansetron (500 μ g/kg) in an individual animal. Analysis of the theta periods was conducted blindly and involved counting the frequency of each 3 sec period to calculate the average for all time points. The results are summarized in Figure 2, which displays the relative changes in frequencies (mean $\pm$ SEM) after normalization to average control values. Ondansetron caused a slight but reliable increase in theta frequency at doses (μ g/kg) of 100, 500, and 1000.

The action of the drug started between 5 and 15 min after injection and lasted for approximately 30–60 min. Analysis of variance for repeated measures revealed significant effects of time [$F = 11.5$ (100 μ g), $p < 0.0001$; $F = 15.7$ (500 μ g), $p < 0.0001$; $F = 11.9$ (1000 μ g), $p < 0.0001$]. Post hoc analysis using paired t tests showed that the theta frequency was significantly enhanced at both 15 min and 30 min following administration of 100 μ g/kg ondansetron (15 min: $T = 2.36$, $p < 0.05$; 30 min: $T = 2.98$, $p < 0.03$), at 5 min, 15 min and 30 min after injection of 500 μ g/kg (5 min: $T = 4.12$, $p < 0.02$; 15 min: $T = 4.93$, $p < 0.01$; 30 min: $T = 5.84$, $p < 0.005$), and at 5 min, 15 min, 30 min, and 60 min after injection of 1000 μ g/kg (5 min: $T = 2.69$, $p < 0.04$; 15 min: $T = 3.79$, $p < 0.02$; 30 min: $T = 5.57$, $p < 0.01$, 60 min: $T = 2.53$, $p < 0.05$). All comparisons were made relative to saline rather than baseline values, since the injection itself tended to slightly, though not significantly, increase the frequency of the rhythm above baseline. Lower concentrations of ondansetron (10 and 50 μ g/kg) caused no apparent alterations in theta rhythm, and the highest concentration tested (5 mg/kg) appeared to depress theta frequency in addition to having slightly desynchronizing effects; analysis of variance did not yield significant effects of time ($F = 2.3$, $p < 0.08$).

LTP induction in vivo. Based on the findings of the previous experiment two concentrations of ondansetron (100 μ g/kg and 500 μ g/kg) were tested for their effect on LTP induction in two consecutive studies involving separate groups of six rats. Five pairs of theta bursts temporally spaced at 30 sec caused a modest and transient LTP effect under control conditions in both groups of animals (see Fig. 3B,C). This effect was slightly but significantly enhanced by ondansetron at 100 μ g/kg, both with respect to slope and amplitude of potentiated responses (Fig. 3B). The paired t test revealed significant differences between saline and drug condition at 1 hr ($T = 2.28$, $p < 0.04$), 2 hr ($T = 2.37$, $p < 0.03$), 3 hr ($T = 2.55$, $p < 0.03$), and 24 hr ($T = 1.95$, $p < 0.05$) after LTP induction. Examples of LTP records from one animal tested under vehicle and drug (100 μ g/kg) condition are shown in Figure 3, D and D'.

The second LTP study which was conducted blindly and involved a new group of six animals yielded results that were virtually indistinguishable from those obtained in the first study (Fig. 3C); that is, LTP induced in presence of 500 μ g/kg ondansetron was greater in magnitude and duration compared to the LTP effect obtained in the same animals after saline injection. Analysis using the paired t tests showed significant differ-

Figure 1. Ondansetron increases the frequency of the hippocampal theta rhythm in the awake, freely moving rat. Each EEG excerpt illustrates a 3 sec representative example of theta rhythm recorded at the time points indicated above, that is, during baseline, after an intraperi-

ences at 1 hr ($T = 2.63$, $p < 0.02$), 2 hr ($T = 3.25$, $p < 0.02$), 3 hr ($T = 2.73$, $p < 0.02$), and 24 hr ($T = 3.01$, $p < 0.02$).

