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Preferential neurotoxicity of colchicine for granule cells of the dentate gyrus of the adult rat*

(selective toxicity/neuronal degeneration)

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ABSTRACT **Injections of 5–7 μg (6–9 nmol) of colchicine into the dentate gyrus of the hippocampus of mature rats result in widespread destruction of dentate granule cells with little, if any, damage to other cell populations, including hippocampal pyramidal cells. Selective destruction of dentate granule cells is also observed after intraventricular injections. The destructive effects of colchicine appear as soon as 12 hr after the injection and lead to the disappearance of the granule cells over a period of days. Whereas the effects on nongranule cell populations in the hippocampus appear to be reversed by approximately 11 days after injection, the granule cells are almost completely absent at long intervals after injection. At the long postinjection survival intervals the disappearance of the granule cells is accompanied by elimination of their terminal projections, the mossy fibers, as revealed by Timm staining for heavy metals. Because the preferential neurotoxic effects of colchicine do not result in morbidity or obvious behavioral debilitation, the toxicity may prove useful for studying the functional consequences of removing specific cell populations in the central nervous system.**

In recent years, the ablation technique for studying brain function has received much criticism due to the lack of selectivity of the electrolytic or aspiration techniques traditionally used. This lack of selectivity is a particular problem when one attempts to dissociate the effects of the removal of specific neurons from the interruption of fibers having distant origins and terminations (fibers of passage), or when one attempts to ascribe the effects of lesions to the removal of one specific population of cells contained within a structure composed of heterogeneous cell types. Because of these problems, considerable recent interest has been generated by drugs or other treatments that are selective in their toxic effects on given neuronal populations or that can destroy neuronal cell bodies without interrupting fibers of passage. Several means of inducing more or less selective lesions have been described, including x-irradiation during development (1–3) and injections of toxic analogs of presumed neurotransmitters, including norepinephrine and dopamine (4–8), serotonin (9), and glutamic acid (10–12). These selective methods have provided new insights into the behavioral, biochemical, and physiological changes associated with the removal of specific cell populations in situations in which more traditional methods for producing lesions have largely reached the limits of their usefulness (13–19). Because of the tremendous potential of methods with a selective neurotoxic effect, particularly when these effects can be wrought in mature animals, we were intrigued by an apparently selective toxicity of colchicine for granule cells of the dentate gyrus of the hippocampal formation. The present study describes this effect.

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MATERIALS AND METHODS

Over 50 adult (250- to 400-g) male Sprague–Dawley rats received injections of amounts of colchicine ranging from 0.18 to 25 μg (0.22–30 nmol) dissolved in 0.5–1.0 μl of deionized water that were stereotaxically localized either in the dentate gyrus itself or in the rostral lateral ventricle. For most cases, intradentate injections were made at two rostrocaudal locations, the rostral being 1.8 mm lateral to the midline, 3.3 mm posterior to bregma, and 3.3 mm deep to the cortical surface, and the caudal being 4.7 mm lateral, 5.3 mm posterior, and 5.5 mm deep. For most of the intradentate injections, including these analyzing the time course of cell death, two doses of 2.5–3.5 μg (3–4.5 nmol of colchicine) dissolved in 0.5–0.7 μl of water were placed at the two rostrocaudal locations. Five additional animals received intraventricular injections of 2.5–20 μg (3–24 nmol) of colchicine dissolved in distilled water. The coordinates for the intraventricular injection were 0.3 mm posterior to bregma, 2.3 mm lateral to the midline, and 4.8 mm deep to the cortical surface. Control injections consisted of equivalent volumes of distilled water or physiological saline.

Postinjection survival times ranged from 12 hr to approximately 120 days. All but nine of the animals were perfused with 10% formalin in saline while deeply anesthetized with sodium pentobarbital. Forty-micrometer frozen sections from these animals were stained with cresyl violet to reveal the Nissl substance. Three additional animals received intradentate injections of 3.5 μg of colchicine and were sacrificed approximately 60–120 days after injection. These animals were processed according to a modified Timms staining procedure (20) for heavy metals, which reveals the distribution of the terminal projections of the dentate granule cells (the mossy fibers). Finally, three animals that had received intradentate injections of 3.5 μg (4.5 nmol) of colchicine at two rostrocaudal locations, and three animals that had received intraventricular injections of 5, 10, or 20 μg (6, 12, or 24 nmol) of colchicine in 1 μl of H_2O were sacrificed 3–4 days after injection and were processed by the cupric silver method (21), which results in the deposition of silver in the somata of degenerating neurons.

