Columnar distribution of serotonin-dependent plasticity within kitten striate cortex

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Recent studies have identified the potential for an important role for serotonin (5-HT) receptors in the developmental plasticity of the kitten visual cortex. 5-HT_{2C} receptors are transiently expressed in a patchy fashion in the visual cortex of kittens between 30–80 days of age complementary to patches demarcated by cytochrome oxidase staining. 5-HT, operating via 5-HT_{2C} receptors, increases cortical synaptic plasticity as assessed both in brain slices and *in vivo*. Herein, we report that bath application of 5-HT substantially increases the probability of long-term potentiation within 5-HT_{2C} receptor-rich zones of cortex, but this effect is not observed in the 5-HT_{2C} receptor-poor zones. Instead, in these zones, 5-HT application increases the probability of long-term depression. These location-specific effects of 5-HT may promote the formation of compartment-specific cortical responses.

Specific, transient, regional, laminar, and columnar changes in the distribution of neurotransmitter-specific afferents and receptors have been shown to occur during the critical period of increased visual cortex plasticity (1–4). Fig. 1 summarizes autoradiographic and histochemical studies showing that, during the critical period, the kitten visual cortex transiently expresses a number of neurochemical markers in a columnar fashion. This columnar, biochemical architecture consists of two sets of complementary columns: one set is enriched in 5-HT_{2C} and 5-HT_{2A} receptors along with synaptic zinc, whereas the other expresses increased levels of CO and acetylcholinesterase (3, 4). Among over 30 neurotransmitter receptors that have been studied within the visual cortex (1, 2), only the 5-HT receptors noted above have been found to concentrate into columns within the developing cortex.

A special relationship between this 5-HT receptor system and the use-dependent plasticity of visual cortex is reinforced by studies showing that these receptor columns do not form in kittens in which one eye was removed early in life or in animals that were deprived of vision by dark rearing (1, 5). In addition, studies in developing kittens have shown that blockade of 5-HT_{2C} receptors with mesulergine reduces the activity-dependent binocular competition that normally occurs when one eye is deprived of vision (6).

Working in brain slices taken from visual cortex of 60- to 80-day-old kittens, we found that low-frequency stimulation (LFS) of the white matter (1 Hz, 15 min) rarely induced long-term potentiation (LTP) or long-term depression (LTD) in layer IV (7). At these ages, however, bath application of 5-HT markedly facilitated the induction of both LTP and LTD. The effects of 5-HT on both LTP and LTD were blocked by the 5-HT_{2C} antagonist mesulergine (7). Although the general effect of 5-HT application was clearly to increase the probability of long-term changes in response to electrical stimulation, we could not predict the variety of effects that were mediated by 5-HT (sometimes LTP, sometimes LTD, and sometimes no change).

We hypothesized that the effects of 5-HT might depend on the location of the recording site with respect to the 5-HT_{2C} receptor columns and therefore on the density of these receptors. To address this possibility, we tried to visualize the 5-HT receptor-rich and receptor-poor zones by retrograde labeling of the complementary (3) cytochrome C oxidase-rich columns. It has been shown that a subset of neurons in the cytochrome C oxidase-rich columns project selectively to extrastriate visual areas, lateral suprasylvian cortex, and area 21a (8). Accordingly (Fig. 2), we visualized the 5-HT_{2C} columnar system as regions of neurons that were not retrogradely labeled with CTX-gold tracer injected into area 21a.

Materials and Methods

To visualize 5-HT_{2C} receptor patches in living brain slices, eight 60- to 80-day-old kittens were injected in area 21a with a retrograde tracer (1% CTX-gold) to label the projection neurons in layer II/III of area 17 (8).

