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Interneurons Targeting Similar Layers Receive Synaptic Inputs with Similar Kinetics

Rosa Cossart, Zdravko Petanjek, Dani Dumitriu, June C. Hirsch, Yehezkel Ben-Ari, Monique Esclapez, and Christophe Bernard*

ABSTRACT: GABAergic interneurons play diverse and important roles in controlling neuronal network dynamics. They are characterized by an extreme heterogeneity morphologically, neurochemically, and physiologically, but a functionally relevant classification is still lacking. Present taxonomy is essentially based on their postsynaptic targets, but a physiological counterpart to this classification has not yet been determined. Using a quantitative analysis based on multidimensional clustering of morphological and physiological variables, we now demonstrate a strong correlation between the kinetics of glutamate and GABA miniature synaptic currents received by CA1 hippocampal interneurons and the laminar distribution of their axons: neurons that project to the same layer(s) receive synaptic inputs with similar kinetics distributions. In contrast, the kinetics distributions of GABAergic and glutamatergic synaptic events received by a given interneuron do not depend upon its somatic location or dendritic arborization. Although the mechanisms responsible for this unexpected observation are still unclear, our results suggest that interneurons may be programmed to receive synaptic currents with specific temporal dynamics depending on their targets and the local networks in which they operate. © 2005 Wiley-Liss Inc.

KEY WORDS: hippocampus; CA1; GABA; glutamate; neuronal network

INTRODUCTION

GABAergic interneurons are local circuit neurons involved in numerous functions, ranging from the control of signal integration, action potential firing, and synaptic plasticity (Freund and Buzsáki, 1996; Miles et al., 1996; Hoffman et al., 1997; Cohen and Miles, 2000; McBain and Fisahn, 2001; Somogyi and Klausberger, 2005) to network oscillations and epileptic synchronization (Cobb et al., 1995; Whittington et al., 1997; Klausberger et al., 2003; Whittington and Traub, 2003). One central issue is to understand how interneuronal networks are organized to perform these multiple functions, in particular whether they are organized according to specific rules. The hippocampus provides an excellent model to study GABAergic networks, because of the wealth of morphological information available on their organization (Freund and Buzsáki, 1996; McBain and Fisahn, 2001). Morphological studies have unraveled a high degree of selectivity for the target zones of interneuron axons that form synapses with the axon initial segment, the perisomatic region, the proximal or distal dendrites of principal cells, or other interneurons (Freund and Buzsáki, 1996; Somogyi et al., 1998). This organization principle has a strong functional significance because of the location-specific nature of information processing along the somato-dendritic axis of neurons (Miles et al., 1996; Klausberger et al., 2003, 2004; Hajos et al., 2004; Pouille and Scanziani, 2004; Tamas et al., 2004). Interneurons can also be classified according to physiological and biochemical criteria, including their firing pattern (Cauli et al., 1997; Parra et al., 1998; Gupta et al., 2000), transmitter release probability, short-term plasticity (Toth et al., 2000), receptor expression (Mody and Pearce, 2004), and neurochemical content (Freund and Buzsáki, 1996; Cauli et al., 1997, 2000). When all these parameters are considered, interneurons cannot be lumped into distinct neuronal populations (Maccaferri and Lacaille, 2003; Baraban and Tallent, 2004; Jonas et al., 2004). Yet, depending on the function under study, a clear division of labor can emerge, since cell-type-specific firing patterns have been described during network oscillations (Klausberger et al., 2003, 2004; Hajos et al., 2004). There is thus a correlation between the physiology (here the cell output) and the morphology. Since neuron firing activity is primarily driven by the properties of their synaptic inputs (Traub et al., 1993; Fuchs et al., 2001; Pouille and Scanziani, 2004), we have now determined the properties of inhibitory and excitatory postsynaptic currents (IPSCs and EPSCs) received by interneurons. We specifically tested the hypothesis that interneurons projecting to the same target and thus possibly exerting a similar function may have a common physiological signature. To aim this, using a quantitative method (cluster analysis of morphometric and physiological variables) to identify families of interneurons without a priori assignment of possible cell classes, we compared the properties of synaptic currents in some

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of the best-described types of hippocampal interneurons. We report that CA1 interneurons cluster into four different families, the primary segregating set of variables being the laminar distribution of their axon along the hippocampal layers and the kinetics of the synaptic currents they receive. Although single interneurons receive a large collection of synaptic inputs in terms of kinetics (Hajos and Mody, 1997), the distributions of single event kinetics are specific to interneuron-families; a family being characterized by interneurons whose axons target the same hippocampal layer(s). A simple mechanistic explanation for this property has proven difficult to be provided at this stage. However, our results suggest that there might be a precise organizational rule for interneurons in the hippocampus, the synaptic current kinetics providing a physiological signature for cells sharing similar targets.