RESULTS

The selectivity of the neurotoxic effects of colchicine can be clearly seen in Fig. 1, which illustrates a horizontal section through the dorsal (*A*) and more ventral (*B* and *C*) hippocampal formation in a rat that had received two injections of 3.5 μg of colchicine, one localized in the dorsal and the other in the ventral hippocampal formation 7 days prior to sacrifice. We found this double injection procedure to be particularly ef-

* A preliminary report of some of these results has appeared in abstract form (42).

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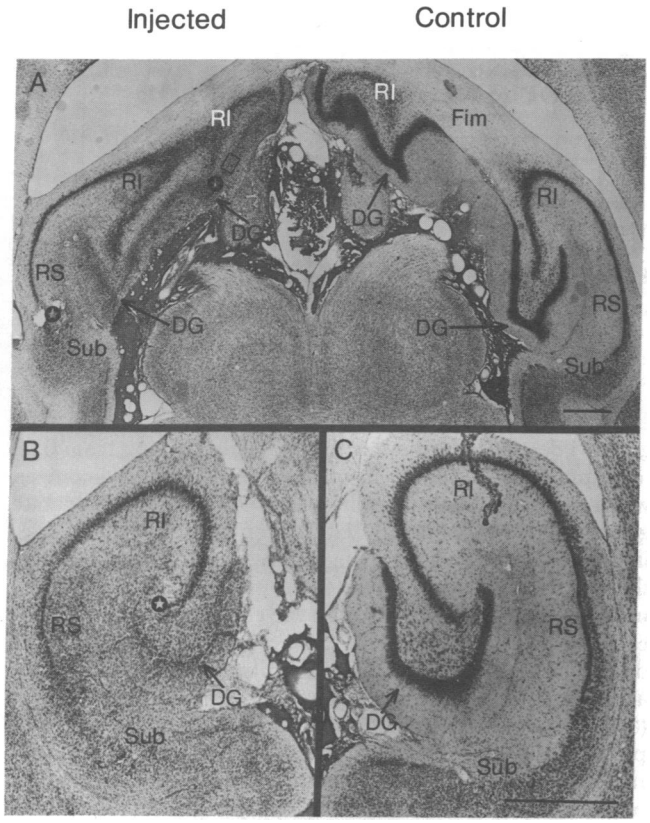

FIG. 1. Comparison of injected and uninjected (control) sides of the hippocampus in horizontal sections at dorsal (*A*) and ventral (*B* and *C*) levels 7 days after injection of 3.5 μg of colchicine near each of these levels. Stars indicate path of injection needle. Arrows indicate the dentate gyrus (DG), which is extensively damaged on the injected side. There appears to be damage to pyramidal cells (compare *B* and *C*), especially in the regio superior (RS) and subiculum (Sub), but the vast majority of pyramidal cells in all subfields survive, whereas almost none of the granule cells do. The box in *A* corresponds roughly to the region shown in Fig. 4, 7 days after injection. Fim, fimbria–fornix; RI, regio inferior. Calibration bars represent 1 mm.

fective in destroying granule cells all along the rostrocaudal axis of the hippocampal formation, while sparing most pyramidal cells in the hippocampus ipsilaterally and almost all of the granule cells and pyramidal cells in the contralateral hippocampus. As is evident, the granule cell layer (arrows) on the side of the injection appears totally disrupted in comparison with the contralateral control, whereas the layers of hippocampal pyramidal cells appear largely unaffected, except for some evidence of cell disruption near the path of the syringe in the subicular area and the regio superior. Despite the disruption of the granule cell layer, there remains at 7 days after the lesion a conspicuous cellularity on the side of the injection. These cells, as illustrated in the higher power photomicrographs of Fig. 4, appear to be phagocytic glial cells, perhaps microglia.

To further document the selectivity of the destruction, the cupric silver method for degeneration was particularly useful. With this stain, neurons undergoing degenerative changes appear argyrophilic against a light staining background. As is evident in Fig. 2, from an animal that had received injections of colchicine comparable to those of the animal in Fig. 1, the granule cells of the dentate gyrus are conspicuously argyrophilic, whereas only a few pyramidal cells near the path of the injection syringe appear dark. In addition, except for a few neurons near the border of the entorhinal cortex and neocortex, no argyrophilic neurons were found in other brain regions.

