The effects of median raphé electrical stimulation on serotonin release in the dorsal hippocampal formation of prenatally protein malnourished rats

David J. Mokler a,⁎, Joseph D. Bronzino b, Janina R. Galler c, Peter J. Morgane a,c

a Department of Pharmacology, College of Osteopathic Medicine, University of New England, Biddeford, ME 04005, USA
b Department of Engineering, Trinity College, Hartford, CT 06106, USA
c Center for Behavioral Department and Mental Retardation, Boston University School of Medicine, Boston, MA 02118, USA

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Abstract

Our previous work had shown an enhanced inhibition in the hippocampal formation of prenatally protein malnourished rats. We have also found a diminishment in 5-hydroxytryptamine (5-HT) fibers in the hippocampal formation of malnourished rats as well as increased levels of 5-HT in the brain. The purpose of the present study was to determine 5-HT release in the dorsal hippocampal formation following electrical stimulation of the median raphé nucleus (MRN) in unanesthetized prenatally malnourished rats. Stimulation of this nucleus at 20 Hz in malnourished rats resulted in a significantly diminished release of 5-HT compared to well-nourished rats. The latter group showed a lesser, though still significant, decrease in 5-HT release following raphé stimulation. Basal release of 5-HT prior to stimulation was significantly higher in malnourished rats as compared to well-nourished controls. This may be the result of a decreased density of 5-HT neurons leading to a diminished control of release. Stimulation of the MRN in behaving malnourished animals may markedly affect the recurrent negative feedback collaterals onto somatodendritic 5-HT and 5-HT autoreceptors thus enhancing the inhibitory effects of stimulation of the median raphé on 5-HT release. Studies are underway to examine the sensitivity of both the somatodendritic and terminal 5-HT autoreceptors in malnourished animals, in order to understand possible mechanisms for our findings.

Keywords: In vivo microdialysis; 5-HT autoreceptors; 5-Hydroxytryptamine; Hippocampal formation; Vigilance state plasticity; Raphé–hippocampal system

1. Introduction

The present studies are aimed at gaining insights into possible mechanisms of the effects of prenatal protein malnutrition on GABA-mediated inhibitory processes in the hippocampal formation. By examining the release of serotonin in the hippocampal formation following raphé stimulation in well-nourished compared to malnourished rats, we can assess how prenatal malnutrition affects the functional integrity of the raphé–hippocampal system. The hippocampal formation offers a unique model system for evaluation of brain insults such as prenatal malnutrition on synaptic function and plasticity. In previous studies [31] we found that electrical stimulation of the midbrain raphé in behaving (awake) rats results in a significant decrease in serotonin release in the dorsal hippocampal formation. These findings in awake rats are opposed to previous studies using anesthetized rats in which electrical stimulation of the midbrain raphé nuclei resulted in increased serotonin release in the hippocampal formation [29,31,41,42].

In our earlier study [31] we described the considerable complexities of the raphé–hippocampal serotonergic system including the multiple feedback fibers onto somatodendritic 5-HTIA and 5-HTID autoreceptors in the raphé nuclei, various types of 5-hydroxytryptamine (5-HT) receptors on terminals of 5-HT neurons (5-HT1A and 5-HT1D), including details of the circuitry of both midbrain raphé nuclei and synapses on interneurons in the hippocampal formation (Figs. 1 and 2). In the present study we have concentrated primarily on the MRN which projects largely to the dorsal hippocampal formation and medial septal nucleus.

In our series of electrophysiological studies we have clearly shown a diminished plasticity in the hippocampal formation of prenatally malnourished rats by examining
Fig. 1. Simplified schema of midbrain raphe–hippocampal serotonergic system. The nucleus raphe dorsalis predominately innervates the ventral hippocampal formation. The MRN predominately innervates the dorsal hippocampal formation. Raphe inputs differentially modulate information processing in the hippocampal formation by innervating specialized subsets of inhibitory interneurons. Interneurons are crucial in determining which excitatory synapses will undergo changes in synaptic strength. The midbrain raphe system modulates activity in intrinsic hippocampal circuits primarily through regulation of activity of GABA interneurons. These input systems regulate hippocampal plasticity by regulating the strength of inhibition on hippocampal principal cells, dentate granule cells, and pyramidal neurons of the hippocampus proper. Modulation of activity in groups of inhibitory interneurons with different functional roles appears to mediate a behavioral control of hippocampal information processing. Axon collaterals form powerful self-regulating systems serotonergic neurons in the raphe nuclei.