MATERIALS AND METHODS

Electrophysiology

Transverse 400-μm-thick slices were cut from the hippocampus of 14- to 21-day-old Wistar rats, using a Leica VT 1000S tissue slicer (Leica, Bensheim, Germany). Animals were sacrificed under chloral hydrate (350 mg/kg) anesthesia in accordance with institutional guidelines. Slices were superfused continuously with a solution containing 124 NaCl, 3 KCl, 1.25 KH_2PO_4, 26 $NaHCO_3$, 1.3 $MgSO_4$-$7H_2O$, 2 $CaCl_2$, 10 D-glucose, bubbled with 95% O_2 and 5% CO_2. Interneurons were visualized by infrared video microscopy, using an upright Leica DM low-frequency stimulation microscope equipped with a 40× objective (Leica, Bensheim, Germany). Patch pipettes were pulled from borosilicate glass tubing (2.0 mm outer diameter, 0.5 mm wall thickness) and filled with internal solutions containing 135 Cs-gluconate, 10 $MgCl_2$, 0.1 $CaCl_2$, 1 EGTA, 2 Na_2 adenosine triphosphate, 10 HEPES, 0.5% biocytin pH 7.25. For whole-cell recordings, the resistance was 3–8 MΩ. Uncompensated series resistances were 6–30 MΩ. The recording temperature was 30–32°C. Signals were fed to an EPC9 (HEKA, Heidelberg, Germany), digitized (10 kHz) with a Labmaster interface card to a personal computer and analyzed with MiniAnalysis 5.1 program (Synaptosoft, Decatur, GA). Spontaneous $GABA_A$ receptor-mediated currents were measured at the reversal potential for glutamatergic events (+10 mV). Bicuculline, a $GABA_A$ receptor antagonist, was applied at the end of the experiments to verify that the currents were indeed GABAergic. Spontaneous glutamatergic currents were measured at the reversal potential for $GABA_A$ receptor-mediated events (−60 mV) and were blocked by CNQX/D-APV. The frequency of spontaneous events was calculated (number of events in a 180-s time period) and TTX was added to the saline to record miniature events. The frequency of miniature events was calculated (number of events in a 180-s time period). Single and averaged miniature events recorded (∼200 events per cell) were fully characterized: rise times (10–90%), amplitudes (maximum and amplitudes of first and second decay components), and decay time constants were calculated using MiniAnalysis 5.1. To discriminate between single events best characterized by single or double decay phases, we used the following criterion implemented in the software: if the root square value obtained with a double exponential fit differed by more than 5% from the one obtained when fitting with a single exponential decay, then the event was characterized by two taus (tau1 and tau2), otherwise a single tau was taken into account. However, for the purpose of cluster analysis of the physiological dataset, we fitted all averaged events with double exponential functions (as the cluster analysis requires events to be fitted in the same way). Minimal stimulation of GABAergic inputs (50% failure rate) was performed with a glass electrode filled with saline placed near the cell body of interneurons (around 100 μm) to evoked IPSCs.

Experimental values are given as means ± SEMs (or standard deviations when indicated otherwise). Student's *t*-test and Chi-square test were used for statistical comparisons. $P < 0.01$ was considered significant, taking into account the correction for multiple comparisons. Distributions of decay time constants were determined for each identified cell. Any given distribution was compared with all others by using the chi-square test.

Morphology

Slices were processed for the detection of biocytin-filled neurons. They were fixed overnight at 4°C in a solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). After fixation, slices were rinsed in PB, cryoprotected in sucrose, and frozen quickly on dry ice. To neutralize endogenous peroxidase, slices were pretreated for 30 min in 1% H_2O_2. After several rinses in saline phosphate buffer (0.01 M phosphate buffered saline (PBS), pH 7.4), slices were incubated for 24 h at room temperature in 1/100 avidin-biotin peroxidase complex (Vector Laboratories, Inc, Burlingame, CA) diluted in PBS containing 0.3% Triton X-100. After 30 min rinses in PBS, slices were processed with 0.04% 3-3′-diaminobenzidine-HCl (DAB, Sigma, St. Louis, MO) and 0.006% H_2O_2 diluted in PBS. One hundred and eleven biocytin-filled neurons were identified as interneurons. Among these 111 interneurons, we could obtain both a complete characterization of mEPSCs/mIPSCs and a complete axonal and dendritic labeling from 31 interneurons. These interneurons were reconstructed for morphometric analysis with a computer-assisted system (Neurolucida, Microbrightfield Inc, Williston, VT) attached to a Nikon microscope (Tokyo, Japan). Morphological variables thus obtained included somatic area, total dendritic and axonal lengths, total dendritic and axonal surfaces, total dendritic and axonal volumes, total number of dendritic and axonal branches, percentage of dendritic and axonal lengths per layer, and sholl parameters of the dendrite tree. The total surfaces and total volumes of dendrites or axons (branched structures) are estimated values calculated by the software. They are computed by modeling each piece of each branch as a frustum. A frustum is the shape formed by a right circular cone that has been truncated. The total length of dendrites or axons is the sum of the lengths of all the branches.

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Multivariate Analysis

Cluster analysis and correlation matrices for morphological and physiological data were performed using Statistica software (StatSoft, Inc.). Factor analysis using the principal component method (PCA) was performed using the same software.

Cluster analysis

The term "cluster analysis" refers to a set of multivariate exploratory statistical methods that group objects (cases) of a data set based on their degree of similarity. All cases are first plotted in a multidimensional space defined by all the measured variables [we included in this study 25 physiological variables: input resistance, membrane capacitance, series resistance, 10–90% rise time for averaged mEPSC and mIPSC, absolute and relative amplitude of first and second exponential components for averaged mEPSC and mIPSC, decay time values for first and second exponential components fitting averaged mEPSC and mIPSC, averaged frequencies for mEPSCs, mIPSCs, sEPSCs, sIPSCs; and 24 morphological variables: absolute and relative dendritic lengths in all hippocampal layers (O, P, R, and LM), absolute and relative axonal lengths in all hippocampal layers (O, P, R, and LM), total axonal and dendritic lengths, number of dendritic segments, axonal and dendritic surfaces and volumes, cell body area].

A certain measure of proximity is chosen (distance), and clusters are eventually formed by the cases that fulfill the criteria of the clustering method selected. Our analysis was performed with Euclidean distances by using Ward's method. According to Ward's method, cases are assigned to clusters so that the variance (sum of squared deviations from the mean) within each cluster is minimized. This method resulted in well-defined groups in this study.

Principal component analysis (PCA)

We used PCA to outline variables that contribute most to the overall variability, and thus, were more important in distinguishing different cell classes. PCA is a data-mining method. It transforms the original data set into a set with fewer variables. These components are sorted in descending order of their contribution to the overall variance. Variables that account for most of the variability should better reflect differences among different cell types. The goal is to replace the original data set (seen as a matrix) by another matrix with fewer variables (or dimensions) while preserving as much of the original information as possible. Measurements for each variable in our data set were transformed to standard scores across all cells based on the commonly used formula (xi-mean)/s. Standardization leads to unit-less values, thus removing any arbitrary effects due to the choice of variable units. In addition, by conforming all variables to the same range of values, all are weighted equally (Romesburg, 1984). In the correlation matrix, certain variables were highly correlated ($r = 0.8$) with each other, for example, absolute and relative amplitude for averaged miniature currents. This means that these variables reflected similar features. To avoid artificially weighting these variables (or features) in the cluster analysis, one of the two was excluded. Of the pairs of highly correlated variables, the one with the lower value in the principal component loadings table was removed.

