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Use of Excitotoxins to Lesion the Hippocampus: Update

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ABSTRACT: Although many of the problems associated with the use of conventional lesion techniques (aspiration, electrolytic, radiofrequency) can be avoided by employing focal injections of excitotoxins, experience gained over the past 12 years has shown that considerable care must be exercised with this newer method, to limit the cell loss to the intended area or structure. Of the toxins that have been used most often to selectively destroy the cells that comprise the hippocampus, ibotenic acid (IBO) and N-methyl-D-aspartate (NMDA) have proved to be nonspecific in their effects on different cell types and these toxins do not cause seizures. In contrast, focal injections of kainate (KA) and quisqualate result in damage that centers primarily in the CA3 pyramidal cell field and hilar cells in the dentate gyrus. In addition, there are obvious seizures and secondary distant damage involving a number of structures and areas associated with mediating seizure activity. Intrahippocampal injections of the toxin colchicine result in a preferential destruction of dentate granule cells but usually also lead to additional cell loss in adjacent areas. Attempts to limit cell loss to specific hippocampal subfields, using different toxins, have met with mixed success. Both the dosage of the agent and the volume injected are important in determining the extent of cell loss, but the volume of the toxin injected has been shown to be especially important in limiting the damage to the intended area. With the development of newer procedures (e.g., immunotoxins, gene knockouts, antisense) that permit more selective cell loss, it should be possible in the future to achieve a level of lesion control that has been lacking in the past. As with the use of excitotoxins, these newer approaches will require special care to limit the damage to the intended area and interpret the results obtained properly. *Hippocampus 2002;12:405–414.* © 2002 Wiley-Liss, Inc.

KEY WORDS: ibotenic acid; NMDA; kainic acid; quisqualate; colchicine; hippocampal cell fields; seizures

INTRODUCTION

Over the past 12 years, the use of excitotoxins to destroy cells in particular brain loci has increased, replacing the use of conventional lesion approaches (i.e., aspiration, electrolytic, radiofrequency). This shift in controlled brain damage has been due in large part to the recognition that, with conventional lesions, there is often damage to either the adjacent or distant structures, or both; to axons that pass through the area of the lesion; and to the vasculature. With most, but not all, excitotoxins, these problems are minimized, as described below. The increased use of focal injections of toxins in experimental investigations has been especially apparent in research involving the hippocampus and behavior.

In 1989, the author described the use of ibotenic acid (IBO) to destroy selectively the cells that comprise the hippocampus (dentate gyrus, hilar cells, CA1–CA3 pyramidal cells) in the rat (Jarrard, 1989). The approach involved multiple stereotactically guided focal injections of small amounts of IBO. The selectivity of the cell loss was determined histologically using a cresyl violet stain to identify cell loss and the presence of gliosis. Silver stains were used to identify degenerating axons and argyrophilic neurons in distant areas. This research also established that the IBO lesion did not interrupt axons passing through adjacent alveus and fimbria. The volume of IBO injected at each site was found to be particularly important in limiting the damage to the hippocampus because large injections were found to spread into adjacent areas (especially subiculum) and into the ventricles with resulting extrahippocampal damage (see below).

The use of excitotoxins to lesion the hippocampus has been especially useful in behavioral investigations involving the rat (Morris et al., 1990; Jarrard, 1995; Chan et al., 2001), but excitotoxins have also been used to advantage in research with monkeys (Dore et al., 1998; Murray et al., 1998; Murray and Mishkin, 1998). In one of the first published studies involving excitotoxin (IBO) lesions directed at hippocampus in the monkey, Murray and Mishkin (1998) reported that visual recognition in monkeys with combined hippocampus plus amygdala lesions was similar to that in intact controls. This finding differed from the impaired performance found when aspiration lesions were used that included additional damage to adjacent rhinal cortex (Mishkin, 1978). In research with both rats and monkeys, the use of the more selective lesion approach has produced changes in behavior that are more limited than what one finds when the damage is initiated by conventional lesion techniques.

Several toxins have been employed to damage hippocampal cells, and a number of different surgical procedures have been used that affect the selectivity of the resulting damage. It is the intent of this commentary to review the implementation of the different toxins that have most often been employed, and the extent to which they can be used to lesion selectively different cell types in the hippocampus. In addition, consideration is given to

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several variables that have been shown to be especially important in limiting the damage to the intended area.

EXCITOTOXINS AND THE HIPPOCAMPUS

Interest in the effects of different toxins on cells in the hippocampus originally grew from the selective patterns of cell loss that were observed after pathological conditions such as status epilepticus, transient ischemia, hypoglycemia, and viral infection (Auer et al., 1984; Meldrum and Corsellis, 1984; Simon et al., 1986; Schmidt-Kastner and Hossmann, 1988). Subsequent research indicated that the excitotoxic actions of glutamate and/or related compounds could possibly cause similar selective pathology (Benveniste et al., 1984, 1989; Hagberg et al., 1985; Butcher et al., 1987; Heyes et al., 1990). Within the hippocampus there is a high density of high-affinity kainate (KA) receptors around mossy fiber terminals in the CA3 cell field (Patel et al., 1986) and a high density of N-methyl-D-aspartate (NMDA) and quisqualate (AMPA) receptors on the basal and apical dendrites of CA1 pyramidal neurons (Greenamyre et al., 1985). Given this distinctive pattern of glutamate receptor density, an important early question was whether these differences in density could explain the vulnerability of neurons in the different cell fields to excitotoxin damage.