Kindling activity, long-term potentiation and short-term paired-pulse plasticity (see Refs. [34,35] for reviews). In these various studies, we also found that malnourished animals show a markedly enhanced inhibition in the hippocampal formation [4,13,14], thus indicating functional alterations in critical inhibitory circuitry that may account for many of the long-term effects of prenatal malnutrition on hippocampal plasticity.

In order to determine possible mechanisms of the enhanced inhibition in the hippocampal formation of malnourished animals, we have concentrated our studies on critical extra-hippocampal systems which have been shown to modulate both long- and short-term plasticity in the hippocampal formation [4,13,14]. These include inputs from the midbrain raphe nuclei, particularly the ascending serotonergic pathway from the MRN, the noradrenergic pathway from the locus coeruleus, and the GABAergic pathway from the medial septum to the hippocampal formation. In the present study we have examined aspects of the functional integrity of the median raphe–hippocampal pathway following prenatal malnutrition.

The ascending serotonergic pathways that originate in the median and dorsal raphe nuclei project widely to the forebrain in rats (Fig. 1 and Refs. [6,25,32,39,49]). Morphological, electrophysiological, biochemical and behavioral studies have shown that the pathways from median and dorsal raphe are anatomically distinct and are involved in different functions [8,20,22,26,28]. Most of the forebrain regions receive 5-HT afferents almost exclusively from the two midbrain raphe nuclei but a number of brain structures are innervated preferentially by either the nucleus raphe dorsalis or medianus. The dorsal hippocampal formation is innervated predominantly by serotonergic neurons originating in the nucleus raphe medianus [6,32].

In the rat brain, ascending serotonergic neurons are negatively controlled by 5-HT$_{1A}$ and 5-HT$_{1D}$ autoreceptors localized on the soma and dendrites of neurons in the midbrain raphe nuclei (Fig. 2 and Refs. [12,45]). 5-HT$_{1A}$
Fig. 2. Release of 5-HT in the CNS is under control of autoreceptors. Inhibitory autoreceptors can be divided into two principal groups: (1) autoreceptors residing on the 5-HT neuronal cell bodies in the raphe nuclei and; (2) autoreceptors residing on the 5-HT nerve fiber terminals. The somatodendritic autoreceptors inhibit 5-HT release through inhibition of cell firing while the terminal autoreceptors act by direct inhibition of release at the nerve terminals.

The serotonergic neuron has a number of control mechanisms on both the firing rate of the neuron and release of 5-HT at the terminal. 5-HT binding sites are heterogeneously distributed in the mammalian brain with especially high concentrations in the raphe nuclei and in the subfields of the hippocampal formation. Functional 5-HT_{1A} and 5-HT_{1D} autoreceptors have been demonstrated on cell bodies and dendrites of 5-HT neurons in the midbrain raphe nuclei. One function is an inhibition of the spontaneous firing rate of 5-HT neurons which results in a reduction of the amount of 5-HT released in terminal regions of these neurons. Additionally, 5-HT_{1A} autoreceptors mediate a decrease in neuronally released extracellular 5-HT within the raphe nuclei themselves as measured by in vivo microdialysis.

Binding sites have been localized by autoradiography in both nucleus raphé dorsalis and medianus and their density diminishes after neurotoxic lesions of serotonergic neurons [33,52,53]. That somatodendritic 5-HT_{1A} autoreceptors located in the midbrain raphé nuclei play a major role in control of 5-HT neurotransmission has been confirmed by the demonstration that local injection of the 5-HT_{1A} agonist 8-OH-DPAT causes a decrease of extracellular levels of 5-HT in the hippocampal formation of the rat [2,21,23,24,27,30,43].

The hippocampal formation receives two principal classes of afferent fibers. The first originates in the cortex and has long been known to transmit fast and accurate information through the hippocampal formation. Fiber systems of this class include the perforant path of the entorhinal cortex, the mossy fibers of the dentate gyrus and the Schaffer collateral–commissural pathways of the CA_{1} region [40], the hippocampal trisynaptic circuit. Many studies have characterized the morphological and physiological properties of the pathways forming this circuit. The other class of hippocampal afferents originates in subcortical nuclei and has completely different patterns of innervation, physiological function, and modes of action. These include the raphé nuclei, in particular the nucleus raphé medianus, the locus coeruleus and the medial septum, all of which are modulators of hippocampal activity in response to primary cortical inputs. These systems affect hippocampal activity primarily by their actions on inhibitory interneurons [17,19].