Data reduction in PCA was achieved by selecting a subset of principal components based on the Kaiser criterion (Kaiser, 1960) and scree plot (Cattell and Coulter, 1966), according to which, selected principal components have an eigen value greater than or equal to 1. The scree plot is a graph of the eigen values vs. their ordinal value. An acceptable threshold is set where this graph plateaus: factors beyond that threshold do not add significantly to the overall variance and, therefore, can be excluded.

RESULTS

Segregation of CA1 Interneurons According to the Laminar Distribution of their Axon

We performed whole-cell recordings from interneurons in all layers of the CA1 hippocampal region, using biocytin-filled electrodes. In this study, we included the interneurons that could be fully morphologically identified and for which mIPSCs and mEPSCs were characterized (Fig. 1a, $n = 31$): stratum oriens-lacunosum moleculare (O-LM) cells ($n = 5$) (McBain, 1994; Sik et al., 1995), Perforant Pathway-associated interneurons ($n = 5$) (Vida et al., 1998), Perisomatic projecting cells (i.e. Basket and Axo-axonic cells, $n = 7$) (Harris et al., 1985; Kosaka et al., 1985; Maccaferri et al., 2000; McBain and Fisahn, 2001), Bistratified stratum oriens interneurons ($n = 4$) (Sik et al., 1995), Schaffer collateral-associated interneurons ($n = 6$) (Vida et al., 1998; Cope et al., 2002), Trilaminar stratum radiatum interneurons ($n = 3$) (Freund and Buzsáki, 1996), and Septum-projecting stratum oriens interneurons ($n = 1$) (Tóth and Freund, 1992; Gulyas et al., 2003). For simplification, we used the X-AB terminology that takes into account the cell body location and the laminar distribution of the axon. Thus, the X-AB cell type corresponds to interneurons with a soma in stratum X and an axon projecting to strata A and B (Fig. 1a). The projection zone of the axon is defined as the layer(s) in which the synaptic contacts are established (Freund and Buzsáki, 1996). It does not include the regions the axon may cross without making any synaptic contact before reaching its target zone. In this study, we assessed the presence of *boutons en passant*, i.e., presumed synaptic contacts, to define the projection zone. For example, O-LM cells have a soma in stratum oriens and a projection zone in stratum lacunosum moleculare (Fig. 1a). The axon originates from the soma or a proximal dendrite, then descends vertically to stratum lacunosum moleculare, crossing stratum oriens, stratum pyramidale, and stratum radiatum. Varicosities (presumed presynaptic terminals) are present only on the axonal branches in stratum lacunosum moleculare, while the branches in stratum pyramidale and stratum radiatum are smooth (devoid of varisosities). Therefore, although an axon may cross different regions before reaching its target area, the

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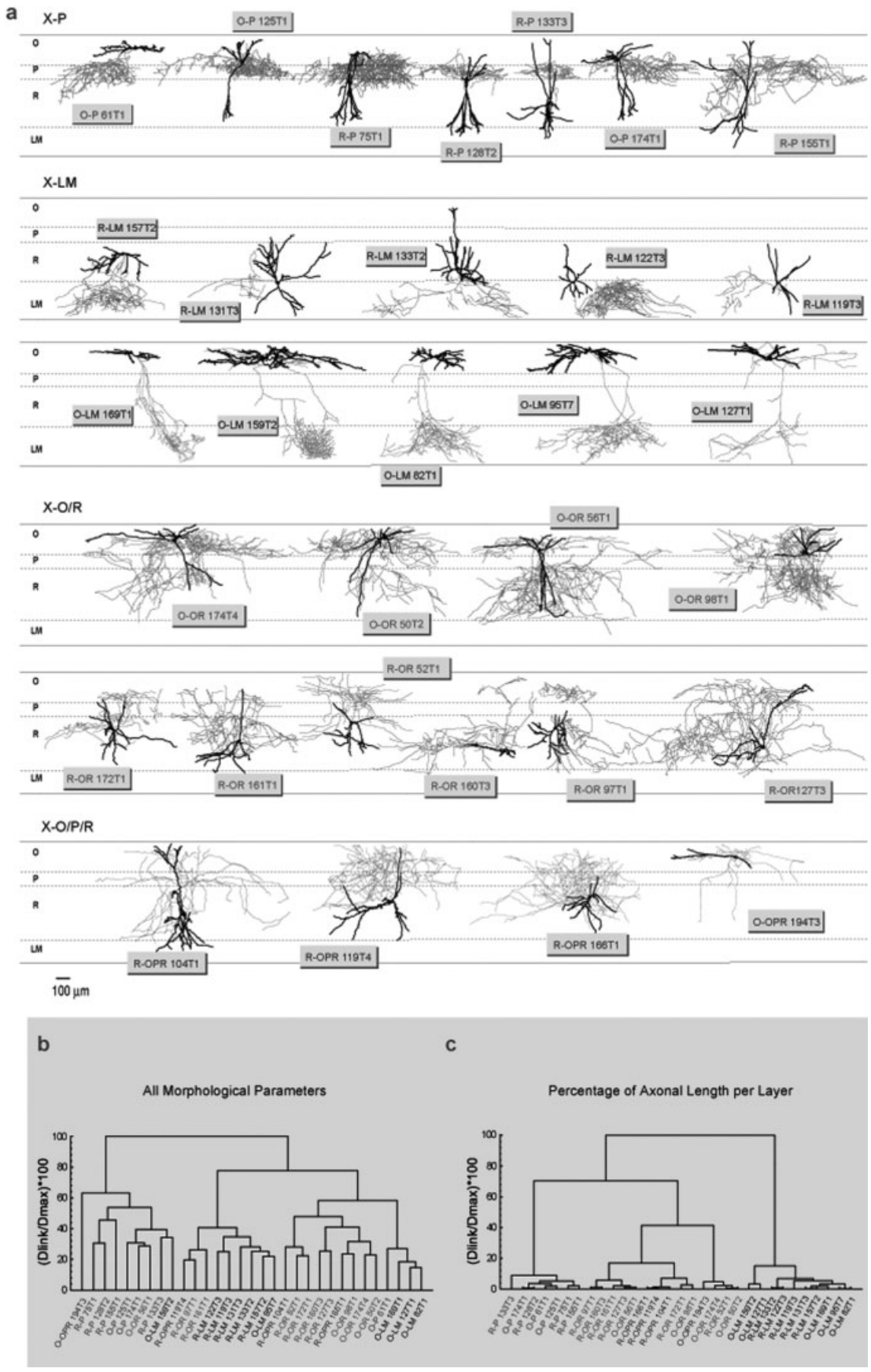