In an attempt to clarify the relationship between patterns of receptor density and the known selective excitotoxic actions of several glutamate-related toxins, the effects of focal injections of the agonists NMDA, IBO, KA, and quisqualate were studied (Jarrard and Meldrum, 1993). To achieve a relatively uniform concentration of each toxin throughout the different hippocampal regions, and to minimize spread into the ventricles and adjacent structures, a procedure involving multiple injections of small amounts (0.05 and 0.10 μl) of each toxin was employed (Jarrard, 1989). Two main findings are relevant to the present discussion. First, focal injections of NMDA and IBO resulted in a similar nonspecific pattern of cell loss within all cell fields of the hippocampus, together with an absence of secondary damage outside the structure. NMDA was tested with progressively decreasing concentrations, all of which failed to show that the cell fields were differentially affected. Second, for both KA and quisqualate, cell loss centered primarily within the CA3 cell field and the hilus of the dentate gyrus. In addition, extensive damage occurred in a number of extrahippocampal structures including the subicular complex, entorhinal cortex, amygdala, and several thalamic nuclei. Damaged cells were also observed in the ventral neocortex from the entorhinal area rostral to the claustrum and anterior olfactory areas. The finding of such extensive secondary cell loss outside hippocampus was attributed to the propagation of seizure activity (see Ben-Ari et al., 1980).

Although IBO has been used most often in behavioral studies as a toxin to remove hippocampal cells, an increasing number of investigators have employed NMDA as a lesioning tool. Given the above results, it would appear that IBO and NMDA are comparable in terms of the cell loss found within the hippocampus after focal injections and also in the absence of pathology outside the structure. Further, intraperitoneal injections of the selective NMDA antagonist, 3-(±)-2-carboxypiperazin-4-yl-proply-1-phosphorate (CPP), served to protect in a similar manner most of the hippocampal cells from the damage caused by focal injections of NMDA and IBO (Jarrard and Meldrum, 1993). It is important to note that injections of each toxin produced nonselective lesioning of all hippocampal regions, including the dentate gyrus, hilar cells, and pyramidal cells (CA3–CA1); that is, the damage was not limited to selective activation of any single receptor type. Thus, the affected cells do not appear to depend on receptor density.

As described above, injections of KA and of quisqualate into the hippocampus result in damage that is especially apparent in the CA3 pyramidal cell field and the hilus of the dentate gyrus. In addition, there is extensive secondary damage to a number of distant structures caused by seizure activity. Given that KA is thought to be one of the most potent of the amino acid neuroexcitants (Cooper et al., 1996), the resulting seizures and extensive additional damage should not be too much of a surprise. Earlier we found that even in KA-injected rats that were pretreated with diazepam there was still a loss of cells in areas some distance from the site of injection (Jarrard, 1983). The existence of obvious behavioral seizures and resulting damage outside hippocampus discouraged most investigators from using this toxin as a lesioning tool in behavioral studies. Given the findings summarized above, it is not surprising that intrahippocampal injections of a mixture of KA plus colchicine (the "cocktail" used in several behavioral studies, e.g., Sutherland and Rudy, 1989; Weisend et al., 1996) lead to secondary cell loss outside the hippocampus, as well as to behavioral impairments that are more significant than those noted when the hippocampus is removed selectively with IBO (Davidson et al., 1993).

Although intrahippocampal injections of KA and quisqualate cause seizures with resulting distant damage, there should be less concern regarding focal injections of IBO and NMDA. This is the case because there are no obvious seizures in rats as they are waking from undergoing injections of either IBO or NMDA, and recordings made up to 12 h after surgery failed to show spiking activity in the cortex (L.E. Jarrard and J. Kant, unpublished results). Further, normal synaptic activation has been reported in dorsal hippocampus up to 9 h (the longest period studied) after IBO injections into ventral hippocampus (Moser et al., 1995). Although similar recordings have not been reported using NMDA, silver stains show an absence of damage outside hippocampus (Jarrard and Meldrum, 1993). Because NMDA and IBO do not cause seizures, the practice of using intraperitoneal injections of anticonvulsants after focal injections of these toxins would appear to be unnecessary.

Questions have been raised regarding the use of excitotoxins to damage the hippocampus, especially the use of such lesions in research involving learning and memory (McClelland et al., 1995; Anagnostaras et al., in press). McClelland et al. (1995) suggested that "the massive and sustained excitatory discharge" resulting from excitotoxic effects on hippocampal neurons could disrupt memory storage sites in structures and areas that receive inputs from the hippocampus. The result would be to produce "catastrophic" interference with the normal consolidation of memory

traces. It is well known that focal injections of KA and quisqualate into the hippocampus do cause "massive excitatory discharge" with resulting damage to subcortical, as well as cortical areas (see above); it seems reasonable to assume that the effect would be to interfere with underlying memory processes. However, our research indicates that this would not be a problem with focal injections of either IBO or NMDA.

