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Functional Interrelations Between Nucleus Raphé Dorsalis and Nucleus Raphé Medianus: A Dual Probe Microdialysis Study of Glutamate-Stimulated Serotonin Release

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Abstract

Dual-probe *in vivo* microdialysis was used to explore the relationships between the two midbrain raphé nuclei, raphé dorsalis (DRN) and raphé medianus (MRN). Infusion of the excitatory neurotransmitter glutamate (10 mM) into the dorsal raphé nucleus produced a large increase in the extracellular 5-HT (5-HT_{ext}) in the dorsal raphé (1400% of control values) that was limited to the time of infusion. This was followed by a significant decrease in extracellular 5-HT below baseline levels that continued for the duration of the experiment (3 hrs). Extracellular 5-HT (5-HT_{ext}) was also increased to 500% of control values in the median raphé nucleus following infusion of 10 mM glutamate (GLU) into the dorsal raphé nucleus. Infusion of the competitive NMDA receptor antagonist AP5 prior to and during infusion of GLU into the DRN resulted in a decrease in the response to GLU in the DRN and an antagonism of the increase of 5-HT_{ext} in the MRN. Infusion of 10 mM GLU into the lateral midbrain tegmentum, an area of the brain just lateral to the DRN, also increased 5-HT_{ext} in the probe in the lateral midbrain tegmentum (900% of control) but did not alter 5-HT_{ext} in the MRN. When glutamate was infused into the MRN, 5-HT_{ext} was also increased to 1400% of control in a time course similar to that seen with infusion of GLU into the DRN. Infusion of glutamate into the MRN, however, did not alter the 5-HT_{ext} in the DRN. These data suggest a serotonergic innervation of the median raphé nucleus by the dorsal raphé nucleus. A reciprocal innervation from the median raphé to the dorsal raphé is not mediated by glutamate, does not appear to be serotonergic, and does not regulate extracellular serotonin in the dorsal raphé.

Keywords

Limbic midbrain; serotonin; glutamate; NMDA receptor; AP-5; *in vivo* microdialysis; 5-HT; 5-hydroxytryptamine

1. Introduction

The serotonergic (5-HT) systems of the forebrain are implicated in a diverse number of homeostatic systems of the brain as well as in many neuropsychiatric disorders. Of particular note is the involvement of the serotonergic systems in depression [26], anxiety [25] and schizophrenia [1]. It is clear from a number of anatomical studies that the two midbrain raphé

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nuclei (dorsalis and medianus) provide the major serotonergic innervation of the forebrain (see Azmitia [4;5], Parent et al. [23], and Steinbusch and Nieuwenhuys [28] for reviews). Each of these nuclei has a distinctive pattern of forebrain innervation. Further, there are major morphological differences in the serotonergic fiber systems from each nucleus. Molliver [21] reviewed considerable evidence of morphological differences between dorsal raphe and median raphe neurons, strengthening the findings that these two nuclei show many anatomical, and probably, functional differences. The nucleus raphe dorsalis has thin fibers with a diffuse distribution, a high density of serotonin transporters (SERT) and a susceptibility to neurotoxic agents such as methamphetamine, whereas the raphe medianus shows thick beaded axons with a more precise topographic distribution, has a low density of SERT and low susceptibility to neurotoxic agents [9;21]. Furthermore, the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN) project to different areas of the forebrain [5;6]. The DRN projects to the dorsal striatum, ventral hippocampus, amygdala, nucleus accumbens and the cerebral cortex (Figure 1). The MRN projects primarily to the septum and dorsal hippocampus. In addition, the DRN and the MRN also project to multiple areas of the lower brainstem (Figure 1). In the cortex the two nuclei project to distinct areas; the projections of the MRN are distributed more uniformly throughout the cortex while the projections of the DRN are much greater to the frontal cortex [21]. Accordingly, these two midbrain serotonergic systems represent distinct functional pathways. To date, as reviewed below, several studies have used various tract-tracing techniques and autoradiography to examine their anatomical relations.

One of the first studies of anatomical relations between raphe dorsalis and medianus was that of Jacobs et al. [11] demonstrating median raphe projections to the dorsal raphe nucleus. This study also indicated that these serotonergic inputs to the dorsal raphe terminated largely on raphe interneurons (probably GABAergic). These authors postulate that impulse flow in 5HT-containing neurons is subject to regulation via negative neuronal feedback as originally proposed by Aghajanian [3]. Kalen et al. [14] reviewed previous studies showing fibers from median raphe to dorsal raphe. Vertes and Martin [36], using autoradiographic techniques, also demonstrated median raphe projections to the dorsal raphe nucleus. The existence of connections between the two-midbrain raphe raises another possibility, i.e., that manipulations, which increase 5-HT concentrations in the synapse, may depress discharge of raphe neurons directly via inhibitory 5-HT synapses on raphe neurons.

Strong projections have also been demonstrated from the DRN to the MRN. Marcinkiewicz [18], using tract-tracing methods, examined inputs to median raphe and showed strong dorsal raphe inputs to the median raphe. Behzadi et al. [6] using retrograde tract-tracing techniques also demonstrated dorsal raphe inputs to median raphe. Vertes and Kocsis [34], using anterograde tracing methods, showed heavy dorsal raphe projections to median raphe. Thus, anatomical studies have demonstrated that the MRN and DRN are connected reciprocally.