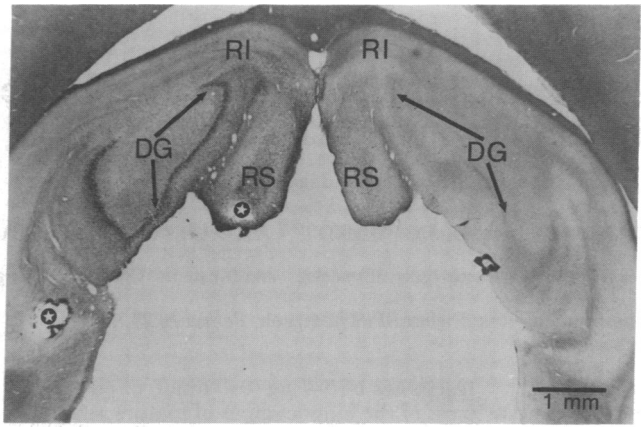

FIG. 2. The ring of dark debris on the left-hand side represents the argyrophilic neurons in the dentate gyrus revealed by the cupric silver stain 3 days after a double injection of 3.5 μg of colchicine into the rostral and caudal hippocampal formation (stars indicate the path of the syringe). Note the absence of argyrophilic neurons elsewhere. Abbreviations are as for Fig. 1.

Additional evidence of the selectivity of the colchicine neurotoxicity is the degeneration of dentate granule cells induced by intraventricular injections of 10 μg (12 nmol) of colchicine. Again, only the dentate granule cells undergo extensive degenerative changes such that they become heavily stained with silver (see the dark arrows in Fig. 3*A*). In general, however, the degeneration induced by the intraventricular injections is not as extensive as that following intradentate injections of approximately comparable amounts of colchicine, and it is not as well restricted to one side as is the case with the intradentate injections.

The conclusions regarding the selectivity of colchicine for dentate granule cells require some qualification. For example, intradentate injections of 25 μg (30 nmol) of colchicine appear to result in more damage to nongranule cell populations than a dose of 2.5–3.5 μg. With the 2.5 to 3.5-μg dose, most pyramidal cells of the hippocampus proper appear normal at long postinjection intervals, but we cannot be certain that they are unaffected by the colchicine, particularly during the early

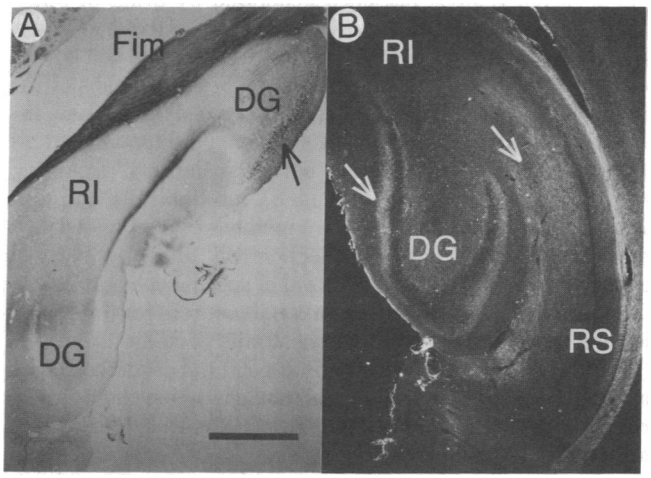

FIG. 3. (*A*) The dark debris (arrow) indicates argyrophilic granule cells 4 days after an intraventricular injection of 10 μg (12 nmol) of colchicine. (*B*) A dark-field photograph indicates the terminal degeneration (bright grains, arrows) of the commissural pathways to the hippocampal formation contralateral to the intraventricular injection of colchicine. The unlabeled calibration bar indicates 1 mm for *A* and 625 μm for *B*.