Anagnostaras et al. (in press) describe using IBO to lesion the hippocampus and finding a substantial loss of cells in cortex after complete hippocampus lesions. Substantial cortical damage was also found when the IBO injections were limited to dorsal hippocampus. Further, these investigators report considerably greater distal cortical damage in rats that were sacrificed at 6 months as compared with 4 days after the lesion. In terms of effects on behavior, excitotoxic lesions of the hippocampus had a greater effect than was observed with selective electrolytic lesions of roughly the same size. These investigators concluded that "…considerable additional work is necessary to develop effective ways of producing large lesions of the hippocampus without producing damage … elsewhere." Details regarding the operating procedures and histological results of this research are not yet available, but it should be pointed out that we do not find cell loss outside the hippocampus when multiple injections of small amounts of IBO are made following the procedures previously described (Jarrard, 1989; Jarrard and Meldrum, 1993). Although any of a number of investigations that employed the use of cell and silver stains to evaluate the damage could be used to support this general conclusion, it is instructive to consider the recent article by Galani et al. (2002). In this study, the effects of several different lesions (including IBO lesions of hippocampus) on the acquisition of a spatial reference and working memory task were compared after short (1-month) versus long (6.5-month) postoperative recovery periods. There was some recovery (of working memory) in the 6.5-month hippocampal group but, more to the point, careful reexamination of the histology and measurement of cortical thickness in rats that were sacrificed 11.5 months after surgery did not display any loss of cortical cells. It should be noted that after long periods of survival and extensive behavioral testing, we have observed spontaneous seizures in several rats in which the hippocampus was removed with IBO. Further, these rats had a pattern of extrahippocampal cell loss (including distal cortical damage) similar to that found in rats after seizures caused by focal injections of KA and quisqualate. The explanation for the development of these seizures remains unknown, but one possibility is that the IBO injections inadvertently spread into the ventricles. Although this could be a point of some concern, it is important to point out that, in our hands, few rats develop seizures after IBO hippocampal lesions, and behavioral data from these animals were always discarded.

Focal injections of colchicine have been used to damage the hippocampus in a number of behavioral studies. Intrahippocampal injections of the toxin result in a preferential destruction of dentate granule cells (Goldschmidt and Steward, 1980; Mundy and Tilson, 1990). The special affinity that the toxin has for cells in the dentate gyrus is thought to be due to the blocking of intracellular transport as a result of binding to and thus prevention of the polymerization of tubulin. Considering the differences in volume and concentration of colchicine used in various experiments, as well as the number and location of injection sites, it is not surprising that the selectivity of the cell loss within hippocampus has varied widely (see below). As a result, there have been a number of conflicting reports regarding the effects on behavior of damaging cells in the hippocampus with colchicine. (For a summary of many of these behavioral studies, see Xavier et al., 1999.) It is unknown whether colchicine disrupts the axons that pass through the area of injection or promotes the development of sustained excitatory discharge (seizures), or both. Xavier et al. (1999) reported that seizures were not observed at any time either during recovery from surgery or behavioral testing, but histological evidence of an absence of secondary damage is lacking.

OTHER CONSIDERATIONS IN DETERMINING SELECTIVITY OF EXCITOTOXIN LESIONS

In addition to the specific toxin used to lesion the hippocampus, and the site and number of sites of the injections, the concentration and volume injected at each site have proved important in affecting the selectivity of the damage. Few investigations have been carried out in which the concentration of an excitotoxin was systematically varied. However, we found that with three different concentrations of NMDA, in which the volume was held constant, the area over which the damage spread was generally similar, but the extent of the cell loss within this area decreased with decreasing concentrations (Jarrard and Meldrum, 1993). A similar conclusion was reached when different concentrations of IBO were used in earlier pilot experiments (L.E. Jarrard, unpublished results). For the most part, with the exception of colchicine, the concentration of various toxins used to damage hippocampus in behavioral experiments have generally been similar—that is not the case for volume injected.

The area of spread of a single injection is affected by a number of variables, including extracellular space, diffusion characteristics of the substance being injected, and the presence of fiber tracts that prevent spread (Alheid, 1990). In determining the most effective volume to use in selective removal of the hippocampus, we found that injections of >0.15 μl of IBO (10 mg/ml) result in cell loss over a wide area, with frequent spread into adjacent structures and the ventricles, together with extrahippocampal damage. Consequently, for a single injection of IBO, we currently use amounts that vary from 0.05 to 0.12 μl. For an injection of 0.10 μl of IBO into hippocampus in the rat, the spread within which all cells are removed is ~1.2 mm in diameter (Jarrard, 1989; Fig. 1B). Even with a smaller injection of 0.05 μl, there is considerable loss of cells over a relatively wide area, including pyramidal cells (CA1–CA3) together with hilar and granule cells in the dentate gyrus.

A number of behavioral research papers were recently published in which investigators used single focal injections of IBO and NMDA into the hippocampus that were considerably larger (in volume) than those described above (Frohardt et al., 2000; Ferbinteanu and McDonald, 2000, 2001; Gilbert, et al., 2001). It is

