

Collateral projections from the median raphe nucleus to the medial septum and hippocampus

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ABSTRACT: It has previously been shown that the median raphe nucleus (MR) is a source of pronounced projections to the septum and hippocampus. The present study examined collateral projections from MR to the medial septum (MS) and to various regions of the hippocampus. The fluorescent retrograde tracers, Fluororuby and Fluorogold, were injected into the septum and hippocampus, respectively, and the median raphe nucleus was examined for the presence of single- and double-labeled neurons. The dorsal raphe nucleus (DR) was also examined for the presence of single- and double-labeled cells and comparisons were made with the MR. The main findings were: (1) pronounced numbers of retrogradely labeled cells (approximately 50 cells/section) were present in MR with injections in the MS or in various regions of the hippocampus; (2) approximately 8–12% of MR cells were double-labeled following paired injections in the MS-CA1, MS-CA3, and MS-dentate gyrus of the dorsal hippocampus, the lateral MS-dentate gyrus, and the MS-ventral hippocampus; (3) single- and double-labeled cells were intermingled throughout MR and present in greater numbers in the rostral than caudal MR; and (4) significantly more single- and double-labeled cells were present in MR than in DR with all combinations of injections. These findings demonstrate that MR projects strongly to the MS and hippocampus, and that a significant population of MR neurons (8–12%) sends collateral projections to both sites. It is well established that the MR nucleus serves a direct role in the desynchronization of the electroencephalographic (EEG) activity of the hippocampus—or the blockade of the hippocampal theta rhythm. The MR neurons that we have identified with collateral projections to the septum and hippocampus may be critically involved in the modulation/control of the hippocampal EEG. A role for the MR in memory associated functions of the hippocampus is discussed. © 2001 Elsevier Science Inc.

KEY WORDS: Dentate gyrus, Brainstem, Serotonin, Memory, Locomotion.

INTRODUCTION

The median raphe nucleus (MR) is a major serotonergic cell group of the brainstem [14,18,48,57], and is a source of pronounced projections to the septum and hippocampus [5,7,26,36,38,55,59,66].

In a recent examination of MR projections in the rat using PHA-L [59], we showed that MR fibers distributing to the septum

terminate selectively within the medial septum-vertical limb of the diagonal band nucleus (MS/DBv) and lateral aspects of the lateral septum, while those to the hippocampal formation (HF) predominantly distribute to stratum lacunosum-molecular of Ammon's horn, and to the granule cell layer and immediately adjacent inner molecular layer of the dentate gyrus (DG).

An extensive body of evidence indicates that the MR is directly involved in the modulation/control of the hippocampal electroencephalogram (EEG), specifically states of hippocampal desynchronization. It has been shown that: (1) MR stimulation desynchronizes the hippocampal EEG [4,23,31,51]; (2) MR lesions generate continuously ongoing theta activity [33,67]; and (3) injections of various pharmacological agents into MR that either inhibit the activity of MR cells [20,23,56,61] or reduce excitatory drive to them [19,50] produce theta at short latencies and for long durations. Based on their findings [23,61] that the activation or suppression of MR desynchronizes or synchronizes the hippocampal EEG, respectively, Vinogradova and colleagues [23] concluded that: “the median raphe nucleus can be regarded as a functional antagonist of the reticular formation, powerfully suppressing theta bursts of the medial septal area neurons and the hippocampal theta rhythm”.

The desynchronizing actions of the MR on the hippocampal EEG appear in part to be mediated by the MS/DBv. Assaf and Miller [4] demonstrated that MR stimulation both disrupted the rhythmical discharge of the septal pacemaking cells and desynchronized the hippocampal EEG, while Kinney et al. [21] showed that injections of the 5-HT_{1A} agonist, 8-OH-DPAT, into MR activated septal pacemaking cells and generated theta. In addition, it has been shown that 5-HT MR fibers selectively contact and form asymmetric (excitatory) [49] connections with GABAergic cells of the MS/DBv [28], and that 5-HT excites putative GABAergic cells of the MS/DBv which, in turn, inhibit subsets of theta pacemaker cholinergic/GABAergic neurons of the MS/DBv [2,27,29,30]. These findings suggest a 5-HT MR activation of GABAergic MS/DBv neurons and a subsequent suppression of septal GABAergic/cholinergic pacemaker cells in the desynchronization of the hippocampal EEG.

Although it appears that the effects of the MR on the hippocampal EEG are primarily routed through the MS/DBv, the MR may exert direct actions on the hippocampus, or possibly even dual actions on the septum and hippocampus, in the desynchronization

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of the hippocampal EEG. MR projects strongly to the hippocampus [36,38,55,59,66] and MR fibers innervating HF, like those to the septum, predominantly contact GABAergic interneurons [11, 13,17], which, in turn, suppress principal cells [10] of the hippocampus. Vinogradova et al. [61] recently demonstrated that microinjections of lidocaine in MR increased the regularity and frequency of discharge of both septal and hippocampal neurons as well as increased the percentage of them showing theta rhythmicity and generated persistent theta.

To further explore the role of MR in the modulation/control of the hippocampal EEG, we examined possible collateral projections from MR to the septum and hippocampus. Specifically, we examined numbers, percentages, and locations of: (1) MR cells projecting to either the MS/DBv or to the hippocampus (single-labeled neurons); (2) MR cells with collateral projections to the MS and to various regions (CA1, CA3, DG) of the dorsal hippocampus; (3) MR cells with collateral projections to MS and DG-CA1 area of the ventral hippocampus; and (4) MR cells with collateral projections to the lateral aspect of the medial septum (LMS) and DG. In addition, we examined the dorsal raphe nucleus for the presence of single- and double-labeled cells following the same series of injections both for comparisons with MR and to determine possible collateral dorsal raphe (DR) projections to the septum and HF involved in DR-associated functions.

A principal finding was that approximately 8–12% of MR cells project, via collaterals, to MS/DBv and the hippocampus. These MR cells may exert dual actions on the MS/DBv and HF, possibly involved in the desynchronization of the hippocampal EEG. A preliminary report has been published previously [34].

MATERIAL AND METHODS

Forty-six male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) weighing 250–400 grams were injected with combinations of the fluorescent retrograde tracers, Fluororuby [42] (TMR-DA, 3,000 MW; Molecular Probes, Eugene, OR, USA) and Fluorogold [41] (Fluorochrome, Denver, CO, USA) into the septum and hippocampus, respectively. These experiments were approved by the Florida Atlantic University Institutional Animal Care and Use Committee and conform to all federal regulations and National Institutes of Health guidelines for the care and use of laboratory animals.