At 2 weeks after the injections, the kittens were deeply anesthetized with an i.v. injection of Brietal (methohexital). After craniotomy and durotomy, tissue blocks containing the primary visual cortex were quickly removed and placed into ice-cold artificial cerebrospinal fluid. The visual cortex was sectioned coronally into 400-μm-thick slices with a vibrating microtome and continuously perfused at a 1-ml/min flow rate in an interface recording chamber with artificial cerebrospinal fluid saturated with 95% O_2/5% CO_2 and warmed to 35°C. The artificial cerebrospinal fluid contained (in mM) 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 1.5 $MgCl_2$, 2.5 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose. Extracellular recording electrodes were filled with 2 M NaCl and were placed in layer IV. Concentric bipolar electrodes were placed in the white matter to activate synaptic inputs in layer IV. Population EPSPs were evoked by using 10- to 200-μA pulses of 0.1-ms duration delivered once every 15 s. After an input–output curve was constructed, a stimulus intensity eliciting approximately one half of the maximal response was used for baseline measurements and LFS (1 Hz, 15 min). Groups of four responses, collected in a 1-min interval, were averaged to calculate the amplitude of the field EPSP during that interval. The change in plasticity was quantified 15–20 min after LFS, by analyzing the amplitude of the response during that period relative to the 5-min baseline preceding LFS. 5-HT (1–10 μM) was bath applied for 15–20 min before LFS was given and was either maintained throughout the experiment or washed out 10 min after the end of LFS. For paired-pulse responses, a 40-ms interval between pulses was used.

After recording, the slices were fixed, resectioned at 50-μm thickness, and processed with the silver-intensified Intense Kit (Amersham Pharmacia) to visualize the patches of area 21a projecting neurons. Labeled cells were charted, and the locations

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Abbreviations: 5-HT, serotonin; CO, cytochrome oxidase; LTP, long-term potentiation; LTD, long-term depression; EPSP, excitatory postsynaptic potential; LFS, low-frequency stimulation.

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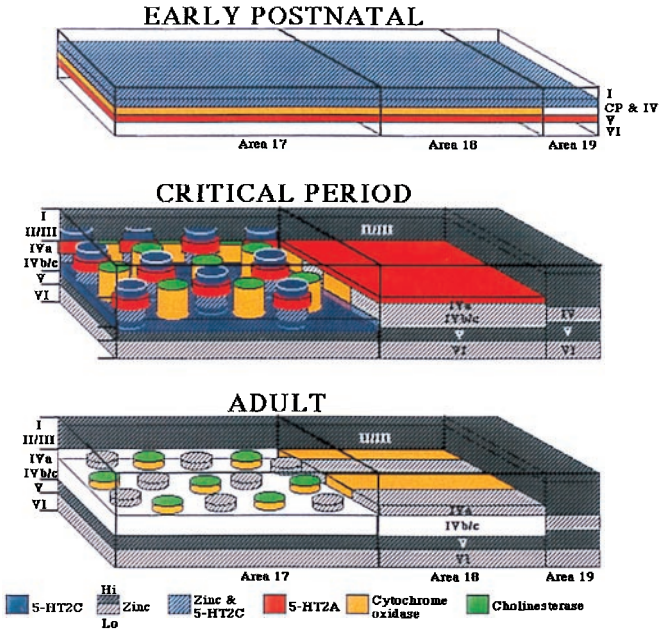

Fig. 1. A schematic diagram that summarizes the temporal and topographic distributions of serotonin (5-HT) receptors, synaptic zinc, cytochrome C oxidase, and acetylcholinesterase in the kitten visual cortex during postnatal development. The columnar mosaic formed by 5-$HT_{2A/2C}$ receptors/zinc and cytochrome oxidase (CO)/acetylcholinesterase becomes prominent between 30 and 80 days of age, after the peak of the critical period, during which the synaptic organization of the visual cortex is susceptible to experience-dependent modifications. The columnar expression of 5-$HT_{2A/2C}$ receptors and acetylcholinesterase is present only during this period of development. The column-specific expression of CO and Zn^{2+} is retained in an attenuated form into adulthood.