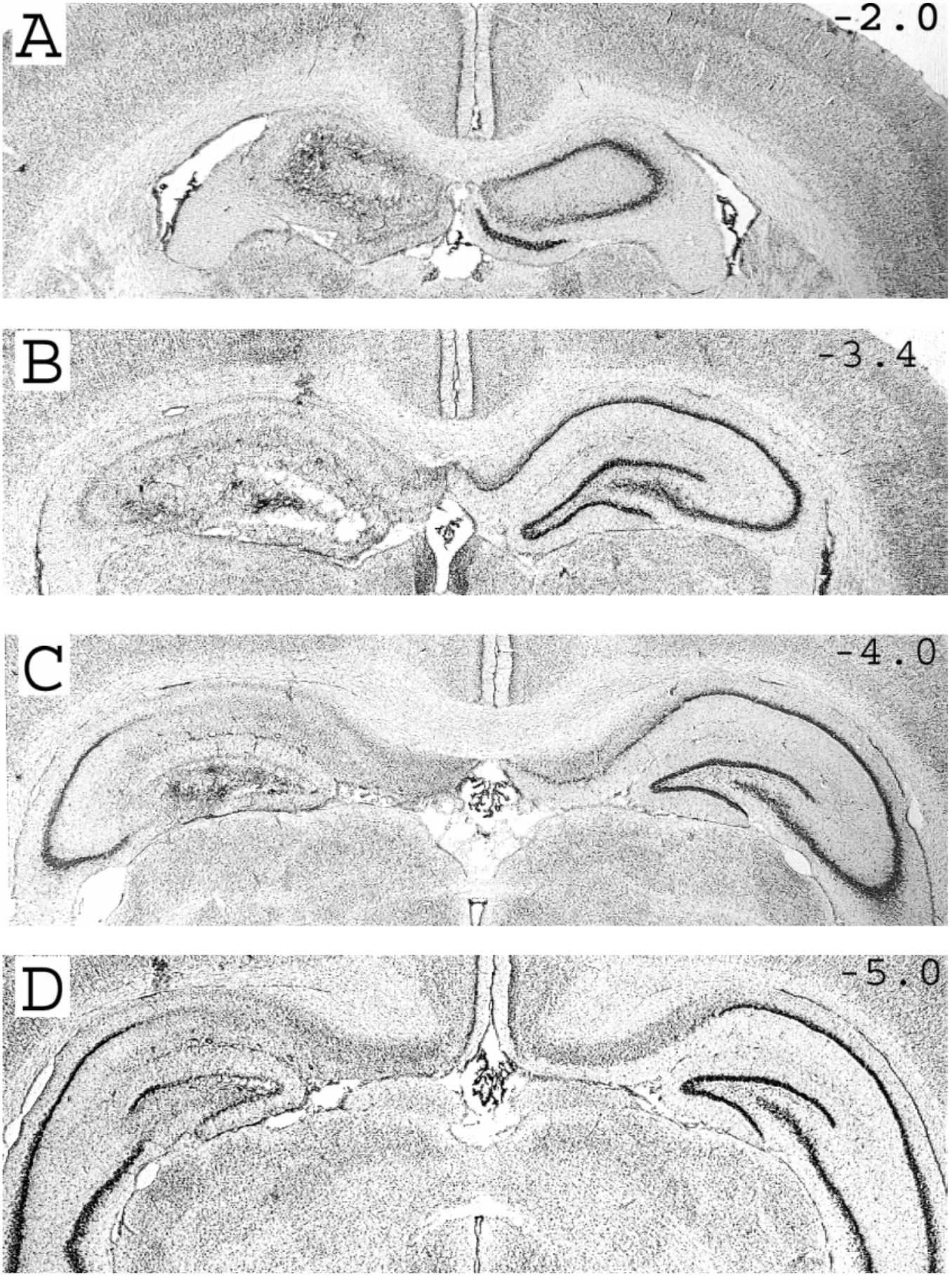

FIGURE 1

especially instructive to consider the procedure used by Gilbert et al. (2002), as these investigators made a single large injection into the dorsal hippocampus, consisting of 0.70 μl of IBO (8 mg/ml, injected over 3.5 min). Because we routinely use single injections that are considerably smaller (0.05–0.12 μl), and have consistently found that injections of larger volumes result in spread out from the intended area, we recently carried out an operation on several rats according to the procedure described by Gilbert et al. (2001). The same amounts (concentration, volume) were injected over a similar period of time, and at the same stereotactic coordinates (e.g., anteroposterior [AP]–3.6 mm from the bregma, mediolateral [ML] −2.0 mm, dorsoventral [DV] −3.2 mm ventral from the skull). The resulting spread and cell loss in hippocampus found with this single injection of IBO are shown in Figure 1. Comparison of the injected hippocampus (left) with the noninjected hemisphere (right) shows that with the large volume of the toxin, there is spread from the injection site at AP −3.6 mm to the most anterior tip of hippocampus (Fig. 1A) and in a caudal direction beyond AP −5.0 mm (Fig. 1D). Inspection of the coronal brain sections shows that the damage was nonspecific in terms of cell type; that is, all hippocampal pyramidal cells, including dentate granule and hilar cells, were damaged extensively. With such a large injection, there is concern that the toxin also spread into adjacent areas and into the ventricles (see below).

Given the spread that occurs even with small volumes (e.g., 0.10 μl) of IBO, it is apparent that a number of well-placed injections are needed for selective removal of all the cells in a large structure such as the hippocampus. Originally, after determining the concentration and volume of IBO to inject, we made single injections in different rats to determine the stereotactic coordinates. The intent was to remove all the cells in the hippocampus, using the fewest number of sites and the smallest possible concentration and volume. We found that injections at 26 sites (13 in each hemisphere) were required (see Jarrard, 1989). (Since that time, we have increased the total number of sites to 30.) To minimize damage to overlying cortex and seepage of IBO into the ventricles, we attach a fine-diameter tapered glass pipette onto the end of a 5-μl Hamilton syringe and inject the toxin manually at a rate of ~0.10 μl/min, using a Kopf microinjector unit. Given the variability that exists from rat to rat in the location of the bregma and overall shape of the skull, the cell loss is not always as complete or as limited to the hippocampus as one would like; however, careful histological analysis of the damage can eliminate those rats that have unacceptable lesions.