Fluorogold (FG) was dissolved in 0.9% normal saline to yield a concentration of 4.0%; Fluororuby (FR) was dissolved in phosphate buffered saline (PBS) to yield a concentration of 10.0%. In sodium pentobarbital anesthetized rats, FG (0.03 μ l) was injected via a 1- μ l Hamilton syringe into four regions of the hippocampal formation in separate groups of rats: the CA1 and CA3 regions of Ammon's horn of the dorsal hippocampus; the DG of the dorsal hippocampus; and DG/CA1 of the ventral hippocampus (vHF). The needle was left in place for approximately 15 min following injections of FG (and FR) to prevent transport of the fluorescent tracers up the needle tract. Three days following FG injections, FR (0.20 μ l) was injected into medial or lateral regions of the MS/DBv.

Seven days after FG injections (4 days after FR injections), rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with a buffered saline wash (pH 7.4, 300 ml/rat) followed by a fixative containing 4% paraformaldehyde in 0.05 M phosphate buffer (500 ml/rat). The brains were removed and stored overnight at 4°C in 20% sucrose in the same phosphate buffer. On the following day the brains were cut at 50 μ m on a freezing

microtome, and every sixth section was mounted from PBS onto chrome-alum gelatin-coated slides, and cover slipped using DPX. Slides were dried overnight in the dark at 4°C and subsequently viewed with a Zeiss fluorescent microscope using appropriate filters for FG (excitation, 350–395 nm; emission, 530–600 nm) and FR (excitation, 540–560 nm; emission, 580 nm). An adjacent series of sections from each rat was stained with cresyl violet for anatomical reference.

Single-labeled neurons were identified by the presence of FG or FR in cells; both tracers generally filled the soma as well as the proximal dendrites of cells. Double-labeled neurons were identified by the presence of both fluorescent tracers in cells as determined by viewing the cells with both filter sets (FG and FR); i.e., switching between sets of filters. Labeled cells were plotted on a representative series of schematic transverse sections throughout the pons/midbrain [47]. Material judged particularly useful for emphasizing or clarifying points of text was illustrated with photomicrographs. The contrast and sharpness of the retrogradely labeled FG cells of Fig. 4A were enhanced using Adobe Photoshop.

RESULTS

Injections of Fluorescent Retrograde Tracers in MS and CA1 of the Dorsal Hippocampus

Injections of fluorescent retrograde tracers in the MS and CA1 were made in 10 rats. The FR injections were confined to the medial MS, while FG injections were positioned along the medio-lateral axis of CA1 of the dorsal hippocampus.

Figure 1 schematically depicts the pattern of distribution of single- and double-labeled cells in MR and DR for one rat (case 58) following injections in MS and CA1. As shown, single- and double-labeled cells were: (1) intermingled in MR; (2) extended dorsoventrally throughout MR; and (3) were predominantly localized to medial regions of MR. Interestingly, there were greater numbers of FG (CA1) than FR (MS)-labeled cells at rostral (Fig. 1A) and caudal (Fig. 1C) levels of MR, but considerably more FR than FG-labeled cells at the intermediate MR (Fig. 1B). The majority of double-labeled cells were located in the rostral one half of MR.

Figure 2 depicts a double-labeled cell at the rostral MR at two levels of magnification for case 58. As shown, the morphological characteristics of this MR cell, including proximal processes, are identical when retrogradely filled with FG (Figs. 2A,C) or FR (Figs. 2B,D).

Table 1 summarizes the findings for ten cases with injections in MS and CA1. Values represent averages over three rostral-caudal levels of MR/DR. The mean number of single-labeled cells/section in MR was 50.6 ± 17.26 with MS (FR) injections and 52.6 ± 14.54 with CA1 (FG) injections. The mean number of single-labeled cells/section in DR was 13.33 ± 7.86 with MS injections and 20.37 ± 14.6 with CA1 injections. The number of double-labeled cells/section in MR ranged from 4 to 22, with a mean value of 10.87 ± 4.67 ; the number of double-labeled cells in DR ranged from 0 to 11, with a mean value of 2.7 ± 2.85 . The percentage of double-labeled cells in MR was 9.5%, and in DR 7.4%. Double-labeled cells in DR were mainly located ventrally, bordering the isthmus region between DR and MR.

Considerably more labeled neurons were observed in DR with CA1 injections compared to septal injections for case 58 (32.0 vs. 7.67 cells/section), which reflects similar differences across all cases with CA1/MS injections (20.37 ± 14.6 vs. 13.33 ± 7.86 cells/section) (Table 1). In addition, significantly greater numbers of labeled neurons were present in