FIGURE 1

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latter is defined by the presence of synaptic contacts. In the following, a cell type designates a neuron with a morphology that is universally agreed upon, like O-LM cells (only the nomenclature may change as Schaffer collateral-associated interneurons, called R-OR cells in this study). Thus, O-LM, R-LM, and LM-LM cells are three different cell types. A family, a term we introduce below, is a collection of cell types that target the same layer(s), here stratum lacunosum moleculare.

We first asked whether interneurons from our database could be segregated according to the axonal distribution across the different hippocampal layers vs. all other morphological parameters. To address this question, we have performed a multivariate analysis of the morphometric data obtained after three-dimensional reconstruction of the interneurons. Twenty-four morphometric variables, including the length and percentage of axonal and dendritic trees in each hippocampal layer, were analyzed. Out of these variables, 20 were statistically independent (less than 80% correlation between them) and could thus be included in the cluster analysis. When including all the independent morphological variables into the analysis, the data would not successfully cluster along the seven previously described morphological types (Fig. 1b). This observation confirms that there is a considerable heterogeneity in general morphometric properties even within interneurons that belong to a given cell type (for example, O-LM cells). We then divided the 20 morphological variables into subgroups comprising somato-dendritic or axonal morphometric parameters, including the laminar distribution of dendritic or axonal arbors. Four cluster analyses were performed (Ward's method, Euclidian distances) taking into account successively each variable subgroup. The only set of variables that could divide the data into a meaningful cluster structure, with short distances (less than 30%) between any pair of elements within the same cluster, was the percentage of axon within the different layers (Fig. 1c). Each cluster contained different cell types that shared the same axonal laminar distribution. We call each cluster a family. For example, the X-LM family includes the O-LM and R-LM (Perforant pathway associated) cell types. It must be noted that the X-OPR cells clustered together, but within the X-OR family since both cell types cross stratum pyramidale. The major difference is that X-OPR cell axons display *boutons en passant* within stratum pyramidale while X-OR cell axons do not. Therefore, X-OPR and X-OR cells belong to two different morphological families. When taking into account the laminar distribution of the dendritic tree alone, we could also divide our dataset into groups of cells with short Euclidian distances but with no functional significance, since interneurons from a given cell type belonged to different clusters (not shown).

We conclude that the unbiased cluster analysis allows organizing our interneuron database into meaningful families, according to the laminar distribution of the axon.

Averaged Kinetics is Interneuron Family-Specific

We then questioned the physiological relevance of the families emerging from the cluster analysis performed earlier. We thus examined whether interneurons belonging to the same family had a common physiological feature. Since the pre- and postsynaptic properties of neurotransmission depend on both the nature of the source and the target (including the kinetics of postsynaptic currents) (Gupta et al., 2000; Maccaferri et al., 2000; McBain and Fisahn, 2001; Walker et al., 2002; Mori et al., 2004; Pouille and Scanziani, 2004), each cell type could have a unique, specific repertoire of synaptic properties. We focused on action potential-independent miniature inhibitory and excitatory postsynaptic currents (mIPSCs and mEPSCs), which represent quantal events. Miniature IPSCs and EPSCs were recorded in each interneuron in the presence of the sodium channel blocker TTX (1 μM) at the reversal potential for glutamatergic (V_{hold}, +10 mV) and GABAergic currents (V_{hold}, −60 mV), respectively. The average frequency of mEPSCs and mIPSCs was 1.7 ± 0.3 and 6.1 ± 0.6 Hz, respectively ($n = 31$). There was no obvious relationship between the frequency of mEPSCs or mIPSCs measured in a given interneuron and the family it belonged to. The rise times and decay time constants were then calculated for each miniature event recorded from each morphologically identified interneuron. Rise times and decay time constants displayed a high degree of variability around the mean in a given cell, resulting in the dispersion of the probability distribution functions (see Fig. 6). This variability, found in all 31 recorded interneurons, confirms that interneurons are characterized by an extensive repertoire of kinetics for their excitatory and inhibitory inputs (Hajos and Mody, 1997; Nusser et al., 2001; Tamas et al., 2004). Before taking into account this variability, we examined the kinetic properties of averaged mEPSCs and mIPSCs in each interneuron. For the purpose of comparison, all decay phases of averaged mEPSCs and mIPSCs were fitted by double exponential functions (see methods).

FIGURE 1. Cluster analysis of morphometric properties segregates the recorded CA1 interneurons into four groups based on the laminar distribution of their axon. (a) Composite drawings of the 31 biocytin-labeled interneurons, from the CA1 subfield of the rat hippocampus, reconstructed with the neurolucida workstation. Each interneuron is named according to the location of its soma (X), the main layers (ABC) targeted by its axon and an experimental number. The somato-dendritic domains of these interneurons are represented in black. Neurons are divided into four groups as a function of the main layers targeted by their axon (color): stratum pyramidale (X-P, red), stratum lacunosum moleculare (X-LM, blue), strata oriens and radiatum (X-OR, green), and strata oriens, pyramidale, and radiatum (X-OPR, gray). Scale bar: 100 μm. (b,c) Cluster analysis trees of the dataset (Ward's method, D link: Euclidian distances) based on all morphological parameters (b) and the percentage of axonal length per layer (c). Same color code as in (a). Distances were normalized. Interneurons that projected to the same layers clustered together.