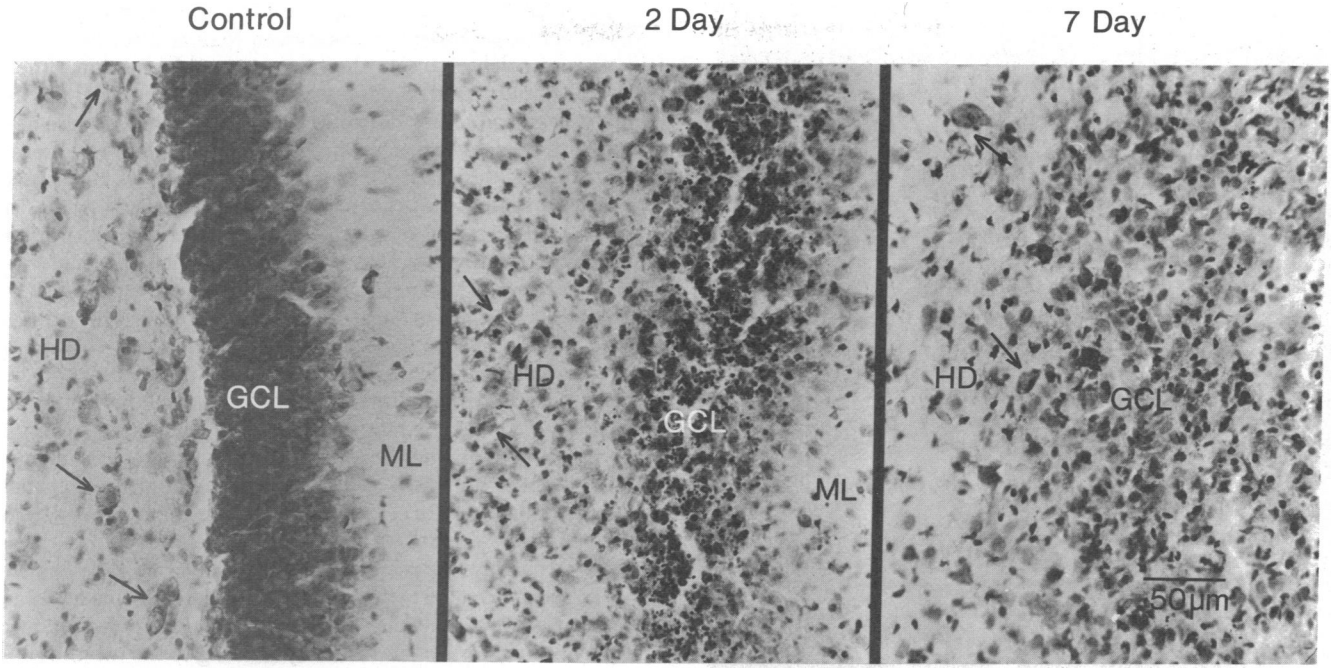

FIG. 4. Comparison of the granule cell layer (GCL) from the uninjected side (control) with the layer 2 days after a 2.5-μg colchicine injection and 7 days after a 3.5-μg colchicine injection. Arrows indicate pyramidal cells in the hilus of the dentate (HD). Note the shrunken granule cells, small particles of cell debris, and large numbers of small densely stained glial cells 2 days after injection. Seven days after injection, there are few, if any, normal-looking granule cells, most of the cell debris is gone, and increased numbers of glial cells remain in the granule cell layer and molecular layer (ML). The region shown here 7 days after injection is taken from approximately the boxed area in Fig. 1*A*.

postinjection period. Two days after the injections, hippocampal pyramidal cells near the injection site appear slightly paler in the Nissl-stained preparations than their contralateral counterparts on the uninjected side, and they are sometimes irregularly shaped. Cell debris (the small darkly stained granules evident at 2 days after injection in Fig. 4) is not found in the pyramidal cell layer, however.

While only the dentate granule cells exhibit dramatic degenerative changes, the cupric silver stain reveals in addition to the degeneration of mossy fiber terminals what appears to be terminal degeneration in several other fiber systems in the hippocampal formation. For example, Fig. 3*B* illustrates a dark-field photograph of silver grains indicative of terminal degeneration in the hippocampus and the dentate gyrus contralateral to an intraventricular injection of 10 μg (12 nmol) of colchicine. Terminal degeneration was also observed in the presubiculum ipsilateral to the injection. Similar patterns of terminal degeneration are apparent after the intradentate injections of colchicine. Because degenerating terminals originate from cell bodies that survive and are not argyrophilic, the possibility exists that these changes are temporary. Colchicine causes degeneration-like changes that are apparently reversible in other systems (22, 23), presumably because of the blockade of axoplasmic transport. The resolution of this possibility will require detailed counts of axons in animals with long-standing lesions. Thus, although some pyramidal cells may be damaged at high doses, it is clear that the destruction of pyramidal cells and, apparently, other nongranule cells is minimal in comparison to the extensive destruction of dentate granule cells at the low doses (2.5–3.5 μg).