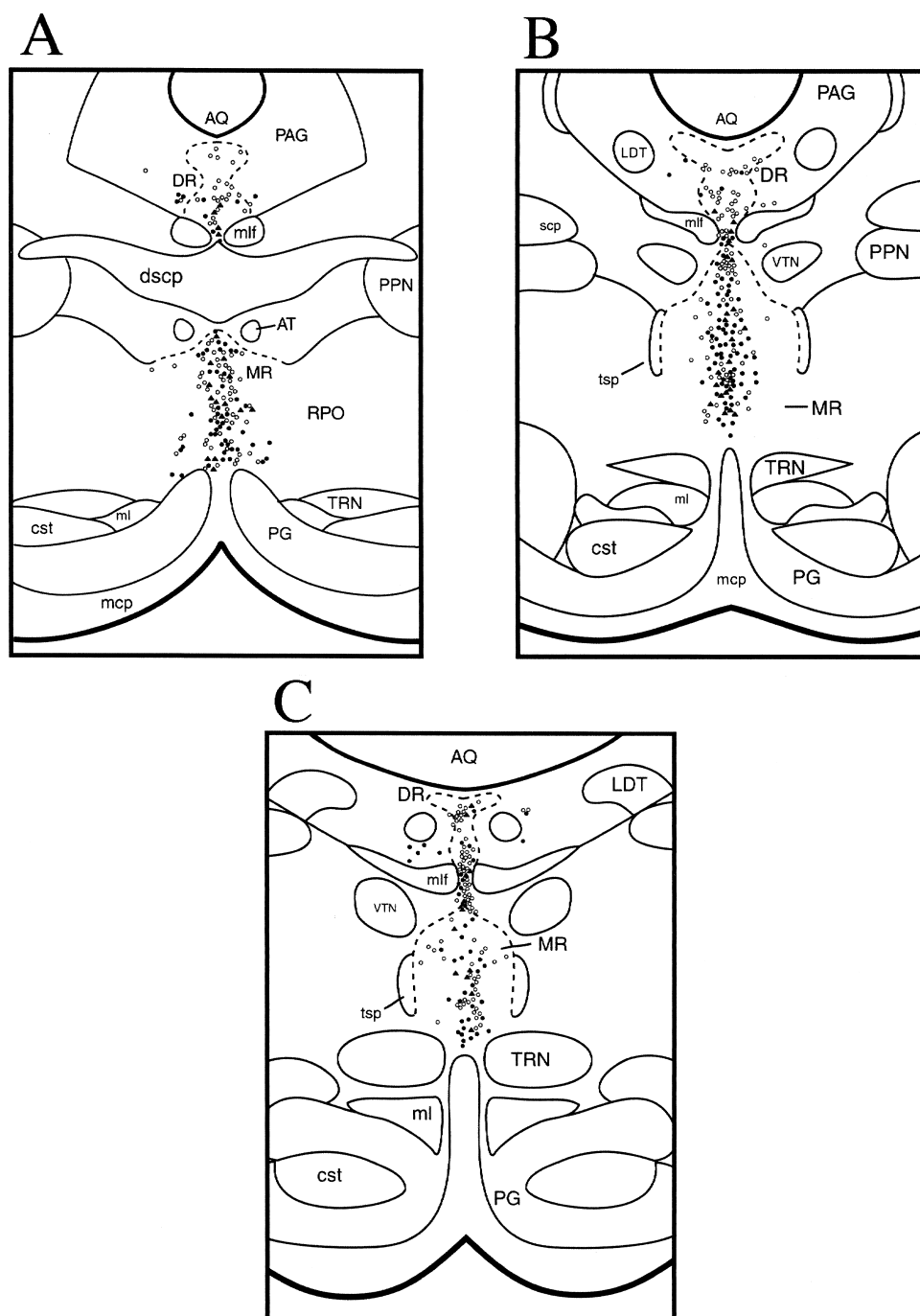


FIG. 1. Series of rostrocaudally aligned (A–C) representative schematic transverse sections through the pons/midbrain depicting the locations of single- and double retrogradely labeled cells in the median and dorsal raphe nuclei for case 58 with medial septum (MS)/CA1 injections. Closed circles represent single-labeled cells with Fluororuby (FR) injections in MS; open circles represent single-labeled cells with Fluorogold (FG) injections in the CA1 area of the hippocampus; and triangles represent double-labeled cells. Note that double-labeled cells are intermingled with single-labeled cells. Abbreviations: AQ, cerebral aqueduct; AT, anterior tegmental nucleus; cst, corticospinal tract; dscp, decussation of the superior cerebellar peduncle; DR, dorsal raphe nucleus; LDT, laterodorsal tegmental nucleus; mcp, middle cerebellar peduncle; ml, medial lemniscus; mlf, medial longitudinal fasciculus; MR, median raphe nucleus; PAG, periaqueductal gray; PG, pontine gray; PPN, pedunculopontine tegmental nucleus; RPO, nucleus reticularis pontis oralis; scp, superior cerebellar peduncle; TRN, tegmental reticular nucleus; tsp, tectospinal tract; VTN, ventral tegmental nucleus of Gudden.

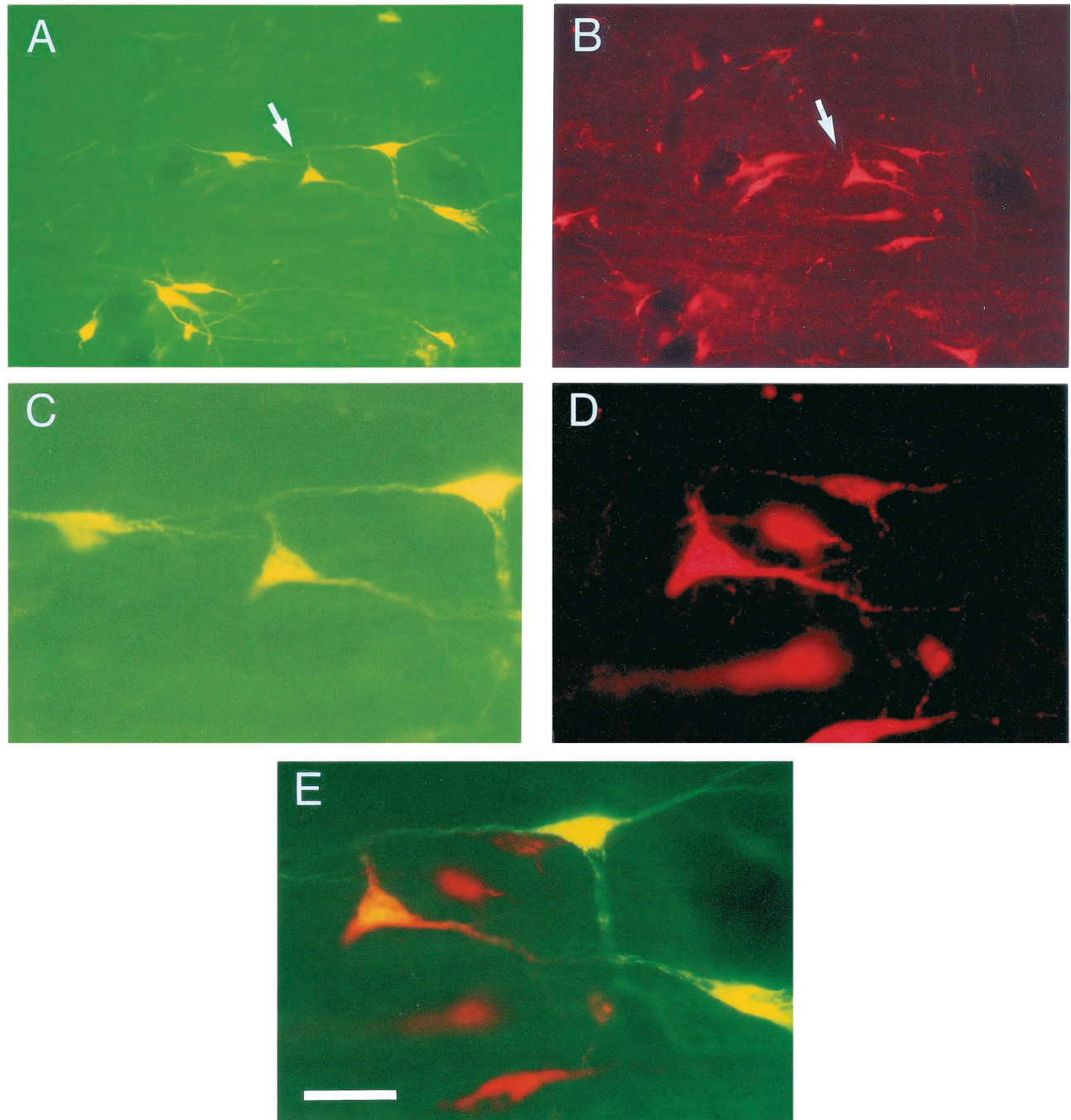


FIG. 2. Low (A,B) and high power (C–E) photomicrographs for case 58 (medial septum [MS]/CA1 injections) depicting a double-labeled cell [arrows in (A,B) and same cell at higher magnification in (C–E)] in the rostral median raphe nucleus (MR). As shown, the morphological characteristics of the double-labeled cell in MR were virtually identical when retrogradely filled with either Fluorogold (FG) (A,C) or Fluororuby (FR) (B,D). (E) Double exposure photomicrograph depicting the combined presence of yellow (FG) and red (FR) fluorescent tags in the double-labeled cell giving it an orange appearance. Note also single FG (CA1) and FR (MS)-labeled cells lying in close proximity to the double-labeled MR neuron. Scale bar for (A,B): 100 μ m; for (C,D,E): 35 μ m.