Olfactory delayed match-to sample task. Results were analyzed using the 16 animals as their own control and comparing performance on drug days to control scores obtained on the days preceding and following the drug day; that is, a total of nine vehicle trials and three drug trials were analyzed for each rat using the paired t test. As illustrated in Figure 4A, the percentage of correct choices made after a six hour delay was significantly greater on the drug versus vehicle injection days [$t(15) = 3.82$; $p < 0.001$].

Radial maze performance. The number of incorrect entries (i.e., arms entered more than once) and the number of correct choices before a reentry occurred were recorded. These results were analyzed using the 12 animals as their own control and comparing performance on drug days to control scores obtained on the days preceding and following the drug day; that is, a total of nine vehicle trials and three drug trials were analyzed for each rats using paired t tests. Figure 4B summarizes the results; the rats exhibited substantially better retention on drug injection days than they did on control injection days ($p < 0.0002$; paired t test); this was equally true for both reentry errors [$t(11) = 6.75$; Fig. 4B'] and number of correct choices before the first error [$t(11) = 5.61$; Fig. 4B''].

Discussion

Substantial evidence indicates that the 5-HT₃ receptor is a ligand-gated ion channel and mediates rapid depolarization (Kilpatrick and Tyers, 1992). Experiments using *in situ* hybridization techniques to determine the distribution in brain of the 5-HT₃ receptor revealed highest densities in the posteroventral region of CA1, particularly in s. lacunosum moleculare, and to a lesser degree in s. radiatum and s. oriens (Tecott et al., 1993). There are numerous potent and selective 5-HT₃ antagonists available; that is, each exhibits at least a 1000-fold selectivity for the 5-HT₃ receptor over other neurotransmitter receptors upon which they have been tested, indicating that the effects observed at reasonable doses can be confidently ascribed to antagonism of the 5-HT₃ receptor (Kilpatrick and Tyers, 1992).

The present experiments investigated the interactions between ondansetron, a drug of this type, with hippocampal LTP and memory using three levels of analysis. First, EEG activity was recorded in the hippocampus of freely moving rats to determine whether manipulations of the 5-HT₃ receptor system affect the probability that neurons will generate activity patterns appropriate for the formation of LTP. Second, the induction of LTP in field CA1 of hippocampus in freely moving rats with chronically implanted electrodes was measured. Given the likelihood that the ascending 5-HT projections—via 5-HT₃ receptors—increase the excitability of a class of defined interneurons (Ropert and Guy, 1991; Oleskevich and LaCaille, 1992) that control the feed-forward, GABA_B-mediated inhibition of CA1 pyramidal cell dendrites (Freund et al., 1990; Buhl et al., 1994), antagonists of the 5-HT₃ receptors were anticipated to increase the frequency of the theta rhythm by reducing the between-wave hyperpolarization caused by slow GABA_B-mediated IPSPs and thereby fa-

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toneal injection of saline and at 5, 15, 30, 60, and 120 min following peripheral administration of ondansetron at 500 μ g/kg. The increase in frequency is most notable at 30 min after injection. Calibration: 50 μ V, 0.5 sec.

Figure 2. Time- and dose-dependent effects of ondansetron on frequency of the hippocampal theta rhythm. A group of four rats were used to test six different concentrations of ondansetron. The order at which different concentrations were tested was randomized across animals. The mean theta frequency for each rat and time point was calculated using the average of four 3 sec theta periods. Each bar represents group averages of individual means ($\pm$ SEM) for a given time point expressed as percentage of the mean frequency during the control period. Individual control frequencies were calculated from the values obtained during baseline and saline periods and normalized to the average frequency during that period.

cilitate LTP obtained with a minimal stimulation paradigm involving pairs of theta bursts. Third, learning and retention in two versions of hippocampally dependent short-term memory tasks were assessed; it was predicted that the drug, at concentrations effective at the former two levels of analysis, would promote memory retention.