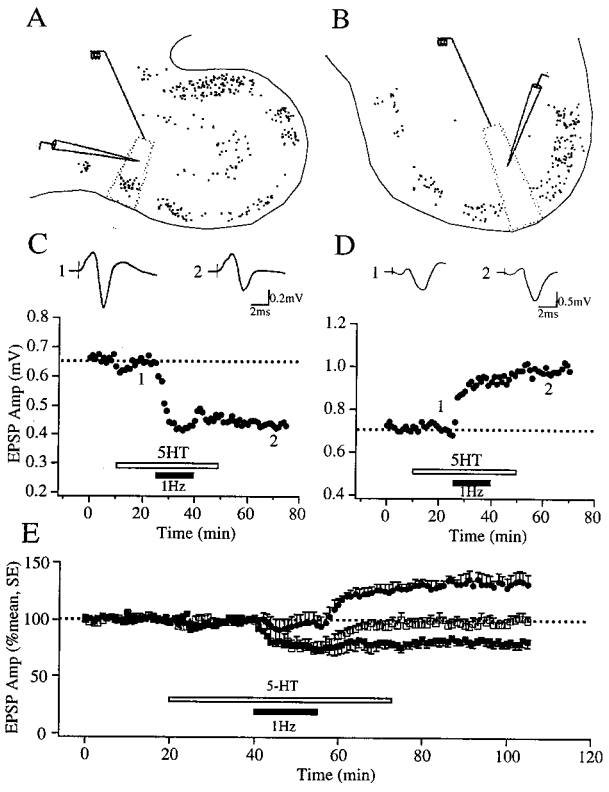

Fig. 2. Serotonergic facilitation of LTD and LTP in relation to CTX-gold-positive patches (which mark CO-rich and 5-HT_{2C} receptor-poor zones). (*A* and *B*) Charts of tissue sections show patches of CTX-gold-labeled cells and the locations of the recording and stimulating electrodes as identified from lesions made after recording sessions. (*C*) LTD occurred in a column marked by a CTX-gold-positive patch. The time course of amplitude changes of population excitatory postsynaptic potentials (EPSPs) recorded from the same slice shown in *A*. Filled circles show the averaged responses before, during, and after LFS (1 Hz, 900 pulses) applied conjointly with 5-HT (1 μM). There was a persistent depression of the tested pathway if 1-Hz conditioning was applied in the presence of 5-HT. (*D*) LTP was evoked in a region falling between patches of CTX-gold-positive neurons. The time course of amplitude changes of population EPSPs recorded from the same slice shown in *B*. There was a persistent facilitation of the tested pathway if 1-Hz conditioning was applied in the presence of 5-HT. (*C* and *D Insets*) Field potentials averaged from 10 consecutive responses taken as indicated on the graphs. (*E*) Summary data (means ± SEM) show the time course of 5-HT-induced facilitation of LTD of the field EPSP amplitude within CTX-gold-positive patches (filled squares; $n = 6$) and LTP within a region falling between patches (filled circles; $n = 5$). There was no persistent change in the control group of slices (open squares; $n = 10$). The solid and open bars indicate the periods of LFS and 5-HT application, respectively.

of patches were compared with lesions marking the recording sites. Adjacent sections were used for CO and Nissl staining to verify the localization of recording electrodes. Statistical analyses were carried out by using paired or independent Student's *t* tests where appropriate.

Results

We used 20 visual cortex slices taken from eight tracer-injected kittens. Bath application of 5-HT markedly facilitated the induction of either LTD (78.2 ± 3.7% of control amplitude; mean percentage ± SEM; $P < 0.01$) in 6 of 20 slices or LTP (124.9 ± 7.2%; $P < 0.05$) in 5 of 20 slices (Fig. 3). Nine slices showed no effect.