Numerous studies have demonstrated a role of glutamate in the regulation of 5-HT release in the raphe nuclei (see Adell et al. [2] for review). Tao and Auerbach [31] have examined the GABAergic and glutamatergic influence on extracellular 5-HT (5-HT_{ext}) in the median and dorsal raphe nuclei using *in vivo* microdialysis. They showed that there were significant differences in the roles of GABA and glutamate in the regulation of 5-HT_{ext} in these two nuclei. Pallotta et al. have also shown that infusion of 100 μ M NMDA into the DRN increases 5-HT_{ext} in the DRN [22]. However, neither group has examined the interactions between these major limbic midbrain nuclei.

To our knowledge, no primarily functional studies of relations between the midbrain raphe nuclei have been carried out. In particular, relations between these two raphe nuclei are needed to examine how their interactions alter serotonin release in key forebrain areas, including the medial prefrontal cortex, hippocampal formation, amygdaloid complex, nucleus accumbens,

anterior cingulate cortex, and other limbic forebrain areas (Figure 1). To date no adequate immunocytochemical studies have revealed the chemical makeup of the interconnecting fibers between the raphé dorsalis and medianus though we have previously proposed [19] they could be serotonergic and/or GABAergic. Many other possibilities exist given the numerous transmitter systems present in both raphé nuclei. From this summary it is clear that functional studies of cross-talk between the midbrain raphé nuclei are in order. An understanding of how the midbrain raphé nuclei communicate may be useful in the design of new pharmacologic treatments for neuropsychiatric disorders such as depression, schizophrenia or sleep disorders.

2. Methods

This study was conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Animals in Research, the Society for Neuroscience Handbook for the Use of Animals in Neuroscience Research, and under protocols approved by the Institutional Animal Care and Use Committee of the University of New England.

2.1 Stereotaxic surgery

Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing between 300 and 325 g were used in these experiments. Animals were housed in a vivarium with a 12:12 light dark cycle (lights on at 0700). Animals had *ad libitum* access to food and water.

For implantation of guide cannulae, animals were anesthetized with 50 mg/kg pentobarbital and 160 mg/kg chloral hydrate i.p. Guide cannulae (CMA 12, CMA/Microdialysis AB, Acton, Mass.) were implanted above both the median raphé and dorsal raphé nuclei using coordinates from Paxinos and Watson [24]. Coordinates for the midpoint of the active membrane of the microdialysis probe in the MRN were AP +0.70; L 0.0; DV +2.0 (Figure 102, Paxinos and Watson, [24]) determined from interaural zero; while the coordinates for the midpoint of the active membrane of the DRN probe were AP +1.70; L 0.0; DV +4.0 from interaural zero (Figure 94, Paxinos and Watson [24]). Guide cannulae were implanted at an angle of 20 degrees from the vertical to avoid the venous sinus and cerebral aqueduct. Figure 2 shows histology demonstrating the guide cannula and the probe tracts for the MRN. In a control study with infusion of GLU into the lateral midbrain tegmentum, an area of the midbrain just lateral to the DRN, the midpoint of the active membrane was AP +1.70, L 2.0 and DV +5.5 with respect to interaural zero.

2.2 In Vivo Microdialysis

Following 3–4 days for recovery from surgery, the animal was placed in a large Plexiglas bowl with a collar attached by a guide wire to a suspension arm (Awake Animal System, CMA/Microdialysis AB, Acton, MA). CMA12 microdialysis probes (3 mm; CMA/Microdialysis AB, Acton, MA) were slowly lowered through the guide cannulae into the MRN and DRN over a 1 min period in the conscious animal under gentle hand restraint. The probes were perfused with artificial cerebrospinal fluid (ACSF) consisting of 147 mM NaCl, 1.26 mM CaCl₂, 2.5 mM KCl, 1.18 mM MgCl in sterile water. ACSF was perfused at a rate of 1 µl/min through the probes using a CMA/Microdialysis Syringe pump and a 1.0 ml Hamilton syringe.

The microdialysis probes used in the present experiment have a recovery rate for 5-HT of 17% (CMA Microdialysis online application notes). Probes were rinsed with distilled water and treated with an enzymatic contact lens cleaner after first use and were reused only once. We have found that a single reuse does not alter recovery rates.

All experiments were started at 08:00–08:30. Following 2 hours of sample collection to allow serotonin release to stabilize [19], three 20 min baseline samples were collected. The control

ACSF was then changed to a solution containing 1 or 10 mM glutamate in ACSF. A liquid switch (CMA Microdialysis) was used to switch between syringes. After 20 min of infusion of glutamate, the perfusion was switched back to control ACSF and samples were collected for an additional 8 samples. In the first set of experiments, GLU (1 or 10 mM) was perfused through the probe into the DRN and dialysis samples were collected from the DRN and the MRN. In a second set of experiments, GLU (10mM) was perfused through the probe into the MRN and dialysis samples were collected from the MRN and the DRN. In a further control experiment, 10 mM GLU was perfused through a probe placed lateral to the DRN and dialysate samples collected from that probe as well as a probe implanted into the MRN.