Since prenatal malnutrition enhances GABA-mediated inhibition of hippocampal activity, we have postulated that diminished 5-HT inputs from the raphé lead to removal of inhibition from GABA interneurons in the hippocampal formation, thus resulting in enhanced inhibition at the level of the principal cells (dentate granule cells and pyramidal neurons). This enhanced inhibition would then relate directly to diminished plasticity in the dentate gyrus that we have repeatedly observed (see Refs. [34,35] for reviews). It has long been known that dentate granule cells gate their inputs and outputs in a manner appropriate to various physiological states [3–5,11,54]. The dynamic plastic gating of information at the level of the hippocampal trisynaptic circuit is clearly related to the amount of inhibition present such that enhanced inhibition is associated with diminished transfer of information through this system, thereby resulting in cognitive deficits seen following insults to the hippocampal formation.

Raphé stimulation approaches may also provide a quantitative measure of the density of 5-HT innervation to the hippocampal formation. This is of special importance since our group [7] showed a one-third reduction in serotonergic fibers in the hippocampal formation of prenatally malnourished rats. McQuade and Sharp [29] noted that the relative size of the 5-HT response in the hippocampal formation to electrical stimulation of the raphé matched approximately quantitative estimates of raphé projections to the region. The change in extracellular 5-HT in response to raphé stimulation reflects not only relative density of 5-HT innervation but several parameters relating to regulation of 5-HT release, including 5-HT terminal autoreceptor sensitivity and 5-HT synthetic rate. 5-HT release measures combined with raphé stimulation offers a means to obtain functional evidence for the integrity of 5-HT projection pathways. Accordingly, using in vivo microdialysis, the extracellular release of hippocampal 5-HT was measured as one index of raphé 5-HT cell activity.
2. Material and methods

2.1. Surgery

Male Sprague–Dawley rats (Charles River-derived) bred in the animal colony at Boston University, and ranging from 275 to 375 g were used in these experiments. Females dams were fed a low protein (6%, malnourished) or a normal protein (25%, well-nourished) diet for 5 weeks prior to breeding. Male rats were then placed with the females. At birth litters were culled to eight rats and cross-fostered with well-nourished dams who had just given birth. Animals were weaned at 21 days and given a normal rat diet. Before surgery, animals were housed with same-sex litter mates in Plexiglass cages in animal rooms with constant temperature (22–24°C) and humidity. Lights were on from 0700 to 1900 h daily. Microdialysis experiments were begun between 0800 and 0900 h. Animals had free access to food and water throughout the experiments.

Animals were implanted with electrodes under pentobarbital (35 mg/kg, i.p.) and chloral hydrate (160 mg/kg i.p.) anesthesia. Stimulating electrodes are bipolar, concentric electrodes with an inner electrode extending 1 mm beyond the outer electrode with a tip 0.25 mm in diameter (Plastics One, Roanoke, VA). Electrodes are insulated except at the tips (1 mm inner electrode). The coordinates for the median raphe nucleus (MRN) are A 1.2; L 0.0; V −1.5 in reference to intraural zero [36]. Electrodes into the MRN were implanted at an angle of 20° from the vertical to avoid blocking the cerebral aqueduct and to prevent damage to the midline blood vessels. Guide cannulae (0.5 mm outer diameter, CMA 10, CMA/Microdialysis, Acton, MA) were implanted at the same time immediately above the dorsal hippocampal formation (AP −3.8, L ±1.5, DV −3.5 from bregma [36]. Once the microdialysis probe was inserted into the guide cannula (0.5 mm outer diameter), the probe was positioned in the dorso-medial hippocampal formation. Animals received intradermal infiltration of bupivacaine, a long-acting local anesthetic, and epinephrine at the site of the incision to decrease bleeding during surgery and pain following surgery. After surgery, animals were housed individually. Animals which lost electrodes were euthanized with an overdose of pentobarbital.