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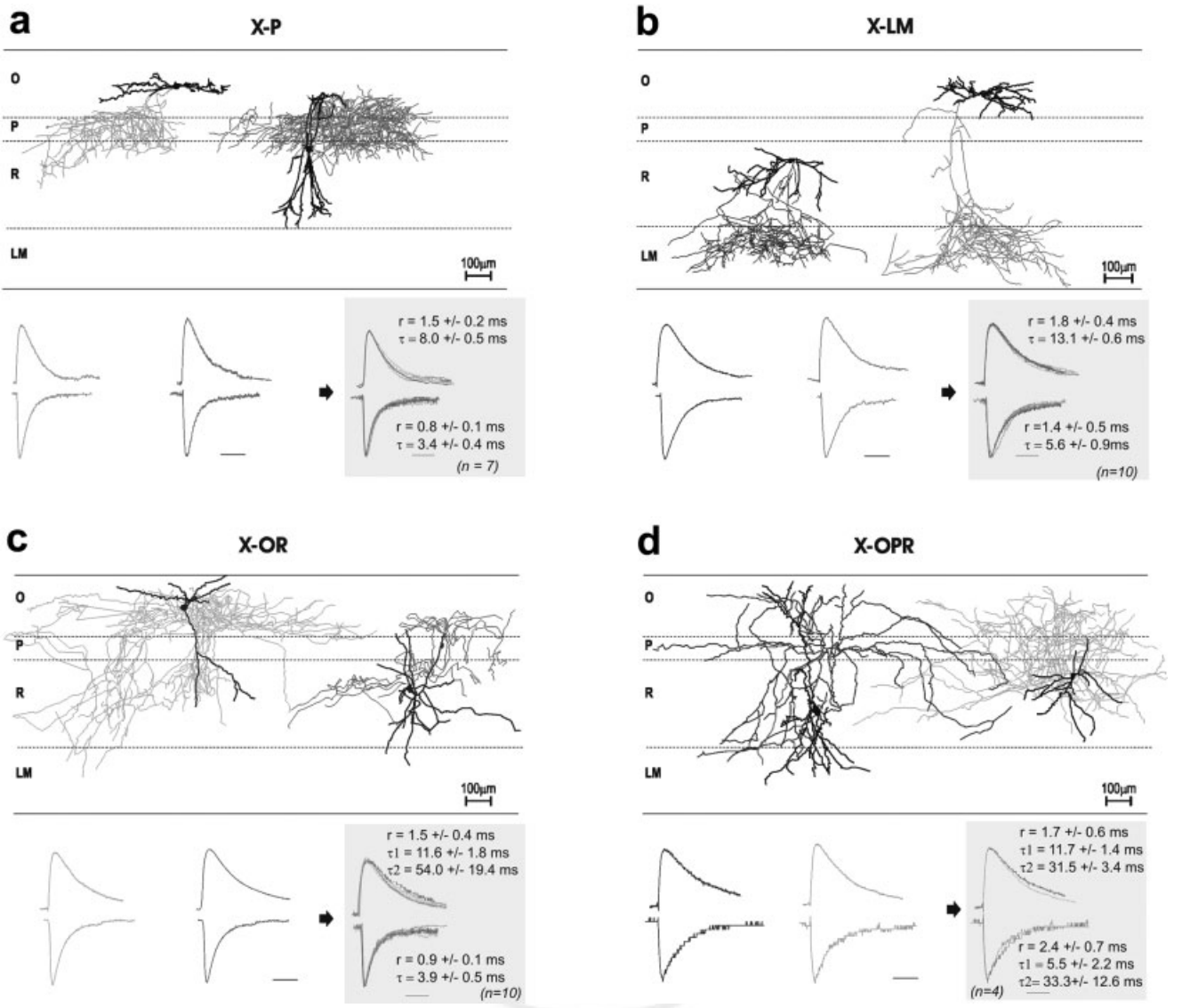

FIGURE 2. Comparison of the kinetics of averaged mIPSCs and mEPSCs recorded in the four different morphological groups of CA1 interneurons. CA1 interneurons with an axon that innervates the same layers receive GABAergic and glutamatergic miniature currents with identical kinetics. Montage of neurolucida reconstructed interneurons illustrating two types of neurons within the four morphological families segregated by the cluster analysis (X-P, X-LM, X-OR, and X-OPR). (a) The axons of the two interneurons illustrated in 1 (orange and red) innervated the stratum pyramidale (P), but their cell body location [stratum oriens (O), P, or stratum radiatum (R)], dendritic morphology (in black) were very different. Miniature IPSCs (outward currents) and EPSCs (inward currents) were recorded at the reversal potential for glutamatergic and GABAergic events, respectively (V_{hold}, +10mV and –60mV) in the presence of TTX. Each trace shows the normalized average, 100–500 single miniature events were averaged) corresponding to each interneuron type illustrated earlier. Averaged mIPSCs (top traces) displayed similar time courses. Averaged mEPSCs also displayed similar time courses (bottom traces). Gray box: superimposition of normalized mIPSCs and mEPSCs recorded from all the interneuron of this family, i.e., stratum pyramidale projecting interneuron. The kinetics is similar within the P-projecting family, regardless of the cell body location. *r* and τ are, respectively, the averaged value of the 10–90% rise time and of the decay time constants calculated for each type of interneuron. Scale bar: 10 µs. Values are given as mean ± standard deviation (SD). (b, c, and d) Same as in (a) for the other families: interneurons with an axon that innervates stratum lacunosum moleculare (LM, blue), strata oriens and radiatum (OR, green), or strata oriens, pyramidale, and radiatum (OPR, gray). The averaged mIPSCs and mEPSCs obtained for all interneurons belonging to these three families overlapped.