The positions of the recording electrodes, which were originally placed in a masked fashion, were later correlated with the patchy distribution of CTX-gold-positive cells. In 8 of 20 slices, the electrodes were located within a CTX-gold-positive patch (Fig. 2), whereas in 12 slices, recordings fell within an interpatch zone. When anatomical and electrophysiological results were compared, we found that in six of eight CTX-gold-patch locations 5-HT application facilitated LTD (two with no effect), whereas LTP was never observed. By contrast, in 5 of 12 interpatch locations, 5-HT application facilitated LTP (7 with no effect), and LTD was never observed. Simultaneous recordings from CTX-gold-patch and interpatch regions in a single-slice preparation (Fig. 3) also showed that serotonergic facilitation of LTD, and LTP depended on electrode position in relation to CTX-gold-positive patches (Fig. 4). To show that the effects in layer IV reported here are synaptic and not a laminar artifact, additional recordings were performed. First, in 12 slices, current source density analysis indicated a weak but distinct current sink in layer IV. In slices with strong field potentials in layer IV, similar to the ones recorded in our original study, the current source density analysis showed that the current sink in layer IV was clearly separated from the current sources in layers II/III and V. Second, if contamination from current sources is a problem, one may expect opposite effects in layer II/III and layer IV when recording from both simultaneously. In 3 of 20 slices, LTD was simultaneously developed in both layers after LFS (layer IV, 81.1 ± 4.1%; layer II/III, 77.9 ± 5.2%; $P < 0.01$), whereas in two slices, LTD was induced only in layer IV. In 3 of 20 slices, LTP was simultaneously induced in layer IV after LFS (127.5 ± 4.8%; $P < 0.01$) and layer II/III (129.5 ± 7.9%; $P < 0.05$), with one slice developing LTP in layer IV only. These results suggest that 5-HT-induced facilitation of synaptic plasticity is consistent with the synaptic activation of layer IV neurons. In addition, we did not see any consistent changes in

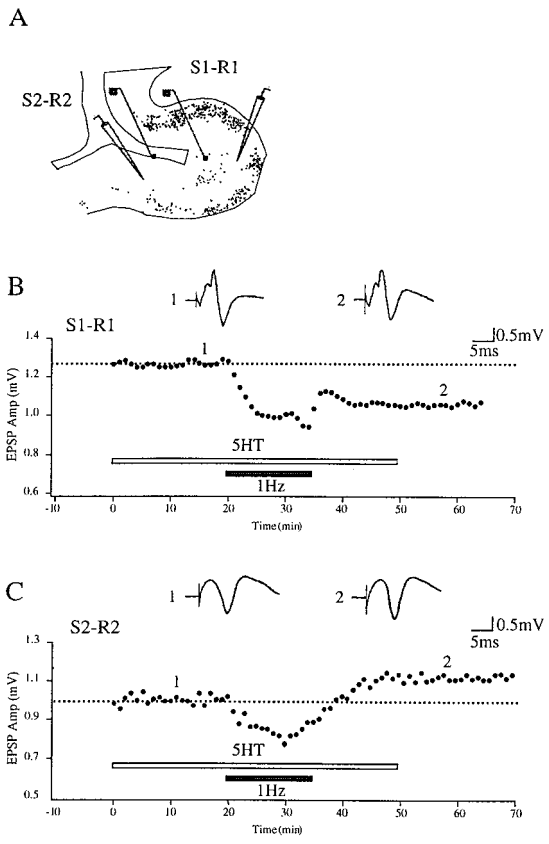

Fig. 3. Serotonergic facilitation of LTD and LTP in a single slice preparation depended on electrode position in relation to CTX-gold-positive patches. (*A*) A chart of tissue sections shows patches of CTX-gold-labeled cells and the locations of recording and stimulating electrodes as identified from lesions made after recording sessions. (*B*) LTD occurred in a column marked by a CTX-gold-positive patch. The time course of amplitude changes of population EPSPs at the S1-R1 pair of electrodes. Filled circles show averaged responses before, during, and after LFS (1 Hz, 900 pulses) applied conjointly with 5-HT (1 μM). There was a persistent depression of the tested pathway if 1-Hz conditioning was applied in the presence of 5-HT. (*C*) LTP was evoked in a region falling between patches of CTX-gold-positive neurons. The time course of amplitude changes of population EPSPs at the S2-R2 pair of electrodes is shown. There was a persistent facilitation of the tested pathway if 1-Hz conditioning was applied in the presence of 5-HT. S1, S2, R1, and R2 represent locations of stimulation and recording electrodes. (*B* and *C Insets*) Field potentials averaged from 10 consecutive responses taken as indicated on the graphs. The solid and open bars indicate the periods of LFS and 5-HT application, respectively.