Finally, in a separate experiment, the competitive n-methyl-d-aspartate (NMDA) antagonist D (-)-2-amino-5-phosphonopentanoic acid (1 mM AP-5, Sigma RBI, St. Louis MO) was infused into the DRN 40min prior to and during infusion of 10 mM GLU into the DRN for 20min to determine if the actions of GLU in the DRN are mediated by the NMDA glutamate receptor. Following the co-perfusion of AP-5 and GLU, the perfusion medium was switched to control ACSF and dialysis samples collected for additional 8 samples.

2.3 Analysis of 5-HT

Samples were analyzed immediately by HPLC with electrochemical detection (HPLC- ECD). The HPLC system was an ESA Choulochem II using a 3 μ M 4.6mm $\times$ 100mm C-18 column (Microsorb MV, Varian, Walnut Creek, CA). The mobile phase consisted of 0.1 M disodium phosphate, 15% acetonitrile, 10 mM EDTA, and 1.5 mM octanesulfonic acid, pH 5.4 at a flow rate of 1 ml/min. Electrodes were set at the following potentials: guard cell +300mV, E1 -150 mV and E2 +200 mV. This allowed for the measurement of 5-HT with a sensitivity of 3 fmol/20 μ l sample. The entire sample (20 μ l) was injected onto the column. The area under the curve for samples was compared using computer software Chromperfect (Justice Laboratory Software, Palo Alto, CA) with a regression analysis of AUC for three authentic standards (10^{-9} , 5×10^{-10} , 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.

2.4 Histology

At the completion of each study, the animal was perfused transcardially with 10% neutral buffered formalin under pentobarbital anesthesia and the brain removed. The brain was sectioned in the frontal plane at 30 μ m and Nissl stained for verification of the guide and probe placement. A photomicrograph of probe placement is shown in Figure 2.

2.5 Statistics

Data was analyzed using SigmaStat 3.1 (Systat Software, www.systat.com). Studies of infusion of glutamate into the DRN or MRN were analyzed using a one-way repeated measures analysis of variance (ANOVA) with a post hoc test using the Student-Neuman-Keuls method. Infusion of AP-5 and AP-5 with glutamate was compared against the infusion of glutamate alone using a two-way ANOVA followed by the Student-Neuman-Keuls test for all pairwise comparisons. A significance level of $p < 0.05$ was used for all analyses.

3. Results

3.1 Histology and basal extracellular levels of 5-HT in the DRN and MRN

Histological verification showed that all probes were located within either the DRN or MRN. A representative photomicrograph is shown in Figure 2. Basal extracellular 5-HT (5-HT_{ext}) in

the DRN was 15.6 ± 2.4 fmoles/20 μ l (n = 16) and in the MRN was 10.6 ± 1.3 fmoles/20 μ l (n=16).

3.1 Effects of glutamate infusion into the dorsal raphe nucleus

The infusion of 1mM GLU into the DRN and collection of 5-HT from the MRN and DRN did not result in a significant change in the 5-HT_{ext} from either nucleus (F (9,10) = 1.58, p = 0.242 for DRN and F (8,9) = 2.38, p = 0.1097 for the MRN; Figure 3).

Increasing the concentration of GLU perfused into the DRN to 10mM GLU resulted in a dramatic effect. Figure 4 shows the result of 10mM GLU infusion into the DRN on 5-HT_{ext} in the DRN. 5-HT_{ext} was significantly increased to 14 times basal release (F (10,27) = 8.30, p<0.0001). Immediately after the infusion was terminated, 5-HT_{ext} decreased until it was at basal levels 40 min after the infusion of GLU was stopped. 5-HT_{ext} continued to decline until, at the end of the study period, 5-HT_{ext} was decreased to 33 ± 2 percent of control levels. Infusion of GLU into the DRN also significantly increased 5-HT_{ext} in the MRN (F(10,36) = 2.13, p < 0.05). The time course of this increase was longer than that seen in the DRN, increasing to a maximum 20 to 40 minutes after the end of the infusion period (Figure 4). 5-HT_{ext} returned to control levels 80 min after the cessation of GLU perfusion of the DRN.

Reverse dialysis of the NMDA antagonist AP-5 (1mM) into the DRN for 40 min did not alter baseline 5-HT_{ext} in the DRN or the MRN. Infusion of AP-5 into the DRN during infusion of 10mM GLU into the DRN resulted in a significant decrease in the response of the DRN to GLU infusion with the increase in 5-HT_{ext} reduced to 400% of control as opposed to 1444% of control with infusion of GLU alone (Fig. 5A; F (8,41) = 4.019, Glutamate alone vs Glutamate + AP-5, p < 0.001). AP-5 infusion antagonized the response of the MRN to infusion of GLU into the DRN (Fig. 5B), reducing the maximal 5-HT_{ext} in the MRN from 500% of control to 150% of control.