DR with CA1 injections in either the dorsal (20.37 ± 14.6 cells/section) or ventral (26.53 ± 21.85 cells/section) hippocampus than with DG injections (5.69 ± 5.44 cells/section for MS/DG cases and 5.21 ± 4.47 cells/sections for LMS/DG cases). The foregoing indicates stronger DR projections to CA1 than to either the MS or to DG.

Injections of Fluorescent Retrograde Tracers in Medial MS and the Dentate Gyrus of the Dorsal Hippocampus

Injections of fluorescent retrograde tracers in the medial MS (FR) and DG (FG) were made in 13 rats. Figure 3 schematically depicts the pattern of distribution of single- and double-labeled

TABLE 1

NUMBERS AND RELATIVE PERCENTAGES OF SINGLE AND DOUBLE FLUORESCENTLY-LABELED NEURONS IN THE MEDIAN AND DORSAL RAPHE NUCLEI FOLLOWING INJECTIONS OF FLUORORUBY (FR) IN THE MEDIAL SEPTUM AND FLUOROGOLD (FG) IN THE HIPPOCAMPAL FORMATION

RAT	MEDIAN RAPHE			DORSAL RAPHE		
	FG	FR	DL	FG	FR	DL
MS/CA1						
3	57.67	62.67	15.67	33.33	24	8
9	51	57.33	12.33	18.67	17.67	3.33
13	46.33	48	10	13.67	14.33	2.33
19	48.33	39.33	10	21.67	14.33	0.67
33	56.33	41.67	6	11.33	8.67	1.33
43	54.67	59.67	13.33	18	13.33	3.67
46	63.33	61.67	12.33	31.67	16.67	4.33
52	57.67	54.67	10.67	17.33	12	1
55	44.33	36.33	5	6	4.67	0.33
58	46.33	44.67	13.33	32	7.67	2
Mean/SD	52.60 $\pm$ 14.54	50.60 $\pm$ 17.26	10.87 $\pm$ 4.67	20.37 $\pm$ 14.60	13.33 $\pm$ 7.86	2.70 $\pm$ 2.85
%	46.1	44.4	9.5	56	36.6	7.4
MS/DG						
2	57	67.67	13	2	9	0
4	54.33	59.33	17.33	13	18.67	3.67
7	50.33	55.67	11.67	1.33	5	0
8	56	77.33	16.33	2	14	0.33
15	48.33	42.67	10	3	6.67	0.33
30	54.33	48.33	11	9.33	14.33	1
32	50.33	31	8	2.33	6.67	0
34	45	32	6.33	4	8	0.67
37	49	40.67	11	9.33	9.33	1.33
39	55.33	39.67	12.33	6	8.33	0.67
41	59	44.67	12.33	4	9.33	0.67
51	58	60.33	13	10.33	14.33	3.67
120	45.67	45	14.67	7.33	13	2.67
Mean/SD	52.51 $\pm$ 15.50	49.56 $\pm$ 18.76	12.08 $\pm$ 5.23	5.69 $\pm$ 5.44	10.38 $\pm$ 6.64	1.15 $\pm$ 1.61
%	46	43.4	10.6	33	60.3	6.7
LMS/DG						
16	51.33	28.67	8.33	9.33	10.33	3.67
31	37	35.17	7	7.67	13.33	0.33
36	55	42.33	10.33	3.67	5.33	0
40	57.33	45	11.33	3	8	0
49	52.67	51	7	5.67	11.67	1
50	38.33	54.67	7	6.67	12	0
53	46.67	33.33	8	5	7.67	0
59	39.33	31.67	10	3	10	0.33
65	58.67	43	10.67	3	9.33	0.67
70	53	33.33	9.33	2.33	8.67	0
71	48.33	40	7.33	6.67	11.67	1
83	59.67	44	8.33	8	13	0.33
126	47.33	54.67	10.33	3.67	4.67	0
Mean/SD	49.59 $\pm$ 17.05	41.29 $\pm$ 13.07	8.85 $\pm$ 3.56	5.21 $\pm$ 4.47	9.67 $\pm$ 4.52	0.56 $\pm$ 1.10
%	49.2	42	8.8	33.7	62.6	3.7
MS/CA3						
6	74	43.67	8.33	8.67	15.33	0.33
10	66.33	59.33	14.67	6.33	21	2.33
23	53.67	58	11	7	23	2.33
82	70.33	73.33	11.67	9.67	15	1
122	53.33	54.67	10.33	14.67	11.67	1
Mean/SD	63.53 $\pm$ 20.09	57.80 $\pm$ 17.74	11.2 $\pm$ 4.00	9.27 $\pm$ 8.70	17.20 $\pm$ 9.78	1.4 $\pm$ 1.68
%	47.9	43.6	8.5	33.3	61.7	5
MS/vHF						
24	51.33	50.33	9.33	29.67	14	3
25	67.33	49	9.67	17	17.33	3.33
28	58.67	50.67	13.67	21	13.33	1.33
29	67.67	52.67	12	57.33	28.33	6.33
88	39.67	49.33	5.67	7.67	9.33	0.33
Mean/SD	56.93 $\pm$ 18.31	50.4 $\pm$ 7.94	10.07 $\pm$ 3.61	26.53 $\pm$ 21.85	16.47 $\pm$ 11.13	2.87 $\pm$ 3.09
%	48.5	42.9	8.6	57.8	35.9	6.3

Combinations of injections: MS/CA1, the medial septum and CA1 region of Ammon's horn; MS/DG, medial part of MS and dentate gyrus of the dorsal hippocampus; LMS/DG, lateral part of MS and DG; MS/CA3, MS and CA3 region of Ammon's horn; MS/vHF, MS and ventral (temporal) hippocampal formation. DL, double-labeled neurons.