FIGURE 1. Cresyl violet-stained coronal sections through dorsal hippocampus at four anteroposterior (AP) levels (A–D) showing the cell loss resulting from a single injection of 0.70 μl of ibotenic acid (IBO) (left) made at AP −3.6 mm with a 5-day survival. The procedure was the same as that described in Gilbert et al. (2001) except the injection was unilateral. Note the loss of cells in all pyramidal cell fields (CA3–CA1) and the hilar and granule cells in dentate gyrus. In addition, there was cell loss in dorsal subiculum and thinning of cells in dorsal thalamus. Numbers in the upper right of each section correspond to the approximate position (in mm) caudal from bregma (Paxinos and Watson, 1997).

Subtle damage to adjacent structures and secondary cell loss in distant areas are extremely difficult to identify in simple cell-stained sections. The silver stain used by Fink and Heimer (1967), although replaced by newer histological procedures for some purposes, is especially useful because it not only permits the identification of degenerating axons that result from the lesion but also helps identify damaged argyrophilic cells and any associated degeneration that may be located some distance from the injection site. Thus, both cell and silver stains have proved useful in evaluating the nature and extent of the damage.

LESIONING SELECTIVELY DIFFERENT HIPPOCAMPAL CELL FIELDS

Recently, there has been considerable interest in the possibility of selective removal of the cells in different hippocampal subfields with the use of focal injections of excitotoxins. This interest arises not only from the finding that there is a loss of hippocampal pyramidal cells in the CA1 region after an ischemic insult, but also because of an interest in how the different cell fields contribute to learning and memory. It is well known that experimental interruption of the blood supply to the brain using four-vessel occlusion (VO) results in the cell death of CA1 cells in the dorsal hippocampus (Pulsinelli and Brierley, 1979; Nunn and Hodges, 1994). It is important, but not often acknowledged, that there is also extrahippocampal damage to other brain structures after an ischemic insult (Nunn and Jarrard, 1994; Nunn et al., 1998). An important question to consider is whether it is possible to remove only the CA1 cells with focal injections of toxins without causing extra-CA1 cell loss as well. Given the anatomical and functional importance of the dentate gyrus, is it possible to lesion the granule cells of the dentate gyrus selectively? No attempt is made in the present work to review this important and growing literature. Rather, the author's comments are limited to several recent behavioral studies in which attempts were made to remove selectively the CA1 cells and the cells that form the dentate gyrus.

Gilbert et al. (2001) reported that different behavioral-cognitive impairments occur when cells in the CA1 cell field of the "dorsal" hippocampus are destroyed compared with removal of cells in the dentate gyrus. The theoretical implications of these results, although potentially important, are compromised by an apparent lack of subfield selectivity as a result of the surgical procedures employed. Specifically, the "CA1 lesion" was made with a single injection into each hemisphere of the large amount of IBO described in the previous section (i.e., 0.70 μl per injection site). Because of a concern regarding the extent of the resulting damage, we made similar unilateral injections of IBO. The nature and extent of the cell loss in different subfields that resulted from this single injection are apparent in the series of cresyl violet-stained coronal sections displayed in Figure 1. Comparing the injected hippocampus with the noninjected control side, it is apparent that there is extensive cell loss in all cell fields, including CA1, CA3, and the dentate gyrus and in all the cells in the hilus of the dentate. As

pointed out above, there is considerable spread along the rostrocaudal extent from AP −2.0 mm and posterior beyond AP −5.0 mm. Although silver stains were not used to help identify distant damage outside the hippocampus, inspection of Figure 1 shows obvious loss of cells in the dorsal subiculum and a possible thinning of cells in underlying thalamus on the injected side. In describing the nature and extent of their lesions, Gilbert et al. (2001) stated that there was minimal damage to hippocampus anterior to AP −2.3 or posterior to −4.3 (the most caudal site examined histologically). In an attempt to quantify the CA1 cell damage, Gilbert and colleagues used a volumetric approach, reporting that there was an 83% reduction in volume of the CA1 pyramidal cell layer in "dorsal" hippocampus, an 18% reduction in dentate gyrus, and an 11% reduction in the CA3 subregion.