The findings of the present experiments confirmed each of the above predictions: ondansetron at concentrations between 100 and 1000 μ g/kg reliably augmented the frequency of the theta rhythm while lower or higher doses had no impact. In fact, a tendency for a decline in theta response (i.e., dominance of non-theta activity alternating with theta rhythm of relatively small amplitude and frequency) was evident at the highest dose tested (5 mg/kg), an observation perhaps related to the "bell shaped dose-response curve" often ascribed to 5-HT₃ receptor antagonists (Kilpatrick and Tyers, 1992; Baumgarten, 1993). Earlier studies have proposed that the ascending serotonergic projections are an important component in the control of rhythmical slow activity (theta) in the hippocampus (Assaf and Miller, 1978; Vanderwolf and Baker, 1986) and in the organization of adaptive behavior (Baumgarten, 1993; Vanderwolf et al., 1990) but the underlying mechanisms had not been elucidated in these studies. Overall, our results confirm and extend these earlier reports; the data indicate that 5-HT₃ antagonists have subtle, yet readily detectable behavioral effects; that is, no overt behavioral changes were observed at any of the concentrations tested, but when the animals were subjected to specific situational contexts, significant effects became apparent. Ondansetron was found to very effectively promote the induction of LTP in freely moving animals and, at the same dosage, substantially extend the duration of transient memory in a spatial and olfactory learning para-

digm. In accord with the former observation is a recent study on facilitation of LTP induced in field CA1 of hippocampal slices following administration of low concentrations of 5-HT₃ receptor blockers (Santamaria and Caille, 1994). Drugs that antagonize 5-HT₃ receptors have also been reported to be effective in facilitating potentiation of the mossy fibers *in vitro* (Maeda et al., 1994). The mechanism whereby the antagonists exert their effect on long-lasting synaptic potentiation is not clear, but it is not unreasonable to assume that their action on theta rhythm and LTP in field CA1 are related. Thus, it has been shown that pharmacological suppression of the GABA_B-mediated hyperpolarization between successive theta bursts increases the ceiling of potentiation by theta stimulation (Arai and Lynch, 1992). It has further been suggested that stimulation of CA1 afferents in stratum radiatum activates a distinct class of interneurons located in s. radiatum/lacunosum border and thought to produce a GABA_B-mediated inhibition of pyramidal cells (Oleskevich and LaCaille, 1992; Williams et al., 1994). The above interneurons may thus correspond to the population of inhibitory cells in s. radiatum/lacunosum that are activated by 5-HT via 5HT₃ receptors and terminate largely in the dendritic fields of pyramidal cells (Freund et al., 1990). These neurons are reported to elicit IPSPs with long rise and decay times and be selectively associated with the Schaffer collateral/commissural pathway (Buhl et al., 1994). The results of the present experiments are consistent with the hypothesis that the between-burst hyperpolarization mediated by GABA_B receptors plays a crucial role in establishing the magnitude of LTP in field CA1 (Arai and Lynch, 1992). Support for this idea was also provided in an earlier study that examined the relationship between the effects of 5-HT on theta burst responses and degree of LTP obtained by the stimulation; that is, it was

Figure 3. Facilitatory effect of ondansetron on induction of LTP in hippocampal field CA1 of the freely moving rat. **A**, Slope of dendritic field EPSP (average of four per data point) recorded in stratum radiatum of field CA1 before and after induction of LTP in a rat with chronically implanted stimulating and recording electrodes in the Schaffer collateral/commissural system. *Open circles*, experiment in which five pairs of theta bursts (5x2 TBS) were delivered following systemic administration of vehicle solution (*inj.*); *solid circles*, five pairs of theta bursts were delivered on a different day after injection of ondansetron (*inj.*) at a concentration 100 $\mu\text{g}/\text{kg}$. Note that in the vehicle control condition the potentiation decays almost back to baseline over 3 hr, but is still evident at 3 hr after injection in the drug condition. The illustrated effects are typical of those obtained in a group of six rats tested with a dose of 100 $\mu\text{g}/\text{kg}$ ondansetron. **B**, Amount and duration of LTP obtained in a group of six rats with a chronically implanted stimulating and recording electrodes in the Schaffer collateral/commissural system and injected with vehicle (*open circles*)