2.2. Electrical stimulation

After allowing 3 days for recovery, animals were placed into a deep Plexiglass bowl (CMA 120 Awake Animal System, CMA/Microdialysis) with clean animal bedding between 0800 and 0900 h. The animals were then connected by an electrode lead to an AM Systems Model 2100 Isolated Pulse Stimulator (AM Systems, Seattle, WA). The electrode lead was connected through a two-lead commutator (Plastics One) allowing the animal to move freely about the plastic cage. Behavioral assessment of vigilance state was made throughout the experiment. Animals were in the quiet waking state at the beginning of stimulation.

The tonic electrical stimulation consisted of constant current bi-phasic pulse pairs at a frequency of 20 Hz with each stimulus being 0.10 ms in duration with a stimulus current of 200 µA. This level of tonic stimulation is not aversive in conscious animals, whereas higher currents of stimulation are aversive in the conscious rat (Mokler, unpublished observations). Studies by Mokler et al. [31] and Sharp et al. [42] have demonstrated that neurons of the median raphe respond continually with increases in the frequency of stimulation producing increased changes in release of 5-HT from 2, 5, 10 and 20 Hz. Lower frequencies of raphe stimulation (2 or 5 Hz) have not shown to change 5-HT release in the hippocampal formation [31,42]. The stimulus frequency of 20 Hz is a moderately high level of stimulation for 5-HT neurons, but a stimulus frequency that can be followed by raphe neurons in terms of 5-HT release [31,42]. Raphé neurons typically fire at 0.5–2.0 Hz in anesthetized animals and up to 5 Hz in active waking states. The firing rate for 5-HT neurons is clearly vigilant-state-dependent, varying from 5 Hz in active waking to almost complete silence during REM sleep [1,18,44].

Microdialysis probes (3 mm CMA 10, CMA/Microdialysis) were perfused at a rate of 1.0 µl/min with artificial CSF (artCSF; 147 mM NaCl, 1.26 mM CaCl2, 2.5 mM KCl, 1.18 mM MgCl2 in sterile Water). ArtCSF contained 1 mM sertraline (Pfizer Pharmaceuticals, Groton, CT), a selective 5-HT uptake inhibitor, to stabilize levels of extracellular 5-HT. Preliminary experiments show that perfusion of the terminal fields in the hippocampal formation with artCSF containing sertraline does not fundamentally alter the dynamics of 5-HT release [41,42] and Mokler, unpublished observations. In an experiment using a 3-mm probe placed in artCSF containing 1 µM 5-HT at 37°C, our recovery of 5-HT has been shown to be between 30 to 40%. Levels of 5-HT in dialysate are unadjusted for recovery in order to avoid underestimates of extracellular levels of 5-HT.

On the day of the experiment, the microdialysis probe was inserted with minimal stress and handling into the guide cannula while the animal was alert. At least six 20-min samples were collected for baseline determination. Baselines were considered stable if microdialysis samples did not differ by more than 20% over three consecutive samples. Experimental manipulation was begun after levels of 5-HT in dialysate had stabilized.

Neurochemical analysis was carried out using liquid chromatography with electrochemical detection (ESA Coulochem II, ESA, Chelmsford, MA) using a C18 3-µm column. The mobile phase consisted of 0.1 M disodium phosphate, 15% acetonitrile, 10 mM EDTA, 1.5 mM octanesulfonic acid at a pH of 5.4. This allowed for the measurement of 5-HT with a sensitivity of 0.5 fmol/20 µl. The area under the curve (AUC) for samples was...
Fig. 3. Basal release of 5-HT was increased in the dorsal hippocampal formation of prenatally protein malnourished compared to well-nourished rats ($t_{27} = 3.01; \ p < 0.001$). Basal release was determined 3 h after probe insertion when 5-HT levels in dialysate had stabilized. Bars represent mean and S.E.M. for well-nourished ($n = 6$) and malnourished ($n = 6$) rats.

compared using computer software (Chromperfect) with a regression analysis of AUC for three authentic standards ($10^{-7}$, $5 \times 10^{-10}$ and $10^{-10}$ M) injected onto the column at the beginning of each experimental day to determine the levels of 5-HT. 5-HT was verified by examining the voltammogram for standards against that determined using microdialysis samples.