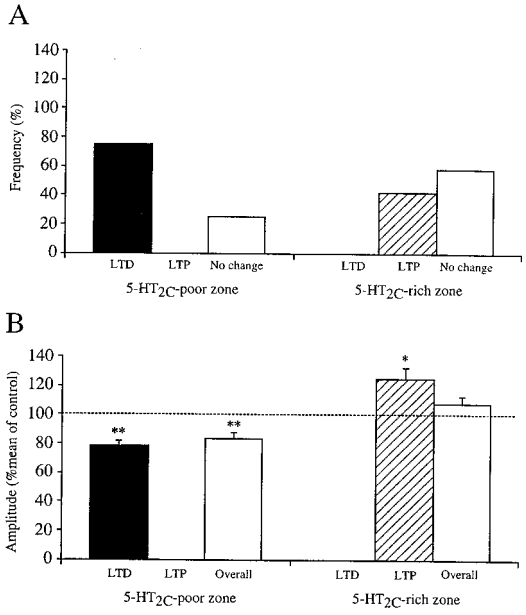

Fig. 4. 5-HT-induced facilitation of synaptic plasticity in the kitten visual cortex has a spatial component. (*A*) Summary data for the frequency of LTP and LTD induced by conjoint application of 5-HT and LFS in layer IV of the kitten visual cortex in relation to CTX-gold retrograde labeling of area 21a projection neurons in layer II/III (complementary to 5-$HT_{2A/2C}$ receptor patches in layer IV). (*B*) Summary data for the magnitude of LTP and LTD in relation to CTX-gold retrograde labeling of area 21a projection neurons in layer II/III (complementary to 5-$HT_{2A/2C}$ receptor columns in layer IV). *, $P < 0.05$; **, $P < 0.01$.

paired-pulse responses in layers IV and II/III after the induction of LTP or LTD. Paired-pulse facilitation was developed in both layers, before LFS (layer IV, 122.3 ± 12.1%; layer II/III, 118.7 ± 6.1%) and after LFS (layer IV, 118.6 ± 6.0%; layer II/III, 125.7 ± 13.1%).

Discussion

Immunohistochemical and autoradiographic analyses have shown a transient expression of serotonergic terminals and receptors in rat and cat visual cortex during early postnatal life (3, 4, 9, 10). The distribution of serotonergic receptors in cat visual cortex also changes in an age-dependent, regional, and laminar-specific manner (3). The most striking and specific pattern of developmentally regulated expression is the distribution of 5-HT_{2C} receptors into patches that separate layer IV of area 17 into 5-HT_{2C} receptor-rich and receptor-poor zones (3, 4). A relationship to plasticity is suggested by the findings that monocular enucleation or dark rearing imposed early in life prevents the appearance of the patches (5), whereas *in vivo* studies have shown that blockade of the 5-HT_{2C} receptor class affects visual cortical activity-dependent plasticity (6, 11). Previous reports have shown that LTP and LTD can be induced reliably in cortical layer IV between 3 and 5 weeks of age (12–14). Thereafter, LTP/LTD susceptibility declines to undetectable levels by 8 weeks of age unless 5-HT is applied (7).