3.2 Effects of glutamate perfusion into the midbrain tegmentum lateral to the DRN

Infusion of 10 mM GLU into the midbrain tegmentum lateral to the DRN resulted in a significant increase in 5-HT_{ext} to 900% of control at the site of infusion (Fig 6). This increase returned to control levels 40 min after cessation of infusion. Infusion of 10 mM GLU into the midbrain tegmentum, however, did not alter 5-HT_{ext} in the MRN.

3.3 Effects of glutamate perfusion of the median raphe nucleus

When 10 mM glutamate was infused into the MRN there was a significant increase in 5-HT_{ext} in the MRN (Fig 6; F (9,48) = 30.9, p < 0.0001). The time course of this increase carried over into the period just following the cessation of GLU perfusion of the MRN. As was the case with infusion into the DRN, infusion of GLU into the MRN caused a significant long-term decrease in 5-HT_{ext} to 20% of control levels by the end of the experiment. Infusion of GLU into the MRN, however, did not result in an alteration in release of 5-HT in the DRN (Fig 6; F (9,48) = 1.81, p = 0.09).

4. Discussion

The present experiments show for the first time that a functional interaction exists between the dorsal raphe and median raphe nuclei. Previous anatomical studies have suggested interactions between these nuclei [7;33]. The infusion by reverse dialysis of the excitatory amino acid glutamate into either the DRN or the MRN results in an increase in extracellular 5-HT (5-HT_{ext}) in that nucleus. Furthermore, these studies have shown that infusion of glutamate into the DRN also increases 5-HT_{ext} in the MRN and that the NMDA antagonist AP-5 blocks this increase.

4.1 GLU infused into the DRN increases 5-HT_{ext} in the DRN

Previous studies have shown that reverse dialysis of the glutamatergic agonist NMDA increases 5-HT_{ext} in the DRN [2;22;29–31]. Tao and Auerbach [29] showed that NMDA (30, 100 or 300 μ M) infused into the DRN increases 5-HT_{ext} in DRN in a dose-dependent manner. These increases were partially blocked by co-infusion of the NMDA antagonist AP-5. Pallotta et al. [22] also used reverse dialysis to infuse NMDA into the DRN, and, in contrast to the findings of Tao and Auerbach [29], they showed that perfusion with 25 μ M NMDA into the DRN decreased 5-HT_{ext} in the DRN while increasing 5-HT_{ext} in the prefrontal cortex. Increasing the concentration of NMDA to 100 μ M did lead to an increase in 5-HT_{ext} in the DRN but a decrease in 5-HT_{ext} in the prefrontal cortex. These effects were also antagonized by the NMDA antagonist AP-5. In a separate study, Tao and Auerbach [29] showed that 300 μ M NMDA increased 5-HT overflow by 380% in the DRN. Our current findings are in agreement with the infusion of the higher concentrations of NMDA into the DRN, as well as the blockade by AP-5. It should be noted here that the concentrations of NMDA used by Tao and Auerbach [29] were 30 times lower than the concentrations of GLU which we required to show an increase in 5-HT and 3 times lower than an inactive (1mM) concentration of GLU. This may reflect the low potency of GLU due to efficiency of removal of GLU from the extracellular environment (see discussion below).

4.2 10 mM glutamate is required to increase 5-HT_{ext} in raphé nuclei

The concentration of GLU used to increase 5-HT_{ext} in the raphé nuclei is an important issue in these experiments. Initial estimates based on previous studies of the concentrations of GLU needed to activate the MRN or DRN led us to an initial concentration of 1mM GLU. The concentrations of GLU used were based on literature values for other glutamatergic drugs infused via reverse dialysis into discrete brain regions (median raphé nucleus, 1mM AP-5 [31]; striatum, 1mM NMDA [38]; MRN, 1 mM NMDA [29]). However, as shown in Figure 3, infusion of 1mM GLU by reverse dialysis into the DRN did not affect the 5-HT_{ext} in either the DRN or the MRN. Increasing the concentration of GLU infused to 10 mM GLU increased 5-HT_{ext} in both the DRN and MRN. If reverse dialysis delivers an estimated 10 to 20 % of the GLU concentration across the dialysis membrane (T. Maher, personal communication) then we would be delivering a concentration of between 1 and 2 mM to the tissue.

A possible explanation for the lack of effect of 1mM glutamate and the need to increase the concentration of GLU to 10 mM to affect 5-HT_{ext} may be the result of the high efficiency reuptake of glutamate in the CNS. Five excitatory amino acid transporters (EAATs) have been identified and are localized on pre- and post-synaptic neurons as well as glia [27]. These transporters modulate extracellular GLU levels below excitotoxic levels by rapidly transporting GLU into the cells. The high efficiency of these transporters may explain the lack of effect seen at 1mM and the high concentration of 10mM needed to elicit a response by reverse dialysis of GLU.