Figure 4. Ondansetron improves retention of memory in both an olfactory and spatial learning task. **A**, Olfactory memory: percentage of trials in which a group of 16 rats selected the test odor from a group of four simultaneously present odors on days in which the animals had been injected with saline (9 d) or drug (3 d) prior to the sample trial in which they were presented with the test odor alone. The interval between the sample trial (test odor alone) and test trial (four odors) was 6 hr. The solid bar on the right indicates the mean group difference between the vehicle and drug condition. The dotted line indicates chance performance. **B**, Spatial memory. **B'**, The number of reentries (errors) into arms already visited made by a group of 12 rats before collecting all four available rewards during the second of two episodes in an eight-arm radial maze. Shown are averages (mean $\pm$ SEM) for a group of 12 rats for days on which saline injections (9 d) or drug injections (3 d) were given 30 min prior to the first of the two episodes. The solid bar on the right summarizes the average within-rat improvement (i.e., reduction in reentry errors) on drug versus saline injection days. **B''**, The number of correct choices made before reentering any of the four arms that had already been visited in a previous (8 hr earlier) episode. Bar denotations are as in left panel; the solid bar on the right indicates the mean of the within-rat differences for drug versus saline days; as shown, the rats made more correct choices before committing an error on drug injection days (for statistical analyses, see text).

found that the facilitatory effects of 5-HT on interneurons acting via GABA_B receptors caused a suppression of the NMDA receptor-mediated currents activated by theta burst stimulation and thereby suppressed LTP (Stäubli and Otaky, 1994). If LTP contributes to the encoding of some forms of memory, then it is reasonable to expect that administration of 5-HT₃ receptor antagonists prior to learning will enhance such variants of memory, which is the result that was obtained. These findings provide the second instance where learning in two tasks known to depend on an intact hippocampus is improved by a compound that facilitates induction of LTP *in vivo*; that is, the first study was conducted with drugs that selectively enhance operation of the AMPA variant of the glutamate receptor and thereby improved both the encoding of memory and LTP (Stäubli et al., 1994a,b). Moreover, as noted above, our findings are consistent with earlier reports on the beneficial action of the 5-HT₃ receptor antagonist ondansetron on cognition in rodents and primates (e.g., Barnes et al., 1990; Costall and Naylor, 1994). In addition, humans chronically treated with ondansetron have been reported to exhibit significant improvements in some tests of cognitive performance compared to placebo controls (Crook and Lakin, 1991).

It is also possible that the above described effects on hippocampal theta rhythm, LTP and memory obtained with ondansetron are to some measure due to factors other than suppression of GABA_B currents. For example, it has been suggested that the cognitive enhancing effects of 5-HT₃ antagonists may be mediated in cortical and hippocampal regions by 5-HT₃ receptors controlling ACh release (Barnes et al., 1989; Kilpatrick and Tyers, 1992). However, detailed hypotheses on how such interactions might occur have not been advanced.

In summary, the above results support the hypothesis that diffuse, ascending systems influence plasticity in forebrain networks by acting on both the rhythms needed to induce changes (e.g., theta) and the response of target cells to afferent activities (e.g., theta bursts) reflecting those rhythms. Further work with the 5-HT system, and its noradrenergic and cholinergic counterparts, will hopefully allow for tests of the broader proposal that interactions of the kind described in the present paper serve to align behavioral psychological states (and their behavioral correlates) with the cellular machinery underlying learning.

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or ondansetron at 100 μ g/kg (solid circles) before delivering five pairs of theta burst stimulation. LTP is expressed as an increase in the slope of the field EPSP (means $\pm$ SEM); each time point contains the average of 16 consecutive responses per animal. Comparisons of LTP after drug versus vehicle injection (intrasubject comparisons) were done using the paired *t* test and revealed that the differences in LTP between the two conditions were significant at all time points measured (see text for exact values). **C**, Same experiment as illustrated in **B**, except that a higher dose of ondansetron (500 μ g/kg) was tested and a different group of six rats used. **D** and **D'**, Averaged field EPSPs (four per average) from one animal run in the experiment shown in **B**, where LTP was induced following injection of vehicle solution (**D**) or ondansetron at 100 μ g/kg (**D'**). Calibration: 1 mV, 5 msec.

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