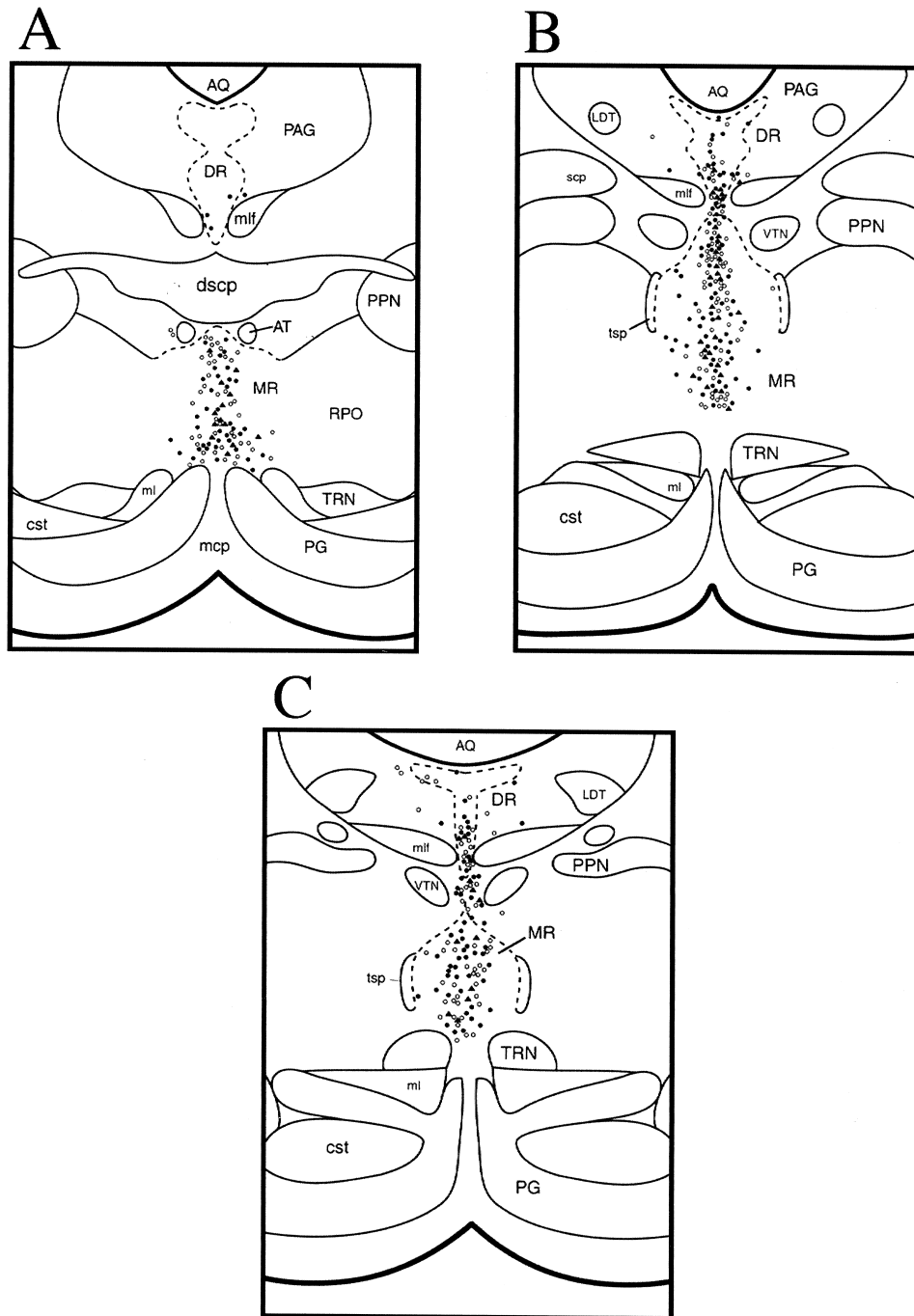


FIG. 3. Series of rostrocaudally aligned (A–C) representative schematic transverse sections through the pons/midbrain depicting the locations of single- and double retrogradely labeled cells in the median and dorsal raphe nuclei for case 120 with medial septum (MS)/dentate gyrus (DG) injections. Closed circles represent single-labeled cells with Fluororuby (FR) injections in MS; open circles represent single-labeled cells with Fluorogold (FG) injections in DG; and triangles represent double-labeled cells. Note that double-labeled cells are intermingled with single-labeled cells. See Fig. 1 for list of abbreviations.

cells in MR and DR for one rat (case 120) with injections in MS and DG. As shown, pronounced numbers of single- and double-labeled cells were present throughout the rostrocaudal extent of MR. There were approximately equal numbers of (single) labeled cells in MR with MS and DG injections. Double-labeled cells

(triangles) were interspersed with single-labeled cells. In general, double-labeled cells were concentrated along the midline in MR, and there was a progressive decline in numbers of double-labeled cells from the rostral to caudal MR.