Some indication of the sequence of morphological changes in the dentate gyrus after colchicine injections can be seen in Fig. 4. One day after the injections small particles of what appears to be densely stained cell debris are present in the granule cell layer near the injection site. By 2 days after the injections almost all the granule cells in the layer appear shrunken, irregularly shaped, or fragmented. This cellular disruption can be seen for more than 1 mm from the injection site. By this time, the increase in the number of small cells (presumably glial phagocytes) is quite evident. By 7 days after injection, the cellular disruption in the granule cell layer has progressed to the point that few, if any, granule cells can be identified, although this identification is made difficult by the continued presence of the presumed glial phagocytes (see Figs. 1 and 4). At all of these survival intervals, the large cells in the hilus of the dentate gyrus appear intact (see Fig. 4, arrows) as do most of the pyramidal cells of the hippocampus proper (see also Figs. 1 and 5).

The degeneration of the dentate granule cells continues to progress until by 120 days after injection little remains of the dentate gyrus (Fig. 5). In addition, at this time, Timm staining, which reveals the terminals of the granule cells (the mossy fibers), indicates that few, if any, mossy fibers survive on the side of the injection (see Fig. 5*C*). The glial hypertrophy that is prominent at the early postinjection survival intervals has also subsided by this time, leaving a hippocampal formation that appears relatively normal except for the absence of the granule cells and their terminal projections, the mossy fibers. There is some indication that the fimbria on the side of the injection is somewhat smaller, although changes in the configuration of the hippocampal formation to accommodate for the removal of the dentate gyrus make a determination of the size of the fimbria impossible without counts of intact axons.

For documentation of the colchicine toxicity, we have attempted to restrict the effect to one side of the dentate gyrus, and our injections have thus been unilateral. Neither these animals nor another group that had received bilateral injections of 3.5 μg in two rostrocaudal levels for behavioral studies exhibited mortality or any physiological or behavioral anomalies that were obvious on casual observation, except for some hyperresponsivity when being handled. This lack of a general debilitation after colchicine injections is required if colchicine is to be useful in behavioral investigations.

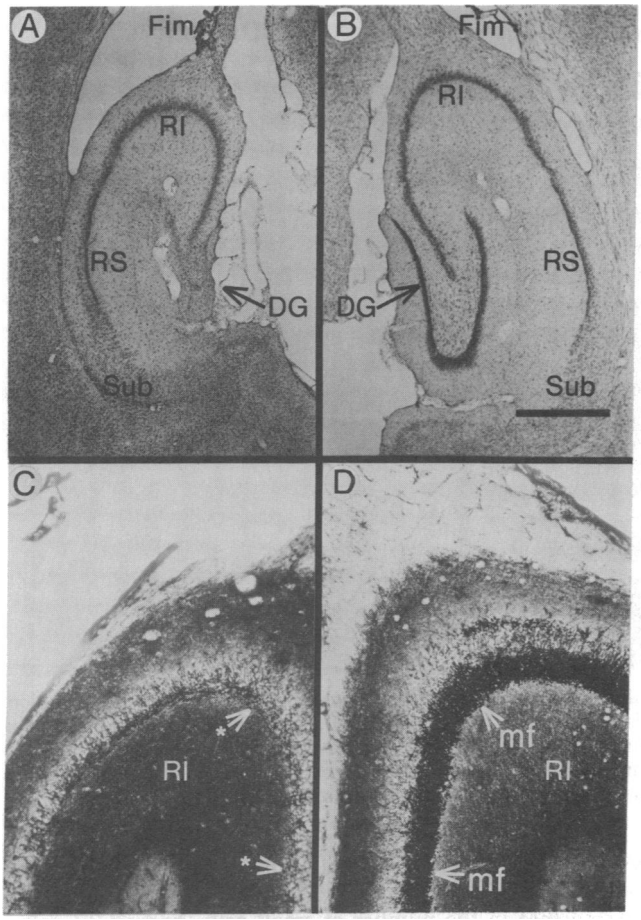

FIG. 5. Comparison of injected (*A* and *C*) and control (*B* and *D*) sides of the hippocampus approximately 120 days after injection. A comparison of *A* and *B* reveals the extent of degeneration of the dentate gyrus while other neuronal populations in the hippocampus have been spared. A comparison of *C* and *D* illustrates the virtual elimination of the dark-staining (Timm stain) mossy fibers (mf) on the side of the injection (*C*). Other abbreviations are as for Fig. 1. Calibration bar represents 625 μm.