The "dentate gyrus lesion" in the study carried out by Gilbert et al. (2001) was made with two injections per hemisphere of colchicine (0.8 μl/site, 2.5 mg/ml concentration), for a total volume of 3.2 μl. (The stereotactic coordinates were AP −2.7 mm, ML ±2.1 mm, DV −4.0 mm ventral from skull, and AP −3.7 mm, ML ±2.3 mm, DV −4.0 mm.) Volumetric analysis was reported to show 95% loss of granule cells, 18% loss in the CA1 pyramidal cell layer, and no cell loss in the CA3 cell field. In an attempt to gain a better understanding of the reported cell loss using this surgical procedure, we made similar injections of colchicine at the same two sites unilaterally in several rats, and with a 5-day survival. The results are shown in Figures 2 and 3. The dentate granule cells were totally removed at the midlevel (AP ∼−3.4 mm), but there was also a complete loss of cells in the hilus of the dentate gyrus and a near-complete loss of CA3 cells. Further, there was a thinning of pyramidal cells in the CA1 cell field and a proliferation of glia throughout the hippocampus on the injected side. The extent of removal of the CA3 cells can be visualized by comparing the injected and noninjected hippocampus in Figure 3B and 3C, in which the field is shown under higher magnification. In contrast to finding no loss of CA3 cells, as reported by Gilbert et al. (2001), it is apparent that there was almost complete removal of the CA3 cells. In terms of the extent in the AP direction, the granule cells were removed from AP −2.0 mm to −5.0 mm, and there is also extensive damage to the other cell fields at all levels. Although the claim of a functional "double dissociation" between dentate gyrus and CA1-lesioned rats reported in the study by Gilbert et al. (2001) is interesting, one cannot help but wonder whether this result is attributable to differential extrahippocampal damage that resulted from the considerable volume of IBO and colchicine that was injected.

It is instructive to compare the lesion procedures used by Gilbert et al. (2001) with those employed in the recent article by Xavier et al. (1999). The latter investigators used multiple injections of small amounts of colchicine (9 sites/hemisphere, 0.06 μl/site, 7 mg/ml) in an attempt to remove all the dentate granule cells, while minimizing extra damage. In addition to cresyl violet stain used to estimate cell loss, and Timm stain to identify loss of mossy fibers, Xavier et al. (1999) employed stereological principles and systematic sampling to determine the number of intact-appearing neurons. The cell counts showed that injected rats exhibited a 90% cell loss of dentate granule cells, 33% loss of hilar cells, 23% neuronal loss of cells in the CA1 layer, and no loss of CA3 cells. In discussing the lesion results reported by Xavier et al. (1999), Gilbert et al. (2001) stated that ". . . as many as 18 bilateral injections of colchicine into DG made little difference with respect to the percentage of damage in volume to the neurons in any hippocampal subregion compared to the four bilateral injections in the present study," and "…the computer-based volumetry with 3-D reconstruction can generate as reliable a set of volumetric data as stereology can produce" (p 633). In attempting to interpret and evaluate these statements, one should note that Xavier et al. (1999) determined the cell loss for all of the hippocampus, whereas Gilbert et al. (2001) were only interested in the "dorsal" hippocampus. Because a concern in these and related studies centering around functional differences between hippocampal cell fields is to provide quantitative information regarding loss of specific cell types, it would seem that the preferred approach would be one that provides unbiased cell counts, e.g., the stereological approach employed by Xavier et al. (1999).

Several studies have been carried out in which multiple injections of small amounts of IBO were used in an attempt to remove selectively neurons in the CA1 cell field in the rat (Johnson and Jarrard, 1986; Nunn et al., 1998). To limit the damage to the intended area, the approach was to inject small amounts of IBO (0.03–0.04 μl, 10 mg/ml) aimed at the CA1 cell field. The surgical procedure in the Johnson and Jarrard study was designed to remove the CA1 cells throughout hippocampus, and this involved injecting IBO at 32 sites (16 in each hemisphere). The research carried out by Nunn et al. (1998) is interesting in that the functional consequences of four VO ischemic lesions were compared with IBO CA1 lesions. The IBO operating procedure was adjusted so that the ischemia and IBO lesions resulted in an equivalent loss of CA1 cells in the dorsal hippocampus. A total of 16 injections (8 on each side) were made using 0.033 μl per site. The finding of greater behavioral deficits and extra-CA1 cell damage in ischemic rats was interpreted as supporting a role for extrahippocampal cell loss in ischemia-induced memory impairments. Thus, unlike the extensive damage that results from a single injection of a large amount of IBO like that used by Gilbert et al. (2001), it is possible to limit the loss of cells primarily to the intended CA1 cell group provided one uses multiple, well-placed injections of small amounts of IBO.

CONCLUSIONS

Although many of the problems associated with the use of conventional lesion procedures can be avoided by employing focal injections of excitotoxins, experience over the past 12 years has indicated that special care must be taken in order to limit the damage to the intended area. The two glutamate-related toxins, IBO and NMDA, are generally nonspecific in terms of their effects on the different hippocampal cell fields, and it has been established that well-placed intrahippocampal injections do not lead to seizure activity. As a result, there is an absence of secondary distant damage