There was a marked overlap in the distribution of FR- and

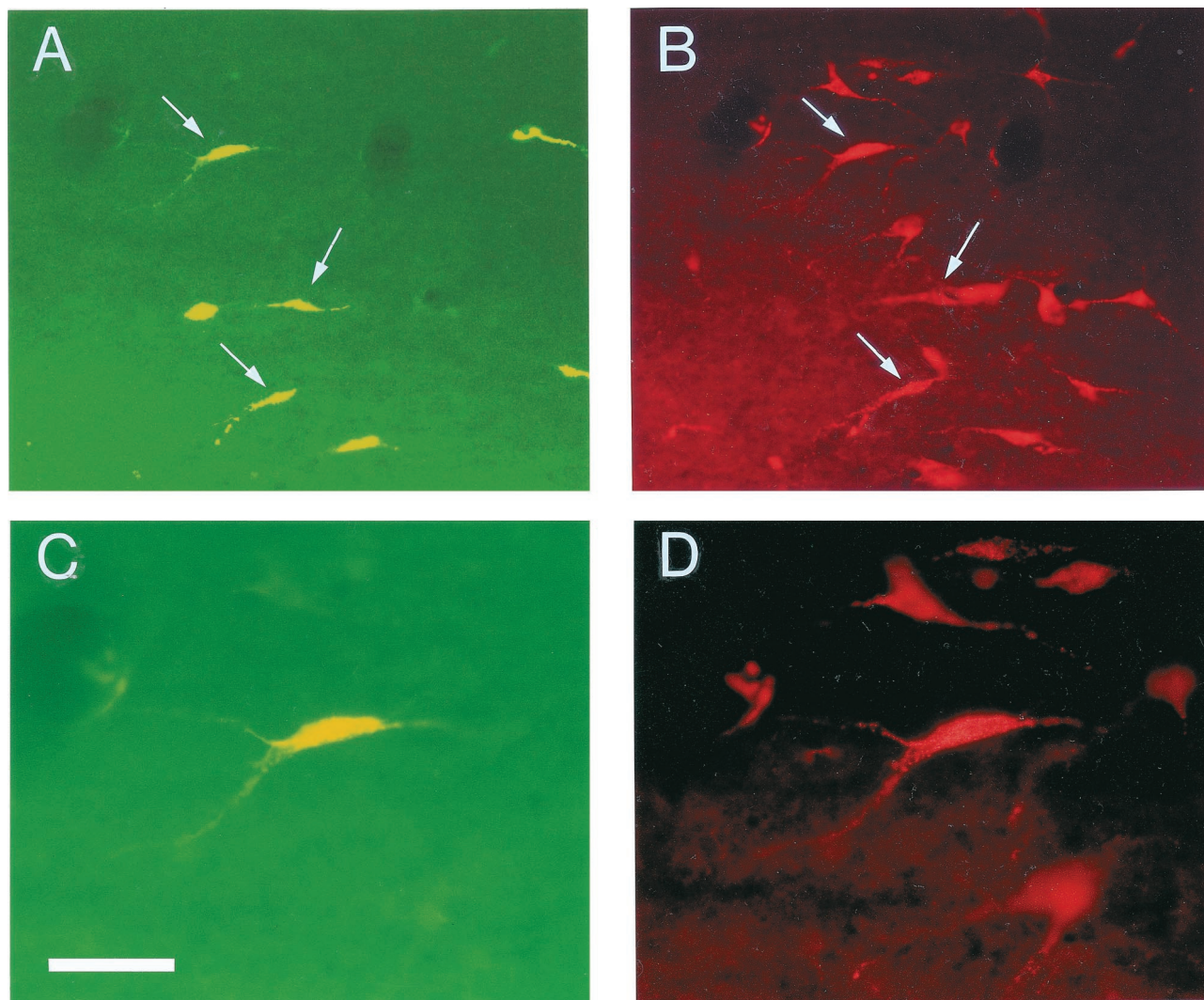


FIG. 4. High power photomicrographs depicting three double-labeled cells in the median raphe nucleus (MR) [arrows in (A,B)] for case 120 (medial septum/dentate gyrus). (C,D) Higher magnification photomicrographs exemplifying the top most double-labeled cell of (A,B). As shown, the cell was virtually identical, morphologically, when retrogradely filled with either Fluorogold (C) or Fluororuby (D). Scale bar for (A,B): 100 μ m; for (C,D): 30 μ m.

FG-labeled neurons in MR, indicating an intermingling of MR cells projecting to MS and hippocampus.

Figure 4 depicts double-labeled cells at the intermediate MR for case 120 at two levels of magnification. As shown, the cells are morphologically identical when retrogradely filled with either FG (Figs. 4A,C) or FR (Figs. 4B,D).

There were significantly fewer single- and double-labeled cells in DR than in MR at all three levels of the brainstem. At the rostral pons (Fig. 3A) a few single FR-labeled cells (MS injections) were present in DR, but no FG-labeled cells (DG injections). At the intermediate and caudal pons (Figs. 3B,C) FR- and FG-labeled cells were intermingled in DR. There were exceedingly few double-labeled cells in the central core of DR (1–2/section). Moderate numbers of double-labeled neurons were present in the isthmus region between DR and MR at the intermediate and caudal pons.

Table 1 summarizes the findings for the 13 cases with injections in MS and DG. The mean number of single-labeled cells/section in MR were 49.56 ± 18.76 with MS (FR) injections and

52.51 ± 15.5 with DG (FG) injections. The mean number of single-labeled cells in DR were 10.38 ± 6.64 with MS injections and 5.69 ± 5.44 with DG injections. The number of double-labeled cells/section in MR ranged from 4 to 27, with a mean value of 12.08 ± 5.23 , and the number of double-labeled cells/section in DR ranged from 0 to 6, with a mean value of 1.15 ± 1.61 . The percentage of double-labeled cells in MR was 10.6%, and in DR 6.7%.

Although not shown in Table 1, more labeled cells were present in rostral than caudal parts of MR with both MS and DG injections: 53.58 vs. 41.54 cells/section in the rostral 2/3 vs. caudal 1/3 of MR with MS injections, and 58.03 vs. 41.46 cells/section in the rostral 2/3 vs. caudal 1/3 of MR with DG injections. By contrast, there were more labeled neurons in the caudal than rostral DR following MS/DG injections: 5.61 vs. 12.77 cells/section for the rostral 1/3 vs. caudal 2/3 of DR with MS injections and 2.38 vs. 7.35 cells/section for the rostral 1/3 vs. caudal 2/3 of DR with DG injections. These findings demonstrate a predominantly rostral

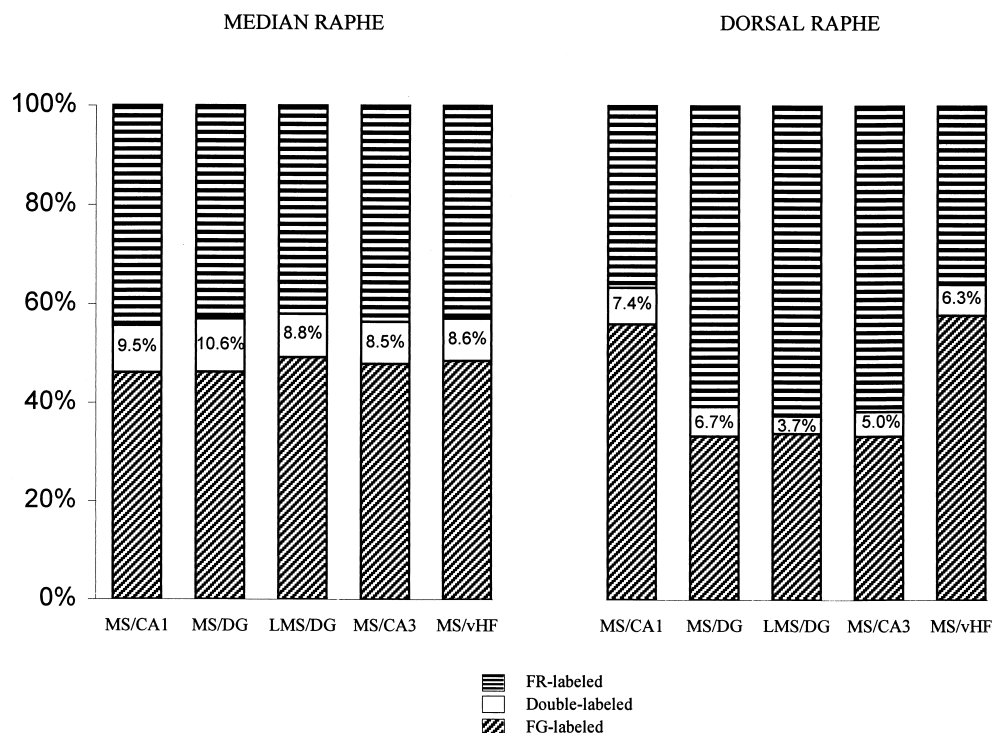


FIG. 5. Summary diagram depicting percentages of single- and double-labeled cells in the median raphe nucleus (MR) and dorsal raphe nucleus (DR) with five combinations of injections: medial part of the medial septum (MS) and the CA1 region of the dorsal hippocampus (MS/CA1); medial part of MS and the dentate gyrus of the dorsal hippocampus (MS/DG); lateral part of MS and DG (LMS/DG); MS and the CA3 region of the dorsal hippocampus (MS/CA3); and MS and the ventral hippocampus (MS/vHF). Fluororuby (FR) cells were labeled following injections in the septum, and Fluorogold (FG) cells were labeled following injections in the hippocampal formation.

origin of MR projections to the MS and DG, and a largely caudal origin of DR projections to these sites.