4.3 Long-term decreases in 5-HT_{ext} following glutamate perfusion of the raphé nuclei

An interesting finding of these experiments is the large decrease in 5-HT_{ext} following infusion over the course of the experiment until 5-HT_{ext} had decreased to 33 percent of the control values in the DRN and 20% in the MRN by the end of the experiment (3 hours post GLU infusion). We have previously shown that in microdialysis experiments, 5-HT_{ext} in the dorsal hippocampus and medial prefrontal cortex does decrease somewhat over the course of the experiment but only to approximately 75% of control after 6 hours of dialysis [19;20]. The present findings reflect a slow inactivation of the raphé nuclei after the initial stimulus, possible via activation of 5-HT_{1A} autoreceptors [8]. We have reported a similar suppression of 5-HT_{ext} after electrical stimulation of the DRN or MRN [19]. This finding of long-term inhibition of the raphé nuclei should be examined in more detail as it may indicate that long-term changes

in serotonin in the forebrain occur after stimulation of these nuclei (such as by stress) and may be indicative of mood changes (depression, psychosis) that may occur after stress.

4.4 Glutamate is directly excitatory on serotonergic neurons

These data demonstrate that the primary effect of GLU is excitatory in the DRN most likely by acting directly on serotonergic neurons. Jolas and Aghajanian [12] have shown that microiontophoresis of NMDA is excitatory to serotonergic cells of the DRN. The glutamatergic cells that synapse on serotonergic cells of the DRN arise from the medial prefrontal cortex, septum, periaqueductal grey (PAG) and the lateral habenular nucleus [12;13], as well as within the DRN itself.

Tao and Auerbach [31] have previously shown that NMDA receptor antagonists alone do not decrease the 5-HT_{ext} in the DRN or MRN, but a combination of AMPA/kainate and NMDA antagonists do decrease 5-HT_{ext} in the raphé. In the present study we have also confirmed that the NMDA antagonist AP-5 does not decrease basal release of 5-HT from the DRN. Furthermore, AP-5 decreases the GLU-stimulated 5-HT_{ext} but does not entirely reverse the increase. This may also reflect the actions of other GLU receptors in the regulation of dialysate 5-HT.

4.5 Glutamate perfusion of the DRN increases 5-HT_{ext} in the MRN

Infusion of GLU into the DRN also produced a significant increase in 5-HT_{ext} in the MRN. This increase was delayed to some extent, reaching a maximum 20 to 40 minutes following the beginning of the infusion of GLU. This also suggests the possibility of 5-HT projections from the DRN to the MRN, though the mechanisms involved in the delay of release are unknown. Alternatively, there is the possibility of a glutamatergic projection from the DRN or the adjacent central gray to the MRN. Thus, infusion of GLU into the DRN may excite glutamatergic neurons projecting to the MRN.

Infusion of GLU into the DRN leads to an increase in 5-HT_{ext} in the MRN that is slower in onset and longer in duration than the direct infusion of GLU into either the DRN or the MRN. This may again demonstrate long-lasting effects of stimulation of the DRN. This may be indicative of differences between the MRN and DRN in terms of SERT and volume transmission [9]. Further work on the long-term sequelae of stimulation of the raphé both in the raphé and in forebrain regions may lead to significant changes in how we view the serotonin system's modulation of the limbic system.

4.6 Infusion of GLU into the lateral midbrain tegmentum

Infusion of 10 mM GLU into the lateral midbrain tegmentum 2 mm lateral to the DRN was used as a control for the possible influence of cells in this area being stimulated by GLU infused into the DRN and increasing 5-HT_{ext} in the MRN. It is clear that infusion of GLU into this lateral tegmentum does not alter 5-HT_{ext} in the MRN thus showing that the influence of GLU is on cells within the DRN. Of considerable interest are the findings that infusion of GLU into this area increases dialysate 5-HT at the site of the probe suggesting either 5-HT cells in this area which is not supported by autoradiographic images of this area or more likely, the existence of serotonergic fibers in this area that are stimulated by GLU receptors. This suggests that fibers arising from the raphé nucleus are excited by glutamate and capable of releasing 5-HT.

4.7 What is the source of 5-HT_{ext} following glutamate stimulation of the raphé?

The source of the rise in extracellular 5-HT in the MRN following perfusion of the DRN with GLU should also be determined in additional studies. If the projection from the DRN to the MRN is serotonergic, then it would be likely that stimulation of this pathway would be

inhibitory on serotonergic neurons of the MRN either by stimulation of 5-HT_{1A} autoreceptors or by stimulation of inhibitory GABA interneurons. If this were the case then the rise in 5-HT_{ext} in the MRN following GLU perfusion of the DRN would be from afferents from the DRN and not from serotonergic cells in the MRN. This would be a different source from the increase in 5-HT_{ext} seen after GLU perfusion of the MRN that is most likely from serotonergic cells within the MRN. This might explain the differences in the time courses of the increases in 5-HT_{ext} in the two experiments. An alternative explanation would be that GLU is stimulating glutamatergic afferents to the MRN and directly exciting 5-HT neurons in this nucleus. This explanation, however, would not account for the differences in the time courses of the increase in 5-HT_{ext} in the two experiments.