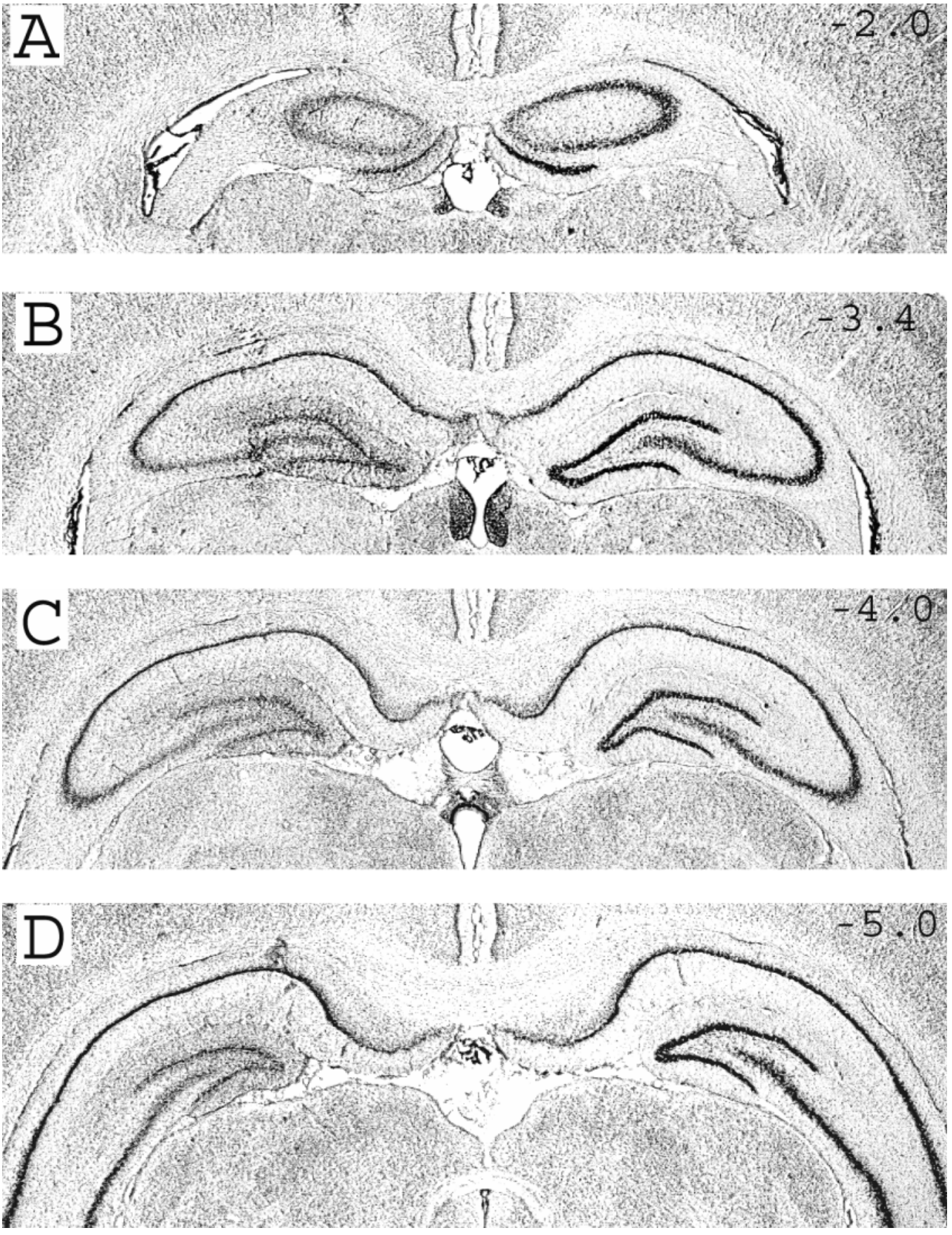

FIGURE 2. Coronal sections (A–D) through dorsal hippocampus showing the cell loss resulting from two unilateral injections of colchicine (0.8 μl/site) (left) made following the procedure described by Gilbert et al. (2001). The dentate granule cells were completely removed at the mid-anteroposterior (AP) level, and there was an extensive loss of hilar and CA3 pyramidal cells, as well as thinning of cells in CA1. There was also a loss of cells in dorsal thalamus.