2.3. Histology

Following microdialysis studies, animals were anesthetized with pentobarbital. After a surgical level of anes-thesia was reached, the heart was exposed and the animal perfused by intracardiac cannula with 100 cm$^3$ buffered saline followed by 100 cm$^3$ of 10% buffered formalin. The brain was removed and stored in buffered formalin. Brains were blocked to include the MRN and dorsal hippocampal formation and sectioned on a vibratome at 50 μm to verify the location of the stimulating electrode and the microdialysis probe. Slices were mounted and stained with Cresyl violet (Nissl stain) followed by photomicroscopy.

2.4. Statistics

Statistics were performed using SYSTAT version 7.0.1 (SPSS). Two-way ANOVAs were used to evaluate the effects of the experimental variables on the release of 5-HT as expressed as a percent of baseline. The transformation to percent of control is done to normalize the data for variability from animal to animal in the basal levels of 5-HT. A least significant differences (lsd) test is used for post-hoc comparisons. A comparison of basal levels of 5-HT was done by a $t$-test. A value of $p < 0.05$ was considered as significant.

3. Results

Probes were inserted through the guide cannula into the dorsal hippocampus on the day of the experiment and were perfused at a rate of 1 μl/min. Within 3 h of probe insertion, the heart was exposed and the animal perfused by intracardiac cannula with 100 cm$^3$ buffered saline followed by 100 cm$^3$ of 10% buffered formalin. The brain was removed and stored in buffered formalin. Brains were blocked to include the MRN and dorsal hippocampal formation and sectioned on a vibratome at 50 μm to verify the location of the stimulating electrode and the microdialysis probe. Slices were mounted and stained with Cresyl violet (Nissl stain) followed by photomicroscopy.

Fig. 4. Time course of 5-HT release in the dorsal hippocampal formation of well-nourished and malnourished rats following electrical stimulation of the MRN. Electrical stimulation (20 Hz, 200 μA, 1 μs biphasic pulse pairs) of the MRN was delivered for 20 min (bar) after stable levels of 5-HT release. No changes in behavioral state were observed during electrical stimulation. Electrical stimulation of the MRN produced a decrease in 5-HT release in well-nourished rats that began after the cessation of stimulation as compared with the immediate decrease in 5-HT release during stimulation in malnourished rats. Bars represent mean ± S.E.M. for an $n$ of 6 animals in each group expressed as the percent of basal release shown in Fig. 3. *Significantly different from basal release in well-nourished rats. **Significantly different from basal release in malnourished rats. $t$-test.

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Fig. 5. Histological frontal section showing electrode tip (1 mm tip exposure) in lateral border of MRN. Electrode inserted at 20° angle to avoid blockage of the cerebral aqueduct (Aq). Arrow points to area of uninsulated tip of the stimulating electrode. Stimulation 1 mm lateral to this tip did not result in altered 5-HT release in dorsal hippocampal formation. Abbreviations: DR: dorsal raphe; MLF: medial longitudinal fasciculus.

Insertion into unrestrained, conscious animals, stable levels of 5-HT were achieved. At the time that these basal levels were achieved, all animals were in a quiet, waking state characterized by lying on their side in the cage with their eyes open, but without activity. We have shown in conscious animals in a quiet, waking state, 3 h after probe insertion, that 5-HT release in the dorsal hippocampus is decreased by addition of the Na\(^+\)-channel blocker tetrodotoxin (TTX) and by deletion of Ca\(^{++}\) from the CSF, respectively, producing declines of 5-HT of 75 and 65% [31].

Basal levels of 5-HT in the dorsal hippocampal formation were significantly different in well-nourished rats as compared to malnourished rats (Fig. 3; \(t_{27} = 3.01; p < 0.001\)). Conscious, behaving, well-nourished animals had basal levels of 5-HT in the dorsal hippocampus of 1.4 ± 0.38 fmol/20 µl, whereas conscious, behaving, protein-malnourished animals had levels of 5-HT of 8.8 ± 2.04 fmol/20 µl. No obvious differences were noted in the behavior of animals in the two groups.

Electrical stimulation was carried out over a period of 20 min after basal levels of 5-HT had stabilized. No behavioral changes were noted in animals at the onset or offset of electrical stimulation, each remaining in a quiet, waking state. Electrical stimulation of the MRN (200 µA, 100 ms, 20 Hz) produced a suppression of 5-HT release in both well-nourished and malnourished rats. Rats in the malnourished group showed a significantly greater suppression of release (\(F_{1,20} = 5.301, p < 0.05\)) than well-nourished controls (Fig. 4). Malnourished rats showed decreased release following stimulation at all time points. Maximal changes in release were seen after 120 min, with malnourished animals decreasing to 40% of control levels and well-nourished animals decreasing to 65% of control.