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Kinetics of averaged miniature currents were strikingly homogenous within interneurons belonging to a given cell type (Fig. 2). For example, all O-LM cells had averaged mEPSCs (or mIPSCs) with similar kinetics (Fig. 2). We then compared the kinetics of averaged miniature currents between any two given types of interneurons. While kinetics differed sharply between some cell types, others completely overlapped. Unexpectedly, cell types that displayed similar kinetic properties

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belonged to the four morphological families previously determined by cluster analysis (i.e., the axon of these interneurons had similar projection zones). Moreover, the decay times of the averaged mIPSCs and mEPSCs were statistically different between any two families of interneurons and very similar within one given family (student *t*-test, $P < 0.01$ and $P > 0.1$; Fig. 2).

Space clamp properties and dendritic morphology might induce an electrotonic filtering specific to a given family that would explain the difference in the kinetic properties between families. A bias due to the quality of our recordings or to the fact that a family had similar membrane properties is very unlikely, since series resistance, membrane capacitance, or input resistance were not correlated with decay time constants of currents (less than 30% correlation in the correlation matrix) and were extremely heterogeneous within a given family. There was no correlation either between the kinetics and the soma location or the basic morphometric properties of the dendrites or the axon. Interneurons that belonged to different families could cluster in the same group when cluster analysis was performed on dendritic variables (e.g., O-P 61T1, O-OR 98T1, and O-LM 169T1). The length of the axonal arborization for various types of interneurons within a same family could also be different (such as O-LM 159T2 and R-LM 131T3). Finally, we computed the dendritic sholl as the percent length of dendrite and the percent number of nodes in 50 μm concentric circles and performed cluster analysis of our interneuron database using independent dendritic sholl variables (25 out of the 30 measured variables were uncorrelated). The cells that clustered together did not match the families previously defined (not shown). Therefore, it is unlikely that the dendritic architecture, membrane properties, or recording conditions should be responsible for the fact that synaptic input kinetics are very similar within each family.

We conclude that the decay time constants of averaged mEPSCs or mIPSCs constitutes a physiological signature for a given family of interneurons.

Kinetics and Axon Laminar Distribution: A Morpho-Physiological Correlation

To determine whether the kinetics of the postsynaptic currents indeed represented an effective classification criterion, we performed a multivariate analysis of the physiological data. We measured 25 physiological variables characterizing membrane and clamp properties, frequency, amplitude and kinetics of miniature currents, as well as frequency of spontaneous events (Fig. 3 and 4, see methods). Out of these variables, 19 were statistically independent (less than 80% correlation between F3,F4

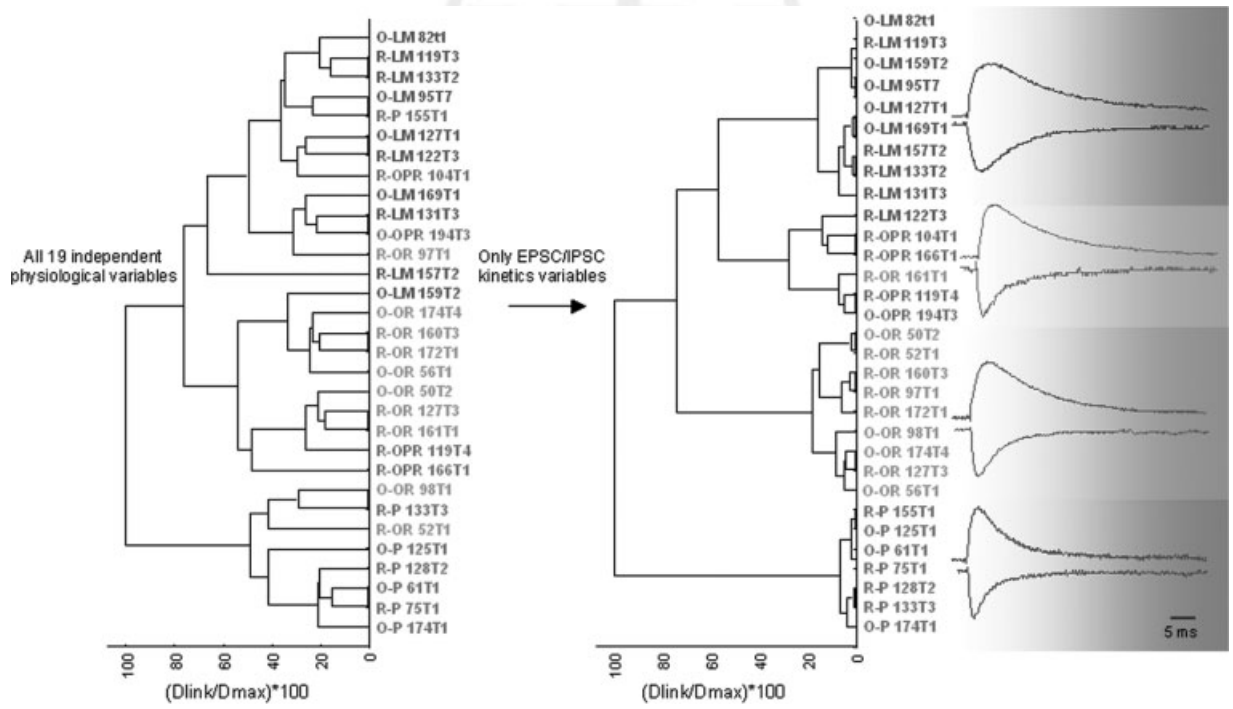

FIGURE 3. Cluster analysis based on synaptic currents kinetics segregates CA1 interneurons into the same families defined by the morphological clustering. Left: cluster analysis tree of all 19 independent physiological variables describing the 31 cells that segregated into four different families (red, X-P; blue, X-LM; green, X-OR; and gray, X-OPR). These variables are presented in Figure 4. They include intrinsic membrane properties (R_m, C_m), series resistance, kinetics ($r_{10–90}$, tau_1, tau_2, and $tau_{90–37}$) and amplitudes of miniature excitatory and inhibitory postsynaptic currents averages (EPSC/IPSC), and frequencies of miniature and spontaneous EPSCs/IPSCs. Each average is composed of 100–300 miniature PSCs. Right: same as left but only miniature EPSC/IPSC kinetics variables are taken into account. When taking into account only kinetics, interneuron segregate into four groups that correspond to the four previously determined morphological families.