Injections of Fluorescent Retrograde Tracers in the LMS and the DG of the Dorsal Hippocampus

The MS is organized into overlapping, largely segregated layers which from inner to outer layers consist of GABAergic, cholinergic (ACh), and calretinin-containing (CR) cells, respectively [22]. The previously discussed injections were made in the medial MS (centered in the GABAergic cell field), while those below were located about 0.3–0.5 mm off the midline in the lateral MS (centered in the ACh/CR cell fields). Lateral MS and DG injections were made on the same side of the brain.

Table 1 summarizes the findings of 13 cases with injections into the LMS and DG. Overall, the pattern of distribution of single- and double-labeled cells in MR and DR with LMS/DG injections was similar to that seen with medial MS/DG injections. As depicted in Table 1, the mean number of single-labeled cells/section in MR was 41.29 ± 13.07 with LMS injections and 49.59 ± 17.05 with DG injections. The mean number of single-labeled cells/section in DR was 9.67 ± 4.52 with LMS injections and 5.21 ± 4.47 with DG injections. The number of double-labeled cells/section in MR ranged from 3 to 19, with a mean value of 8.85 ± 3.56 , and the number of double-labeled cells/section in DR ranged from 0 to 4 with a mean value of 0.56 ± 1.1 . The percentage of double-labeled cells in MR was 8.8% and in DR was 3.7%.

Despite strong similarities in patterns of labeling with LMS and

medial MS injections, there were notable differences. For instance, there were more single-labeled cells in MR with medial than lateral MS injections (49.6 vs. 41.3 cells/section) as well as greater numbers and percentages of double-labeled cells in MR/DR with MS compared to LMS injections (Table 1). These results demonstrate stronger MR projections to medial than to lateral parts of MS.

Figure 5 summarizes percentages of single- and double-labeled cells for each of the five combinations of injections, including MS/CA3, and MS and ventral hippocampal (MS/vHF) injections. As depicted (Fig. 5), injections in the CA1 and CA3 regions of the dorsal hippocampus (MS/CA1, MS/CA3) produced comparable percentages of single- and double-labeled cells in MR. Equivalent percentages were also seen in MR with dorsal (MS/CA1, MS/CA3, MS/DG) and ventral (MS/vHF) hippocampal injections.

DISCUSSION

The main findings of the present report were as follows: (1) pronounced numbers of retrogradely labeled cells (approximately 50 cells/section) were present in MR with injections in the MS or in various regions of the hippocampus; (2) approximately 8–12% of MR cells were double-labeled following paired injections in the MS-CA1, MS-CA3, MS-DG of the dorsal hippocampus, the lateral MS-DG, and the MS-ventral hippocampus; (3) single- and double-labeled cells were intermingled throughout MR and present in greater numbers in rostral vs. caudal MR; and (4) significantly more single- and double-labeled cells were present in MR than in

DR with all combinations of injections. These findings demonstrate that MR projects strongly to the MS and hippocampus, and that a significant population of MR neurons (8–12%) sends collateral projections to both sites.

Methodological Considerations

The possibility exists that the double-labeled cells in MR resulted from the retrograde transport of FR from damaged and/or intact fibers passing through MS to the hippocampus rather than fibers terminating in MS; i.e., the fiber of passage problem. We discount this possibility for the following reasons: (1) FR does not appear to be taken up by intact fibers of passage [42]; (2) the medial and lateral MS injections of the present study were made rostral to the fimbria/fornix; that is, the route taken by the bulk of MR/DR fibers coursing through the septum to the hippocampus; and (3) unlike MR, exceedingly few double-labeled cells (mean = 1–2 cells/section) were present in DR. It would seem if FR were significantly taken up by fibers of passage substantially more double-labeled cells would have been seen in DR.

MR Projections to the Septum: Single-labeled Neurons

We demonstrated that MR projects extensively to the lateral and medial aspects of MS. On average, 40–50 labeled cells/section were identified in MR following FR injections into the medial or lateral MS. Labeled cells were concentrated in the rostral two thirds of MR.

In accord with present findings, several previous reports using retrograde [26,53] or anterograde [5,7,36,55,59] techniques have shown that MR distributes strongly to the septum. In a recent PHA-L analysis in rats [59], we showed that MR fibers projecting to the septum primarily originate from the rostral pole of MR, and terminate in the MS and the lateral part of the lateral septum (LS), but avoid the intermediate LS. By contrast, DR fibers project heavily to the intermediate LS but sparsely to MS and the lateral LS [54]. Morin and Meyer-Bernstein [36] described virtually identical findings for the hamster, showing pronounced terminal labeling in MS and lateral LS with MR, but not with DR, injections of PHA-L; and dense labeling in the intermediate LS with DR, but not with MR, injections.

We identified considerably more labeled cells in MR with medial than lateral MS injections. As discussed, the medial MS mainly contains GABAergic neurons; the lateral MS, cholinergic and CR-containing cells [22]. Taken together, this suggests that MR may more strongly innervate GABAergic than ACh/CR elements of the MS. In line with this, we recently showed that serotonergic MR fibers terminate exclusively on GABAergic cells of MS [28].

MR Projections to the Hippocampus: Single-labeled Neurons

We demonstrated that MR projects densely throughout the hippocampus. Approximately 50–60 labeled cells/section were identified in MR following injections in the dorsal (CA1, CA3, and DG) or ventral hippocampus. Similar to the septum, considerably more labeled neurons were observed in the rostral two thirds (60.16 cells/section) than in caudal one third (39.83 cells/section) of MR with injections in various regions of the hippocampus.

Supporting present results, several previous reports have shown that MR strongly targets the hippocampus [5,7,35,36,38,55,59,66]. Using PHA-L, recent studies have demonstrated that MR fibers distribute throughout the hippocampus, and terminate densely in the stratum lacunosum-moleculare of Ammon's horn and the granule cell layer and overlying molecular layer of DG [36,59]. MR projections to the hippocampus were shown to primarily originate from the rostral pole of MR [59].

