1. INTRODUCTION

Intercellular communication among cells in the central nervous system can occur via surface-surface interactions, the release of neuroactive substances which bind to specialized receptors on other cells, or via the direct exchange of electrical or chemical signals between cells. The latter means of communication utilizes gap junctions, pores comprised of two hemichannels localized in both of two communicating cells. Each hemichannel is formed by six connexin molecules, being integral membrane proteins. The pore formed by the associated hemichannels has a diameter of approximately 1.2 nm and allows the passage of electrical charge, as well as small molecules.¹

In the mature central nervous system of mammals, gap-junctional communication is most pronounced among astrocytes, though gap junctions are also expressed between neurons²⁻³ and oligodendrocytes.⁴⁻⁶ Coupling is not exclusively confined to cells of the same type, but occurs also as heterologous coupling, e.g., between astrocytes and oligodendrocytes.⁷⁻⁸ Unlike the obvious role in providing fast electrical transmission
between some specialized neurons, little is known about the functional implications of gap-junctional communication between glial cells in the mature CNS. Possible functions of gap junctional communication among astrocytes may reside in the propagation of signals, the redistribution of ions, or metabolic interactions among coupled cells. These possibilities will be discussed in more detail below.

In the developing CNS gap junctional communication has been shown to be a widespread phenomenon in neurons. Functional implications for developmental processes which have been proposed are the nonsynaptic propagation of neuronal activity or the diffusion of molecules expressed only in a subpopulation of cells. The latter mechanism may be of importance for the establishment of molecular gradients underlying pattern formation in the brain. The development of gap junctional communication among astrocytes in the mammalian cortex and its significance for putative functions will be addressed below.

2. DYE COUPLING AMONG ASTROCYTES

The existence of gap junctions between cells can be shown by visualizing them on the ultrastructural level or by specific antibodies directed against individual members of the connexin family. Functional gap junctional communication, however, can only be proven by the injection of low-molecular weight dyes into single cells and observing the dye-spread to adjacent cells, by double cell recording to determine the transfer of electrical signals, or by observing the spread of physiological signals, e.g., calcium elevations, between neighboring cells. An advantage of the dye-transfer technique is that the gap-junctional communication can be visualized on the network level and that the technique can be combined with other anatomical techniques, e.g., immunocytochemical analyses of cell types involved in the communicating network.

Today, two low molecular weight dyes are commonly used for dye coupling studies, Lucifer Yellow and biocytin-derivatives. Due to its lower molecular weight, biocytin and its derivatives allow visualization of dye coupling even in weakly coupled cells where the fluorescent compound Lucifer Yellow fails to pass gap junctions. Comparison of the spread observed with dyes of different molecular weight can thereby reveal the strength of coupling. We injected Lucifer Yellow and Neurobiotin (Vector Laboratories) into electrophysiologically identified astrocytes in slices of rat visual cortex and hippocampus. Typically, the injections resulted in a circular area containing labeled cells (Fig. 12.1A) suggesting an omnidirectional and uniform spread of the dye within the functional syncytium of coupled cells. Depending on the injection time and the tracer such areas could reach a diameter of up to 1 mm. Quantification of the density of labeled cells revealed 10,000-40,000 cells/mm². Following application of 2 mM heptanol which strongly reduces gap junctional communication, the dye accumulated in the injected cell (Fig. 12.1B) proving that dye spread occurred via gap junctions.

Counterstaining of the dye-labeled cells with an antiserum against the astroglial marker GFAP showed that the vast majority of cells were astrocytes (Fig. 12.2). The impression of a cell type-specific and quantitative coupling of astrocytes was partially supported by comparing the overall number of dye-coupled cells and the total number of astrocytes in the cortical layers and different subregions of the hippocampal formation. In the stratum radiatum of the hippocampus and in the visual cortex both values were very similar, the number of dye-filled cells slightly exceeding the number of GFAP-positive astrocytes. This finding could either reflect the inclusion of a few non-astrocytic cells in the functional syncytium, e.g., oligodendrocytes, or the presence of GFAP-negative astrocytes. The data from gray matter areas therefore suggest a dominant cell type-specific interaction among astrocytes. A clearcut exception from this generalization emerged from observations in the stratum lacunosum...