Fig. 6. Histological frontal section through the dorsal hippocampal formation showing track of microdialysis probe with tip centered in hilus (H) of dentate gyrus (DG). Median raphe stimulation in malnourished rats resulted in significantly greater diminishment in 5-HT release in this area compared to normal diet controls. The extent of the active membrane of the dialysis probe (3 mm) is indicated by black arrow heads. Abbreviations: HF: hippocampal formation.
Furthermore, the pattern of serotonin release in the dorsal hippocampal formation differed between the two groups (Fig. 4). In malnourished animals, 5-HT levels declined significantly during the period of stimulation ($t_{11} = 2.99, p < 0.01$), whereas well-nourished rats did not show declines until after stimulation had ceased. In both well-nourished and malnourished rats, stimulation produced a long-term depression in the release of 5-HT which lasted for the duration of the study (2 h). Fig. 5 shows the histological frontal section showing electrode tip in lateral border of MRN. Stimulation 1 mm lateral to this tip did not result in altered 5-HT release in the dorsal hippocampal formation. Fig. 6 shows the histological section indicating the microdialysis probe track in the hippocampal formation.

4. Discussion

Malnourished rats showed a significantly higher basal release of 5-HT in the dorsal hippocampal formation as determined by in vivo microdialysis compared to well-nourished controls. This increase was seen in the presence of 1 μM sertraline in the dialysis fluid. Previous studies by our group have shown increased levels of 5-HT in the brains of malnourished rats [38,48]. In those studies select gross anatomical areas of the malnourished brain showed significantly elevated levels of 5-HT and 5-HIAA at postnatal days 0 through 30. This was accompanied by a significant increase in brain tryptophan [38]. These data and the data in the present study suggest a long-term derangement in 5-HT neurotransmission in the brain of prenatally malnourished rats. Given the importance of 5-HT in the development of the brain, this may be a key finding in the pathology of malnutrition.

In contrast to the studies on 5-HT levels and release in the brain, our group has also shown a marked decrease in the serotonin innervation of the dorsal hippocampus in malnourished animals [7]. Diaz-Cintra et al. [16] have also shown that neurons in the dorsal raphé nucleus of malnourished animals have decreased dendritic spines, decreased dendritic branching and decreased dendritic extent. If this pattern holds for the MRN this may then lead to a diminished 5-HT innervation of the hippocampal formation associated with a decreased control of 5-HT release. One possible explanation for an increase in basal release of 5-HT with a decrease in innervation involves a decrease in the regulation of release by autoreceptors in either the raphé nucleus (somatodendritic 5-HT$_{1A/1D}$ receptors) or the dorsal hippocampus (presynaptic 5-HT$_{1B/1D}$ autoreceptors). Thus, the increase in basal release of 5-HT may be compensatory as a result of the early postnatal loss of 5-HT fibers in the raphé–hippocampal system. Studies are presently underway to determine if there is an alteration in the sensitivity of the somatodendritic autoreceptors in the raphé nuclei or presynaptic autoreceptors in the dorsal hippocampal formation in order to determine if receptor pathology may be one component of this derangement in release. Further studies are also needed to determine if the increased basal release of 5-HT in malnourished rats may be the result of increased synthesis or a decrease in reuptake, although the latter is unlikely since the dialysis fluid contains a 5-HT uptake inhibitor.