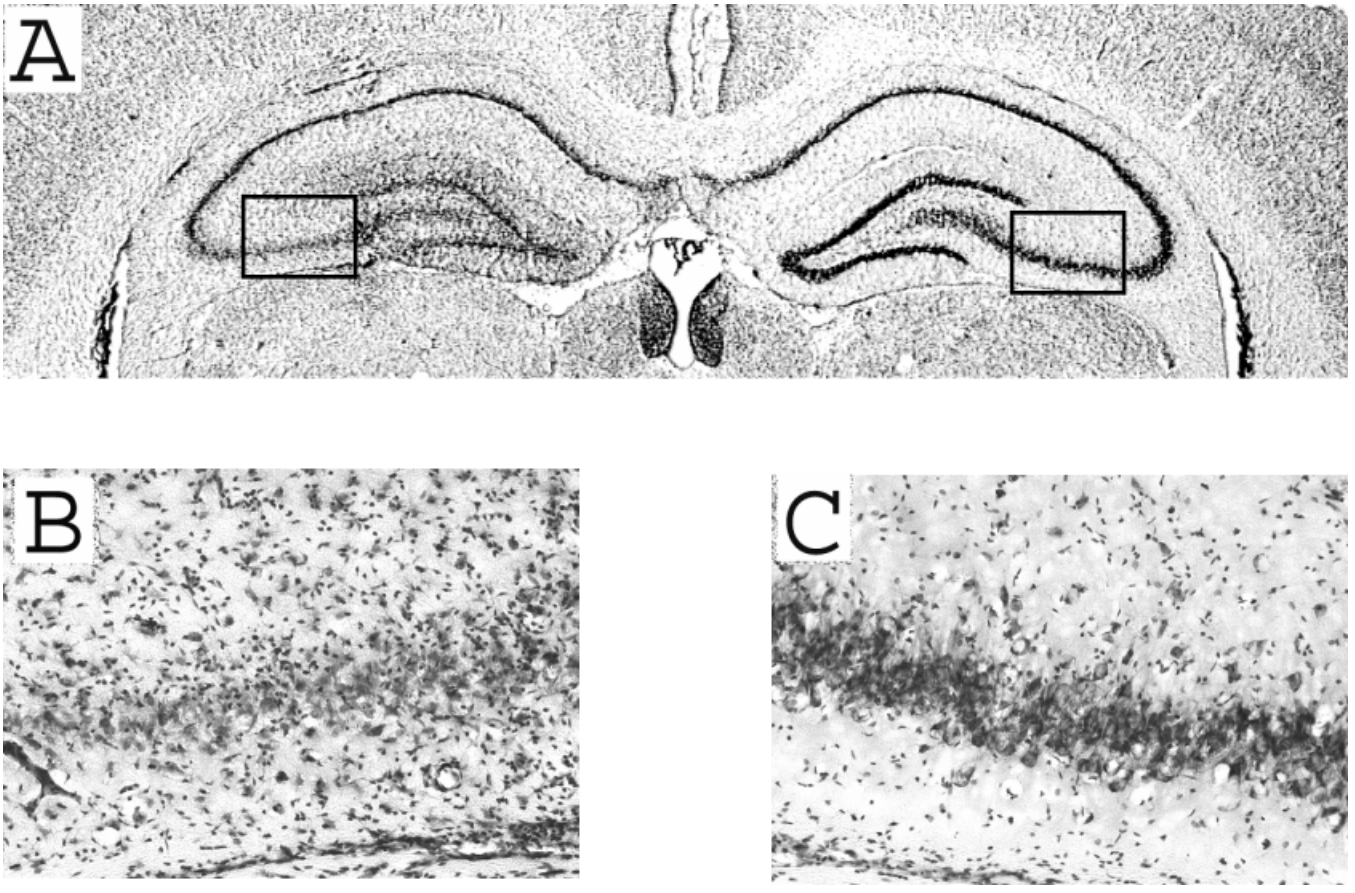

FIGURE 3. A: Coronal section of dorsal hippocampus at anteroposterior (AP) −3.4 mm, showing the cell loss that results from the two unilateral injections of colchicine (left) as described in the text and shown in Figure 2. Higher magnification of the CA3 cell fields outlined by squares are shown for the injected side (B) and the uninjected side (C). Although colchicine has an affinity for dentate granule cells, and these cells are essentially removed at this level, it is apparent by comparing B with C that most of the CA3 pyramidal cells are also removed. Note the proliferation of glial cells on the injected side.

to other structures caused by seizures. When KA and quisqualate are injected into hippocampus, there is preferential damage to the pyramidal CA3 cell field and to cells in the hilus of the dentate gyrus. A problem with using these excitotoxins is that there are obvious behavioral seizures, as well as considerable secondary distant damage. This extrahippocampal damage includes loss of cells in a number of structures and areas, which include the entorhinal cortex, subicular complex, amygdala, several nuclei in the thalamus, and damaged cells in the ventral cortex from the entorhinal area to the claustrum and olfactory-related areas. Given this additional damage, it would appear that IBO and NMDA are the preferred toxins to use if the intent is to remove the cells that form the hippocampus.

Both the concentration and the volume injected are important variables in determining the selectivity of the damage. Of these two variables, the volume of the toxin is especially important. We have found that in the rat hippocampus, single injections of IBO larger than 0.15 μl spread to adjacent structures (like subiculum), and into the ventricles. Thus, with larger injections there is the real possibility that damage to structures in addition to hippocampus will result and thus serve to confound the interpretation of any behavioral changes.

Attempts to lesion selectively the cells within different hippocampal subregions have met with mixed success. Although several toxins do have a preferential affinity for specific cell types (e.g., KA for CA3 pyramidal and hilar cells and colchicine for dentate granule cells), limiting the damage to any one cell type has proved difficult. With carefully selected toxins and sites, and with injections that are small in amount, it does appear that the damage can be limited primarily to specific cell fields, but there is usually additional unwanted damage. Careful histology and quantitative methods that provide details regarding cell loss and sparing of each hippocampal cell type are especially important.

As the main interest in most lesion studies is to gain further insight into the specific contributions to behavior made by cells in identified brain structures or areas, it is extremely important to limit the damage to the intended area. Although such selective damage is not always possible, it is important that the nature and extent of all cell loss be presented in detail, and any unwanted damage described. This is because it is frequently found that additional, unwanted damage can serve to confound interpretation of the results. As an example, in early studies using excitotoxins to lesion the hippocampus, we often found that the damage extended beyond the hippocampus to include loss of cells in the subiculum.

By separately analyzing the data for those rats that exhibited damage to the hippocampus plus subiculum, the effects on behavior were often found to be greater than in rats in which damage was limited to the hippocampus (Hampson et al., 1999; Jarrard et al., 1986; Morris et al., 1990). Only when sophisticated behavioral testing procedures are matched by equally sophisticated lesion procedures and careful histological evaluation will a lesion approach result in greater understanding of how different structures and areas are involved in controlling behavior (Jarrard, 2002).

With the development of new procedures that permit more selective cell loss limited to particular structures, types of neurons, or specific proteins (e.g., immunotoxins, gene knockouts, antisense, suicide transport), it should be possible in the near future to achieve a level of lesion control that has been lacking in the past. Although these newer approaches do offer considerable promise, they will, as with the use of excitotoxins, require special care on the part of investigators to use the procedures in such a way as to limit the damage to the intended cells. Further, special care will be needed to interpret the results obtained properly.

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