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		LM	OR	P	OPR
	Rm (mΩ)	507 ± 294	738 ± 640	621 ± 237	600 ± 318
	Cm (pF)	183 ± 121	103 ± 59	99 ± 46	100 ± 10
	r_{10-90} (ms)	1.4 ± 0.5	0.9 ± 0.1	0.8 ± 0.1	2.4 ± 0.7
	τ_1(ms)	5.6 ± 0.9	3.9 ± 0.5	3.4 ± 0.4	5.5 ± 2.2
	τ_2(ms)	8.4 ± 6.8	6.8 ± 9.2	3.4 ± 0.4	33.3 ± 12.7
	tau_{90-37} (ms)	5.8 ± 1.5	4.0 ± 0.6	3.3 ± 0.5	7.3 ± 3.0
AMPA/KA	amp (p A)	18.7 ± 11.6	12.5 ± 4.3	16.3 ± 4.4	10.1 ± 2.4
	amp 1 (%)	60 ± 12	65 ± 23	47 ± 13	62 ± 8
	amp 2 (%)	40 ± 12	37 ± 22	52 ± 14	38 ± 8
	f_{EPSC} (hz)	5.2 ± 6.1	4.8 ± 3.0	9.5 ± 11.6	1.0 ± 0.5
	f_{mEPSC} (hz)	1.5 ± 2.2	1.5 ± 2.4	1.8 ± 1.5	0.3 ± 0.2
	r_{10-90} (ms)	1.8 ± 0.4	1.5 ± 0.4	1.5 ± 0.2	1.7 ± 0.6
	τ_1(ms)	13.1 ± 0.6	11.6 ± 1.8	8.0 ± 0.5	11.7 ± 1.4
	τ_2(ms)	13.0 ± 0.5	54.0 ± 19.4	8.0 ± 0.5	31.5 ± 3.4
	tau_{90-37}	12.6 ± 0.4	13.4 ± 2.8	8.2 ± 0.8	13.2 ± 1.6
GABA	amp (p A)	16.4 ± 8.9	18.3 ± 8.8	20.2 ± 2.7	20.1 ± 7.8
	amp 1 (%)	52 ± 9	71 ± 13	56 ± 10	71 ± 14
	amp 2 (%)	45 ± 11	29 ± 13	44 ± 10	32 ± 13
	f_{IPSC} (hz)	9.3 ± 6.5	5.9 ± 3.0	14.5 ± 8.9	5.2 ± 5.9
	f_{mIPSC} (hz)	5.1 ± 4.0	3.4 ± 2.0	9.2 ± 6.8	2.8 ± 2.1
n		10	10	7	4

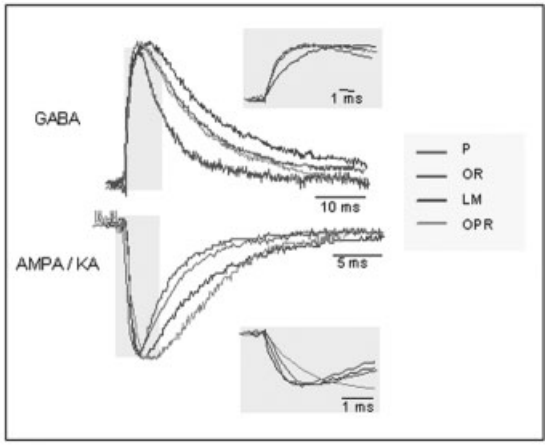

FIGURE 4. Table summarizing for each family the values obtained for the 21 physiological parameters taken into account. R_m, membrane resistance; C_m, membrane capacitance; r_{10-90}, 10–90% rise time; τ_1, first exponential decay; τ_2, second exponential decay; tau_{90-37}, 90–37% decay; amp, amplitude of the average PSC; amp1, fraction of the total amplitude corresponding to the first exponential decay; amp2, fraction of the total amplitude corresponding to the second exponential decay; f_s, average frequency of spontaneous PSCs; f_m, average frequency of miniature PSCs (in the presence of TTX 1 μM); P, pyramidale; O, oriens; R, radiatum; LM, lacunosum moleculare. The averaged mIPSCs (top trace) and mEPSCs (bottom trace) obtained for each family are superimposed, showing the different kinetics between the families. Four families of CA1 interneurons could be defined by cluster analysis based on the projection zone of their axon. Values are given as mean ± SD.

them in the correlation matrix) and could thus be implemented in the cluster analysis.

When including all the independent physiological variables into the analysis, the data would not significantly cluster (long Euclidian distance between cells within the same cluster and dispersion of identical cell types into different clusters, see Fig. 3). Similar to the morphometric analysis, we then divided the physiological variables into subgroups and performed independent cluster analyses. The only group of variables that induced a significant clustering of the data was the kinetics variables (i.e., the first and second exponential decays of mEPSCs and mIPSCs, Fig. 3). The Euclidian distance between cells within the same group was short (less than 30% or less than 5 absolute distance value), indicating that families were defined according to the homogeneity of kinetics parameters. Any other combination of variables was ineffective in obtaining clustering (including membrane properties, spontaneous and miniature currents frequencies or amplitudes). Furthermore, except for two cells, the clusters thus obtained were identical to the families defined by the analysis of the morphological data.

To test for the robustness of the correlation between synaptic input kinetics and axonal laminar distribution, we performed three different tests.

First, we found that interneurons clustered into the same families when the axonal distribution and synaptic kinetics were selected as a new set of analysis variables (Fig. 5). Second, we considered the entire set of 64 independent variables and performed principal component analysis (PCA) on these variables to extract the variables that correlated by more than 70% with the first and second principal components. We found that the key variables were the decay kinetics of mIPSCs (tau1, tau2, and %a1) and the axonal laminar distribution in the LM and the P layers (not shown).