Activation of the 5-HT_{2C} receptor stimulates phospholipase C activity and phosphatidylinositol turnover (15). This intracellular cascade consequently results in an increase of inositol-1,4,5-trisphosphate production, which in turn releases Ca^{2+} from intracellular stores (16). An increase of intracellular Ca^{2+} would activate Ca^{2+}-dependent protein kinases such as protein kinase C and calcium/calmodulin-dependent protein kinase. These kinases have the potential to phosphorylate, among other substrates, *N*-methyl-D-aspartate receptors, which may in turn facilitate a cascade of *N*-methyl-D-aspartate receptor-mediated long-term cellular changes, such as LTP and LTD. Both LTP and LTD have been implicated in the synaptic remodeling and refinement of neuronal connections that occur during development. This suggestion is consistent with evidence showing that blocking phosphatidylinositol turnover or elevating Ca^{2+} levels affects plasticity in the visual cortex (17). In addition, 5-HT has been shown to facilitate the excitatory actions of *N*-methyl-D-aspartate and glutamate in cat and rat neocortical slices (18, 19). The enhanced *N*-methyl-D-aspartate response evoked by 5-HT application was long lasting and irreversible (19).

Our results show that the nature and sign of 5-HT facilitation of synaptic plasticity depends on the columnar expression of 5-HT_{2C} receptors in layer IV. After 5-HT application, cells located in a 5-HT_{2C} receptor-poor zone should respond with smaller changes in phosphatidylinositol turnover and intracel-

lular Ca^{2+} than those of cells located in the center of a 5-HT_{2C} receptor-rich zone. Because intracellular levels of Ca^{2+} have been shown to influence induction of either LTP or LTD (20–22), the effects observed here may depend on whether Ca^{2+} levels are strongly elevated (in the 5-HT_{2C} receptor-rich zones) or more weakly elevated (in the 5-HT_{2C} receptor-poor zones). The cases in which 5-HT did not increase the synaptic plasticity may reflect an intermediate increase of intracellular Ca^{2+} levels in the overall population, such that the overall response of the population recorded is neither LTP nor LTD. This notion is consistent with the Bienenstock–Cooper–Munro model (23), which proposes that the LTD–LTP crossover point would depend on the level of postsynaptic response, and with previous studies by Artola *et al.* (20, 24) showing that either LTP or LTD can be induced by protocols in which Ca^{2+} levels are elevated to various degrees.

It is well known that plasticity in the visual cortex changes over time, but may also vary depending on the cortical layer involved (25, 26). The distribution of signaling molecules involved in regulating plasticity would be the most likely factor determining the multiparametric space of synaptic modifiability.

The importance and the role of column-specific alterations in responsivity during cortical columnar development remains to be elucidated. Why should the same pattern of stimulation result in lowered long-term responsivity in one column of cortex, while promoting increased responsivity in an adjacent cortical column? One possibility is that the different long-term consequences of neuronal activation reflect the different functions of the 5-HT_{2C} receptor-rich and receptor-poor columnar areas. 5-HT_{2C} receptor-poor areas are characterized by higher levels of cytochrome oxidase (3, 4), a metabolic enzyme whose concentration has been correlated with higher levels of neuronal activity. In the monkey cortex, neurons in cytochrome oxidase-rich patches are associated with lower levels of orientation selectivity, a preference for lowered spatial frequencies, and generally increased levels of responsivity relative to cells in the complementary patches (27). In the cat, neurons in CO-rich zones are responsive to lower-spatial and high-temporal frequencies as well (28). Our results suggest that the different response properties of neurons in the two columnar systems may be determined by LTP/LTD-like processes and by serotonergic modulation of these processes. The differential concentration of 5-HT_{2C} receptors and the subsequent differing responses (LTP or LTD) to visual input in the two columnar systems may have the effect of *detuning* the responses of neurons within the 5-HT_{2C} receptor-poor zones during the continuing process of visual development, while simultaneously *sharpening* the tuning of neurons within the 5-HT_{2C} receptor-rich zones, leading to the observed differences in response properties. Numerous theoretical models (29) associate LTP-like alterations with the sharpening of cortical-response parameters, such as orientation selectivity, whereas just the opposite might be expected with enhanced LTD. However, with the exception of Bienenstock–Cooper–Munro model (23), none of the models incorporate LTD as a specific component. The differential concentration of 5-HT_{2C} receptors and the different long-term responses that this concentration engenders to cortical input may be part of the mechanism to achieve differential selectivity and responsivity within the different compartments of the developing visual cortex.

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