4.7 Glutamate perfusion of the MRN does not alter 5-HT_{ext} in the DRN

Finally, infusion of GLU into the MRN does not alter 5-HT_{ext} in the DRN. While previous studies have shown that there are anatomical projections from MRN to DRN these would not appear to be either serotonergic or to regulate the 5-HT_{ext} in the DRN. Another neurotransmitter that is likely to be involved is GABA. Tao and Auerbach [31] have shown that administration of the GABA_A antagonist bicuculline into the DRN increases 5-HT_{ext} within the DRN. Liu et al. [17], however, have shown that the inhibition of 5-HT_{ext} in the dorsal raphe by GABA is by 5-HT excitation of GABA interneurons. Further work is planned to examine the possibility the GABAergic neurons are involved in the interactions between the DRN and MRN.

4.8 Functional significance of dorsal and median raphe interactions

The functional significance of glutamatergic neurotransmission in the MRN has been suggested. Kinney et al. [15] have shown that injections of NMDA or kainate receptor antagonists into the MRN induces theta rhythm in the hippocampal formation. These data are in agreement with other findings that decreases in glutamate in the MRN are associated with theta [32] and that lesions of the MRN also increase theta rhythm in the hippocampus [35]. These data support the current findings and suggest that the glutamatergic influence on MRN serotonergic neurons is excitatory and produce a desynchronization in the hippocampus.

Since the projections arising from the dorsal and median raphe are dissimilar with respect to their anatomical and pharmacological properties, the two serotonergic systems are likely to have different functional roles. The different patterns of innervation by fine and beaded serotonergic axons arising, respectively, from the dorsal and median raphe, strongly exert different functional influences over particular cortical areas. The fine axons from the dorsal raphe are more widespread in most cortical areas whereas beaded axons from median raphe are more restricted to discrete zones and layers of cortex [9;16;37]. Importantly, these median raphe neurons may strongly influence cortical excitability by their direct action on inhibitory neurons [9]. Thus cross talk between the two raphe nuclei would allow for an integrated regulation of 5-HT control of forebrain activity.

4.9 Summary and conclusions

The present data suggest a direct connection from the dorsal raphe nucleus to the median raphe nucleus without reciprocity. Thus, it appears that the DRN directly influences MRN neuronal activity. This has significant consequences in how the serotonergic systems of the midbrain and the forebrain may be coordinated. Stimuli that activate the DRN would also affect the MRN indirectly. Whether the stimulation of the DRN excites MRN serotonergic neurons remains to be determined. These results may explain our previous findings showing an inhibition of 5-HT release in the dorsal hippocampus during and following electrical stimulation of the DRN [19]. Further studies are planned to examine in more detail how the median raphe might influence 5-HT activity in the dorsal raphe. Certainly, an understanding of the interrelations between the two midbrain raphe nuclei will be important in understanding

the mechanisms by which serotonin modulates activity in different forebrain areas and adds to our understanding of limbic midbrain circuitry. In particular, defining the chemical relations between raphé medianus and raphé dorsalis helps in elucidating the regulation of the 5-HT system within the larger interacting limbic forebrain and limbic midbrain. The differences in how these nuclei interact may lead to better therapeutic interventions.

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Reference List

1. Abi-Dargham A. Alterations of serotonin transmission in schizophrenia. *International Review of Neurobiology* Volume 2007;78:133–164.
2. Adell A, Celada P, Abellan MT, Artigas F. Origin and functional role of the extracellular serotonin in the midbrain raphé nuclei. *Brain Res Brain Res Rev* 2002;39:154–180. [PubMed: 12423765]
3. Aghajanian GK, Haigler HJ, Bloom FE. Lysergic acid diethylamide and serotonin: Direct actions on serotonin-containing neurons in rat brain. *Life Sci* 1972;11:615–622.
4. Azmitia, EC. The serotonin-producing neurons of the midbrain median and dorsal raphé nuclei. In: Iverson, LL.; Iverson, SD.; Snyder, SH., editors. *Handbook of Psychopharmacology, Vol. 9 - Chemical Pathways in the Brain*. New York: Plenum Press; 1978. p. 233-314.
5. Azmitia EC, Segal M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphé nuclei in the rat. *J Comp Neurol* 1978;179:641–688. [PubMed: 565370]
6. Behzadi G, Kalen P, Parvopassu F, Wiklund L. Afferents to the median raphé nucleus of the rat: Retrograde cholera toxin and wheat germ conjugated horseradish peroxidase tracing, and selective D-[H3]-aspartate labeling of possible amino acid inputs. *Neuroscience* 1990;37:77–100. [PubMed: 2243599]
7. Bosker FJ, deWinter TYCE, Klompmakers AA, Westenberg HGM. Flesinoxan dose-dependently reduces extracellular 5-hydroxytryptamine (5-HT) in rat median raphé and dorsal hippocampus through activation of 5-HT_{1A} receptors. *J Neurochem* 1996;66:2546–2555. [PubMed: 8632181]
8. Harsing LG Jr, Prauda I, Barkoczy J, Matyus P, Juranyi Z. A 5-HT₇ heteroreceptor-mediated inhibition of [3H]serotonin release in raphé nuclei slices of the rat: evidence for a serotonergic-glutamatergic interaction. *Neurochem Res* 2004;29:1487–1497. [PubMed: 15260125]
9. Hensler JG. Serotonergic modulation of the limbic system. *Neurosci Biobehav Rev* 2006;30:203–214. [PubMed: 16157378]
10. Imai H, Steindler DA, Kitai ST. The organization of divergent axonal projections from the midbrain raphé nuclei in the rat. *J Comp Neurol* 1986;243:363–380. [PubMed: 2419370]
11. Jacobs, BL.; Mosko, SS.; Trulson, ME. The investigation of the role of serotonin in mammalian behavior. In: Drucker-Colin, RR.; McGaugh, JL., editors. *Neurobiology of Sleep and Memory*. New York: Academic Press; 1977. p. 99-133.
12. Jolas T, Aghajanian GK. Opioids suppress spontaneous and NMDA-induced inhibitory postsynaptic currents in the dorsal raphe nucleus of the rat in vitro. *Brain Res* 1997;755:229–245. [PubMed: 9175891]
13. Kalen P, Karlson M, Wiklund L. Possible excitatory amino acid afferents to nucleus raphé dorsalis of the rat investigated with retrograde wheat germ agglutinin and -[3H]aspartate tracing. *Brain Res* 1985;360:285–297. [PubMed: 2866825]
14. Kalen P, Skagerberg G, Lindvall O. Projections from the ventral tegmental area and the mesencephalic raphé to the dorsal raphé nucleus in the rat. *Exper Brain Res* 1988;73:69–77. [PubMed: 3208862]
15. Kinney GG, Kocsis B, Vertes RP. Injections of excitatory amino acid antagonists into the median raphé nucleus produce hippocampal theta rhythm in the urethane-anesthetized rat. *Brain Res* 1994;654:96–104. [PubMed: 7982102]
16. Köhler C, Chan-Palay V, Haglund L, Steinbusch H. Immunohistochemical localization of serotonin nerve terminals in the lateral entorhinal cortex of the rat: demonstration of two separate patterns of innervation from the midbrain raphé. *Anat Embryol (Berl)* 1980;160:121–129. [PubMed: 7006458]