DISCUSSION

The preferential destruction of granule cells of the dentate gyrus as a result of direct injections of colchicine may have several immediate applications. For example, the method offers opportunities for studies of the behavioral effects of damaged hippocampal circuitry. The potential benefits of such studies have already been amply demonstrated by the analyses of the anatomical and behavioral effects of the destruction of the granule cells of developing animals by x-irradiation (1–3, 24). However, these studies are complicated somewhat because the manipulations can only be performed in developing animals, prohibiting pretreatment behavioral analyses. In addition to a possible contribution to behavioral studies, the selective removal of dentate granule cells in adult animals may provide a means to further define the capabilities of the adult nervous system for plastic adjustments in comparison with an immature nervous system, because the elimination of the granule cells in developing animals by x-irradiation results in the establishment of new patterns of afferent connectivity in the hippocampus (24). In all of these approaches, the combined use of colchicine with other neurotoxins (such as kainic acid) may be quite useful, because colchicine appears to have a complementary pattern of selectivity for cells in the hippocampal formation in comparison with kainic acid (12) with similar routes of administration.

It is important to point out that although we have not observed cellular damage in other brain regions after direct injections of colchicine into the hippocampal formation, other studies (unpublished) demonstrate that some neuronal populations in the olfactory bulb and cerebellar cortex are also destroyed by colchicine after direct injections into these areas. Nevertheless, despite the fact that direct injections can affect cells other than dentate granule cells, it is noteworthy that, in a recent study, intracerebral injections of colchicine not aimed at the hippocampus also appeared to affect dentate granule cells most severely (25). A precise definition of all of the populations of neurons that might be affected by colchicine will require direct injections into a variety of brain regions, along with more detailed analyses utilizing intraventricular injections and perhaps other routes of administration. Whatever the degree of selectivity of colchicine for different cell types, it is clear from the present results that the effects of colchicine can be devastating to some cell types, and this fact makes it quite clear that any study that utilizes colchicine in the central nervous system must carefully control for such destructive effects.

Unfortunately, the mechanism of neurotoxicity of colchicine has not been revealed by the present results. Colchicine has been widely used to inhibit the rapid phase of axonal transport, and it presumably exerts this effect by binding to the tubulin subunits of the microtubules (26). However, colchicine is also known to affect other cellular processes, including a variety of enzymatic reactions and transmembrane transport systems, as well as mitosis (27, 28). Colchicine also apparently binds to components of plasma and nuclear membranes as well as to free tubulin (29–31). Although some studies using doses of colchicine similar to those of the present study report only reversible effects (22, 32), several other papers suggest lasting morphological changes in neurons and glia (23, 33–36).

One particularly interesting study suggests that some neurons may have an uptake system with an affinity for colchicine. Intraventricular injections of colchicine damaged serotinergic bulbospinal neurons in much the same way as neurotoxic indolamines (37). While the investigators suggested the axonal membrane as a possible site for the action of colchicine, another possibility is the dendrite. Dendrites are not only rich in microtubules but also contain specific receptors and perhaps uptake systems. Colchicine might irreversibly damage dendrites by disrupting the microtubular network that supports them.

One interesting possibility that is raised regarding the selective toxic effects of colchicine relates to the well-known ability of dentate granule cells to participate in various phenomena of short- and long-term neuroplasticity. For example, after denervation of the granule cells they can be rapidly reinnervated through a number of sources; this presumably requires the synthesis of new postsynaptic specializations, because the specialized postsynaptic elements [spines (38) and postsynaptic densities (39)] disappear after denervation and reappear with reinnervation. In addition, the dentate gyrus also exhibits dramatic forms of long-term physiological plasticity that appear to require associative interactions between synapses (40), and these may be due to postsynaptic changes (41). Although dentate granule cells are not unique in their capacity to accept new synapses or alter existing ones, they may possess an exaggerated capacity for such plastic change, in comparison with other neurons. To the extent that these sorts of changes require plastic alterations in the postsynaptic cells, and to the extent that such plastic alterations are manifestations of processes in which the granule cells normally engage, it may be that the mechanisms that permit plasticity also make the granule cells particularly susceptible to compounds that disrupt intracellular transport mechanisms. The elucidation of these and

other possibilities will require more detailed analyses of the mode of action of colchicine and of its interactions with granule cells.

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