It has been shown that serotonin-containing neurons of MR selectively contact (and putatively excite) GABAergic cells of both the septum [28,29] and hippocampus [11,13,17]. These findings, together with the demonstration that GABAergic cells of the septum and hippocampus inhibit projection cells of respective structures [10,30], suggest that 5-HT MR neurons exert a net suppressive effect on the output of the septum/hippocampus [28,29].

DR Projections to the Septum and Hippocampus: Comparison with MR Projections

We observed significantly fewer labeled cells in DR than in MR with injections in the septum or in the hippocampus; that is, 3–4-fold fewer cells in DR than in MR with MS injections, and 2–10-fold fewer cells in DR than in MR with HF injections.

In accord with our findings, several early reports largely using retrograde techniques demonstrated stronger MR than DR projections to the septum and hippocampus [5,6,25,26,35,53]. For instance, Kohler and associates identified significantly more labeled cells in MR than in DR following HRP injections in the MS [26] or hippocampus [25]. In like manner, recent reports in the rat and hamster using PHA-L [36,54,59] have described stronger MR than DR projections to both MS and HF and further showed, like here, that DR projections to HF almost exclusively originate from the caudal DR.

Similar to the case with single-labeled cells, significantly fewer double-labeled cells were identified in DR than in MR following all combinations of injections (i.e., approximately 1–2 cells/section in DR compared to 10–12 cells/section in MR across the various injections). Double-labeled cells in DR were mainly located in the caudal two thirds of DR. The low numbers of double-labeled neurons in DR would indicate that the DR largely exerts independent, as opposed to dual, actions on the MS and HF.

To our knowledge only a single previous report has examined collateral MR and DR projections to the septum and hippocampus [1]. In general accord with present findings, Acsady et al. [1] reported that injections of retrograde tracers in the septum and hippocampus of two rats gave rise to the following percentages of double-labeled cells: 12% and 14% in MR and DR for one rat and 28% and 30% in MR and DR for the other rat. Although their percentages may be slightly inflated based on their method of calculating double-labeled cells [1], their percentage of double-labeled neurons in MR for one rat (12%) was comparable to our percentages, but that for the second rat (28%) was higher than our percentages for any septal-HF pairings. This latter difference could involve larger injections in their study than in ours. Finally, Acsady et al. [1] reported that all double-labeled MR cells (i.e., those with collateral projections to the septum and HF) stained positively for serotonin.

Double-labeled Cells in MR and Possible Functional Significance

We showed that approximately 8–12% of MR neurons were double-labeled following combinations of injections in the septum and hippocampus. Although MR cells with collateral projections to the MS and HF were located throughout the rostrocaudal extent of MR, they were concentrated in the rostral one half of MR.

The MR is directly involved in the desynchronization of the hippocampal EEG (or blockade of the hippocampal theta rhythm) ([58] for review). MR stimulation desynchronizes the hippocampal EEG [4,23,51]; MR lesions produce continuous theta [33,67]; and various pharmacological agents injected into MR that either suppress the activity of MR neurons or reduce excitatory drive to them

generate theta at short latencies and long durations [19,20,23,32,50,56,61].

The desynchronizing actions of MR on the hippocampal EEG are thought to be primarily mediated by the MS. MR stimulation disrupts the rhythmical discharge of septal pacemaking cells and desynchronizes the hippocampal EEG [4,23]; injections of 5-HT_{1A} agonists into MR activate septal pacemaking cells and generate theta [21]; and 5-HT MR cells selectively innervate [28] and excite GABAergic cells of the MS, which, in turn, inhibit septohippocampal MS [2,27,29,30] neurons, possibly involved in the desynchronization of the hippocampal EEG [58]. Although it appears that the MR primarily affects the MS in the modulation of the hippocampal EEG, it is also possible that the MR may exert a direct influence on the HF or, as suggested by the present findings of collateral MR projections to MS and HF, dual actions on the septum and hippocampus in the control of the hippocampal EEG.

We have recently identified two major classes of MR cells with activity related to the hippocampal EEG in urethane anesthetized rats: fast firing cells (21–42 Hz) that discharged at increased rates of activity with theta, and slow and regular firing neurons (1–4 Hz) that slowed in activity with theta [24,60]. We tentatively suggest that the fast firing cells are GABAergic neurons, the slow firing cells are 5-HT cells, and that these two populations mutually interact in the modulation of the hippocampal EEG. The slow firing MR neurons with reduced discharge with theta constituted about 9% of our population of MR cells. It is possible that the presently identified MR cells with collateral projections to the MS/DBv and HF may be those (or a subset of them) that fire at decreased rates with theta and may be uniquely involved in the desynchronization of the hippocampal EEG.

An accumulating body of evidence indicates that the theta rhythm of the hippocampus is critically involved in memory processing functions of the hippocampus ([52,58] for review). If, as indicated, theta serves an important role in long-term potentiation (LTP)/memory, it would seem that a 5-HT MR mediated disruption of theta (hippocampal desynchronization) might suppress LTP and memory. Consistent with this possibility, several reports have shown that serotonergic agents block LTP and that 5-HT antagonists (mainly 5-HT₃ antagonists) enhance LTP and/or memory [8,9,45,46,58]. Ascending 5-HT MR fibers, by disrupting theta (or desynchronizing the hippocampal EEG), may block or temporarily suspend mnemonic processes in the hippocampus. The MR may be an important part of a system of connections that directs the hippocampus to essentially disregard insignificant environmental events.

In addition to its effects on the hippocampal EEG, the MR serves a well documented role in the control of locomotion [3,15,16,43,62,63]. MR stimulation suppresses locomotor behavior [12,39] and various manipulations that reversibly or irreversibly inhibit the activity of MR cells produce locomotion [15,16,37,40,43,63–65]. The demonstration that the MR affects both locomotion and the hippocampal EEG suggests common MR mechanisms controlling both functions. In this regard, Sinnamon et al. [44] recently demonstrated the important findings that injections of GABA in MR both enhanced hypothalamic-induced stepping behavior (locomotion) and increased the power of theta in the 4–5 Hz band.

The possible dual MR control of locomotion and states of the hippocampal EEG would appear to have important functional consequences. It is obviously critical for a rat (or other species) moving through its environment to commit relevant aspects of it to memory, hence a coupling of motor behavior with theta to encode information when moving. On the other hand, there may be less of a demand to commit information to memory when an animal is stationary or engaged in automatic behaviors, such as grooming or

consumatory acts and hence the absence of theta during these conditions.

In summary, we have shown that 8–12% of cells of the median raphe nucleus send collateral projections to the MS and hippocampus. These cells may serve a unique role in the modulation of the septum and hippocampus in the desynchronization of the hippocampal EEG.

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