17. Liu R, Jolas T, Aghajanian G. Serotonin 5-HT(2) receptors activate local GABA inhibitory inputs to serotonergic neurons of the dorsal raphe nucleus. *Brain Res* 2000;873:34–45. [PubMed: 10915808]
18. Marcinkiewicz M, Morcos R, Chrétien M. CNS connections with the median raphe nucleus: Retrograde tracing with WGA-apoHRP-Gold complex in the rat. *J Comp Neurol* 1989;289:11–35. [PubMed: 2478595]
19. Mokler DJ, LaRivière D, Johnson DW, Theriault NL, Bronzino JD, Dixon M, Morgane PJ. Serotonin neuronal release from dorsal hippocampus following electrical stimulation of the dorsal and median raphe nuclei in conscious rats. *Hippocampus* 1998;8:262–273. [PubMed: 9662140]
20. Mokler DJ, Torres OI, Galler JR, Morgane PJ. Stress-induced changes in extracellular dopamine and serotonin in the medial prefrontal cortex and dorsal hippocampus of prenatal protein malnourished rats. *Brain Res* 2007;1148:226–233. [PubMed: 17368432]
21. Molliver ME. Serotonergic neuronal systems: What their anatomic organization tells us about function. *J Clin Psychopharmacol* 1987;7:3S–23S. [PubMed: 3323265]
22. Pallotta M, Segieth J, Whitton PS. N-Methyl--aspartate receptors regulate 5-HT release in the raphe nuclei and frontal cortex of freely moving rats: differential role of 5-HT1A autoreceptors. *Brain Res* 1998;783:173–178. [PubMed: 9507110]
23. Parent A, Descarries L, Beaudet A. Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of [3H]5-hydroxytryptamine. *Neuroscience* 1981;6:115–138. [PubMed: 6164006]
24. Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Vol. 5. Amsterdam: Elsevier Academic Press; 2005.
25. Sheehan DV, Sheehan KH. Current approaches to the pharmacologic treatment of anxiety disorders. *Psychopharm Bull* 2007;40:98–109.
26. Shelton RC. The molecular neurobiology of depression. *Psychiatric Clinics of North America* 2007;30:1–11. [PubMed: 17362799]
27. Shigeri Y, Seal RP, Shimamoto K. Molecular pharmacology of glutamate transporters, EAATs and VGLUTs. *Brain Res Brain Res Rev* 2004;45:250–265. [PubMed: 15210307]
28. Steinbusch, HWM.; Nieuwenhuys, R. The raphe nuclei of the rat brainstem: A cytoarchitectonic and immunohistochemical study. In: Emson, PC., editor. *Chemical Neuroanatomy*. New York: Raven Press; 1983. p. 131–208.
29. Tao R, Auerbach SB. Differential effect of NMDA on extracellular serotonin in rat midbrain raphe and forebrain sites. *J Neurochem* 1996;66:1067–1075. [PubMed: 8769868]
30. Tao R, Auerbach SB. Regulation of serotonin release by GABA and excitatory amino acids. *J Psychopharmacol* 2000;14:100–113. [PubMed: 10890306]
31. Tao R, Auerbach SB. Influence of inhibitory and excitatory inputs on serotonin efflux differs in the dorsal and median raphe nuclei. *Brain Res* 2003;961:109–120. [PubMed: 12535783]
32. Varga V, Kekesi A, Juhasz G, Kocsis B. Reduction of the extracellular level of glutamate in the median raphe nucleus associated with hippocampal theta activity in the anaesthetized rat. *Neuroscience* 1998;84:49–57. [PubMed: 9522361]
33. Vertes RP, Fortin WJ, Crane AM. Projections of the median raphe nucleus in the rat. *J Comp Neurol* 1999;407:555–582. [PubMed: 10235645]
34. Vertes RP, Kocsis B. Projections of the dorsal raphe nucleus to the brainstem: PHA-L analysis in the rat. *J Comp Neurol* 1994;340:11–26. [PubMed: 8176000]
35. Vertes RP, Kocsis B. Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus [Review]. *Neuroscience* 1997;81:893–926. [PubMed: 9330355]
36. Vertes RP, Martin GF. Autoradiographic analysis of ascending projections from the pontine and mesencephalic reticular formation and the median raphe nucleus in the rat. *J Comp Neurol* 1988;275:511–541. [PubMed: 3192756]
37. Waterhouse BD, Mihailoff GA, Baack JC, Woodward DJ. Topographical distribution of dorsal and median raphe neurons projecting to motor, sensorimotor, and visual cortical areas in the rat. *J Comp Neurol* 1986;249:460–476. [PubMed: 3528236]
38. Westerink BHC, Enrico P, Feimann J, De Vries JB. The pharmacology of mesocortical dopamine neurons: A dual-probe microdialysis study in the ventral tegmental area and prefrontal cortex of the rat brain. *J Pharmacol Exp Ther* 1998;285:143–154. [PubMed: 9536004]