In malnourished rats, electrical stimulation of the median raphé results in a decreased release of 5-HT in the dorsal hippocampus. In this regard, we [31] and Sharp et al. [42] have shown that stimulation mimicking the frequency of raphé cell firing (2 and 5 Hz) does not significantly alter 5-HT release in the hippocampus. Furthermore, the decrease in release is seen only in the conscious behaving rat whereas increases are seen following raphé stimulation in the anesthetized animal [31,42]. The relative decrease in release in malnourished animals is greater in magnitude than the decreased release evoked by electrical stimulation of the MRN in well-nourished controls. This greater inhibition of the release in malnourished animals may, again, be the result of a greater sensitivity of the somatodendritic or terminal 5-HT autoreceptors in these animals. Examination of the release of 5-HT from the MRN itself and the dorsal hippocampal formation in malnourished rats after local administration of the 5-HT$_{1A}$ agonist 8-OH-DPAT will help determine if there is a difference in the sensitivity of the somatodendritic autoreceptor in malnourished rats. In preliminary studies, we have recently found that infusion of 8-OH-DPAT into the MRN suppresses 5-HT release in the dorsal hippocampal formation in a manner similar to controls [30], initially suggesting that the sensitivity of 5-HT$_{1A}$ receptors may not be different between the two groups. In this regard, central serotonergic pathways show a striking diversity and complexity of receptor heterogeneity (Fig. 2). There is extensive evidence that 5-HT neuronal activity is tightly controlled through feedback mechanisms (Fig. 1). At the terminal level stimulation of 5-HT$_{1B}$ and 5-HT$_{1D}$ autoreceptors results in a reduction of the amount of 5-HT released from nerve endings. At the somatodendritic level 5-HT$_{1A}$ and 5-HT$_{1D}$ receptors, which are found in high density in the midbrain raphé nuclei on 5-HT neurons [45,47], modulate the spontaneous firing rate of 5-HT neurons [46] and, thereby, the amount of 5-HT released from nerve endings [9,10].

The raphé–hippocampal pathways represent a complex control system and stimulation studies in relation to release of transmitters have to be viewed in terms of the organization of anatomical projections, various receptor subtypes and the degree to which 5-HT neurons terminate on different families of GABA interneurons in the hippocampal formation. Only through concurrent morphological, physiological, pharmacological and biochemical studies can we better understand the nature of malnutrition-induced neuropathology and its relation to cognitive impairments. This relates to altered inhibition in the hippocampal formation resulting from the diminishment of 5-HT inhibi-
tion of GABA interneurons. Inhibitory neurons are crucially involved in shaping normal hippocampal functions and are thought to be important elements in the development of hippocampal pathologies following malnutrition or alcohol insults [34,35].

A key issue in understanding the function of hippocampal circuits is to reveal the conditions and processes that govern the reverberation of neuronal impulses through the hippocampal trisynaptic circuit. It is clear that extra-hippocampal inputs, such as the raphé–hippocampal serotonergic pathway, play a major role and need to be examined as to their functional integrity following neuronal insults. As Buzsaki [15] points out, the activity of ascending subcortical inputs, e.g., from the raphé, locus coeruleus or medial septum, can reorganize the functional connectivity of the circuitry of the hippocampal formation into different modes of operation and thereby alter its output patterns. This also points out the necessity of studying the activity of the intact brain which is able to oscillate through the behavioral states of waking, slow-wave sleep and REM sleep. Importantly, the raphé–hippocampal system is part of a more complex control system which must be considered as a whole in behaving animals (Fig. 1). Consequently, studies should also examine the role of behavioral state control of other neurotransmitters on raphé afferent systems, including the corticotrophin releasing factor (CRF) system, as well as norepinephrine, excitatory amino acids and, particularly, inhibitory neurotransmitters such as GABA. It is now clear that CRF fibers innervate the midbrain raphé nuclei and thus comprise a part of a larger GABAergic circuitry. Its presumed role in the noradrenergic nucleus locus coeruleus [37,50,51].

The raphé stimulation approach may also provide a quantitative measure of the density of 5-HT innervation to specific forebrain areas [29]. In light of the fact that our group found an approximately one-third reduction in the 5-HT plexus in the hippocampal formation of malnourished rats [7], this may reflect itself in diminished evoked release we have reported here. The decrease in extracellular 5-HT in response to raphé simulation is likely to reflect not only the relative density of the 5-HT innervation but also other parameters involved in regulation of 5-HT release such as 5-HT synthesis rate, terminal 5-HT autoreceptor sensitivity and size of the 5-HT release and storage pools. In any event, 5-HT release measurements combined with raphé stimulation offers one means to gather functional evidence regarding the integrity of 5-HT pathways from particular raphé nuclei following prenatal protein malnutrition.

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References


[47] S.J. Starkey, M. Skingle, 5-HT_{1A} as well as 5-HT_{1A} autoreceptors modulate 5-HT release in the guinea pig dorsal raphé nucleus, Neuropharmacology 33 (1994) 393–402.