Figure 1.

Schema representing the differential projection systems of the dorsal and median raphe nuclei. This diagram also shows the differential innervation of the forebrain by the rostral and caudal dorsal raphe nucleus (from [10]). Abbreviations (see [28]): AM – amygdaloid complex, EC – entorhinal cortex, HF – hippocampal formation, HYP - hypothalamus, IO – inferior olive nucleus, LMT – lateral midbrain tegmentum, MS – medial septum, NGC – nucleus gigantocellularis, NTS – nucleus tractus solitarius, PFC – prefrontal cortex, RM – nucleus raphe magnus, RO – nucleus raphe obscurus, RP – nucleus raphe pallidus, RPO – nucleus raphe pontis, ST – striatum.

Figure 2. Photomicrograph of probe placement into the median raphe nucleus (DRN). This shows the continuity of the guide cannula and microdialysis probe track. Also shown is part of the guide cannula track for the DRN (dorsal raphe nucleus) probe.

Figure 3.

Effects of 1 mM glutamate infused into the dorsal raphe nucleus on extracellular 5-HT in the DRN and MRN. Points represent mean $\pm$ s.e.m. ($n = 3$) for 5-HT release expressed as a percent of the individual control periods ($-40, -20, 0$ min). Glutamate was infused by reverse dialysis through the DRN probe for 20min (GLU).

Figure 4.

Effects of 10mM glutamate infused into the DRN on extracellular 5-HT in the DRN (n=5) and MRN (n=5). Points represent mean $\pm$ s.e.m. for 5-HT release expressed as a percent of the individual control dialysate concentrations (-40, -20, 0 min). Glutamate was infused for 20min through the probe (GLU). Note logarithmic scale on vertical axis. Infusion of GLU into the DRN produced a significant increase in 5-HT_{ext} in both brain areas, $p < 0.05$, two-way ANOVA. * = significantly different from time 0 min

Figure 5.

Effects of AP5 and AP5 + GLU infused into the DRN on extracellular 5-HT in the DRN and MRN (n = 3). AP5 (1 mM) was infused for two 20 min periods prior to co-infusion of AP5 + GLU (10 mM). Note logarithmic scale on vertical axis.

Figure 6.

Effects of reverse dialysis of 10mM glutamate into the midbrain tegmentum lateral to the DRN on extracellular 5-HT in the lateral midbrain tegmentum and the MRN. Glutamate was infused for 20 min through the probe (GLU). Note logarithmic scale on vertical axis. Points represent mean $\pm$ s.e.m. (n = 5) for 5-HT release expressed as a percent of the individual control periods (-40, -20, 0 min).

Figure 7.

Effects of 10 mM glutamate infused into the MRN on extracellular 5-HT in the MRN (n = 6) and DRN (n = 6). Bars represent mean $\pm$ s.e.m. for 5-HT release expressed as a percent of the individual control concentrations (-40, -20, 0 min). Glutamate was infused for 20min through the probe (GLU). Note logarithmic scale on vertical axis.

Figure 8.

Schema of the interactions of the dorsal (DRN) and median raphe nuclei (MRN). See text for discussion. LMT – lateral midbrain tegmentum, MFB – medial forebrain bundle, PFC – prefrontal cortex, RD – dorsal raphe nucleus, RM – median raphe nucleus