To test if the specificity we describe can be generalized to other types of synaptic activities, we measured the kinetics of action-potential-dependent IPSCs and EPSCs, which can result from the simultaneous activation of multiple synapses. Spontaneous glutamatergic activity (in the absence of TTX) is a mixture of action-potential-dependent and -independent events. The mean frequency of sEPSCs was 4.8 ± 0.8 Hz ($n = 17$ interneurons). At this frequency, sEPSCs seldom overlapped. This allowed to resolve single events and to measure their kinetics. As for mEPSCs, the shape of averaged and scaled sEPSCs as well as the distributions of their decay time constants were similar for interneurons belonging to the same family (not shown, $n = 10$ X-LM, $n = 5$ X-O/R, and $n = 2$ X-P). The mean frequency of sIPSCs was 11.1 ± 1.2 Hz ($n = 17$ interneurons). Since single, nonoverlapping events are difficult to sample at this frequency, we examined IPSCs evoked by minimal stimulation of GABAergic fibers near the soma of the recorded interneuron. We found that the averaged and scaled traces of evoked IPSCs were similar within each family of interneurons and that they fitted the shape of averaged mIPSCs (not shown).

We conclude that the 4 families in our interneuron database (X-P, X-LM, X-OR, and X-OPR) share homogeneous morpho-physiological properties.

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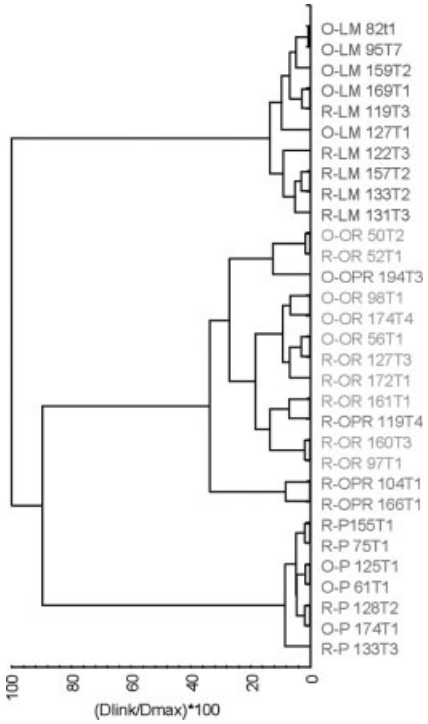

FIGURE 5. Cluster analysis based on synaptic currents kinetics and axonal laminar distribution. Cluster analysis tree of the dataset based on six independent morpho-physiological variables describing the percentage of axonal length per layer and the averaged synaptic inputs kinetics (Ward's method; D link, Euclidian distances). Distances are normalized. Same color code as in previous figures. Significant clustering was obtained when taking into account the axonal projection zone and the synaptic input kinetics.

The Repertoires of Kinetics are Family-Specific

Although the average of miniature synaptic currents was a useful parameter to tentatively propose a morphological–physiological organizational rule for CA1 interneurons, it did not cover the extent of the distribution of kinetics found in each interneuron. Indeed, kinetics of synaptic currents display great variability within a given cell (Hajos and Mody, 1997; Nusser et al., 2001; Tamas et al., 2004), even at unitary connections (Biro et al., 2005), reflecting different receptor subunit composition, dendritic filtering, space clamp problems, etc. To take into account this variability, we thus fitted the rise and decay phases of all single miniature events recorded from each interneuron in our database. Rise times and decay time constants displayed a high degree of variability around the mean in a given cell, resulting in the dispersion of the probability distribution plots for these two parameters (Fig. 6). This variability shows that interneurons are characterized by an extensive repertoire of kinetics for their glutamatergic and GABAergic inputs measured at the soma in keeping with previous reports (Hajos and Mody, 1997). Despite this variability within a single cell, cumulative histograms for decay times overlapped in interneurons of a given family and were statistically different between any two families (Fig. 6, Chi-square test, $P > 0.8$ and $P < 0.01$, respectively). Therefore, interneurons with similar axonal projection zones display the same repertoire of decay times for miniature currents measured at the soma.

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DISCUSSION

Our results show a correlation between the repertoire of decay times of miniature synaptic currents measured at the soma in an interneuron and the layer distribution of its axon. We propose that a precise organizational rule links the axonal target zone to the synaptic inputs on interneurons, probably reflecting a spatio-temporal precision in the construction of hippocampal GABAergic networks.

We have used interneurons for which the morphological classification was unequivocal, i.e., with an extensive arborization in the slice. In vitro labeling of the axonal arborization of interneurons shows only quantitative differences with in vivo labeling in terms of number of axonal branches, total length, etc, but no qualitative difference in terms of projection zone (Sik et al., 1995; Klausberger et al., 2003). The projection zone of the axon is the region where the synaptic contacts are established. It excludes the layers the axon may cross without making any synaptic contact before reaching its target zone. Thus, O-LM cell axonal branches are smooth in stratum pyramidale and stratum radiatum (devoid of *boutons en passant* and thus of presumed synaptic contacts), while covered with varicosities in stratum lacunosum moleculare (Freund and Buzsáki, 1996). Using a nonbiased quantitative method, we found that the different CA1 interneuron cell types included in our database clustered into four different families according to the laminar distribution of their axon. This result was expected since the morphological characterization of interneurons according to the target zone of their axon has proven a very meaningful classification in several studies (Freund and Buzsáki, 1996). The cluster analysis we performed in this study, however, shows that this qualitative classification can be quantified using a statistical approach.

The physiological characterization is based on the recording of synaptic currents. The kinetics of synaptic events recorded at the soma is different from its actual value at the postsynaptic site, as it is altered by two factors: (i) space clamp problems (inherent to the measurement) and (ii) dendritic filtering (a cell property). (i) Space clamp problems arise from the distance between the recording electrode and the active synapses, and by the quality of the recording. As a consequence, space clamp problems and series resistance prevent an accurate measurement of the absolute values of decay time constants, and the values reported here should be considered as relative. The classification we provide could be biased if the quality of our recordings was specific to each interneuron family and if cells within a family had similar membrane properties. However, series resistance, membrane capacitance, or input resistance were not correlated with decay times of currents and were heterogeneous within a given family as shown by the cluster analysis. These arguments suggest that space clamp problems (including series

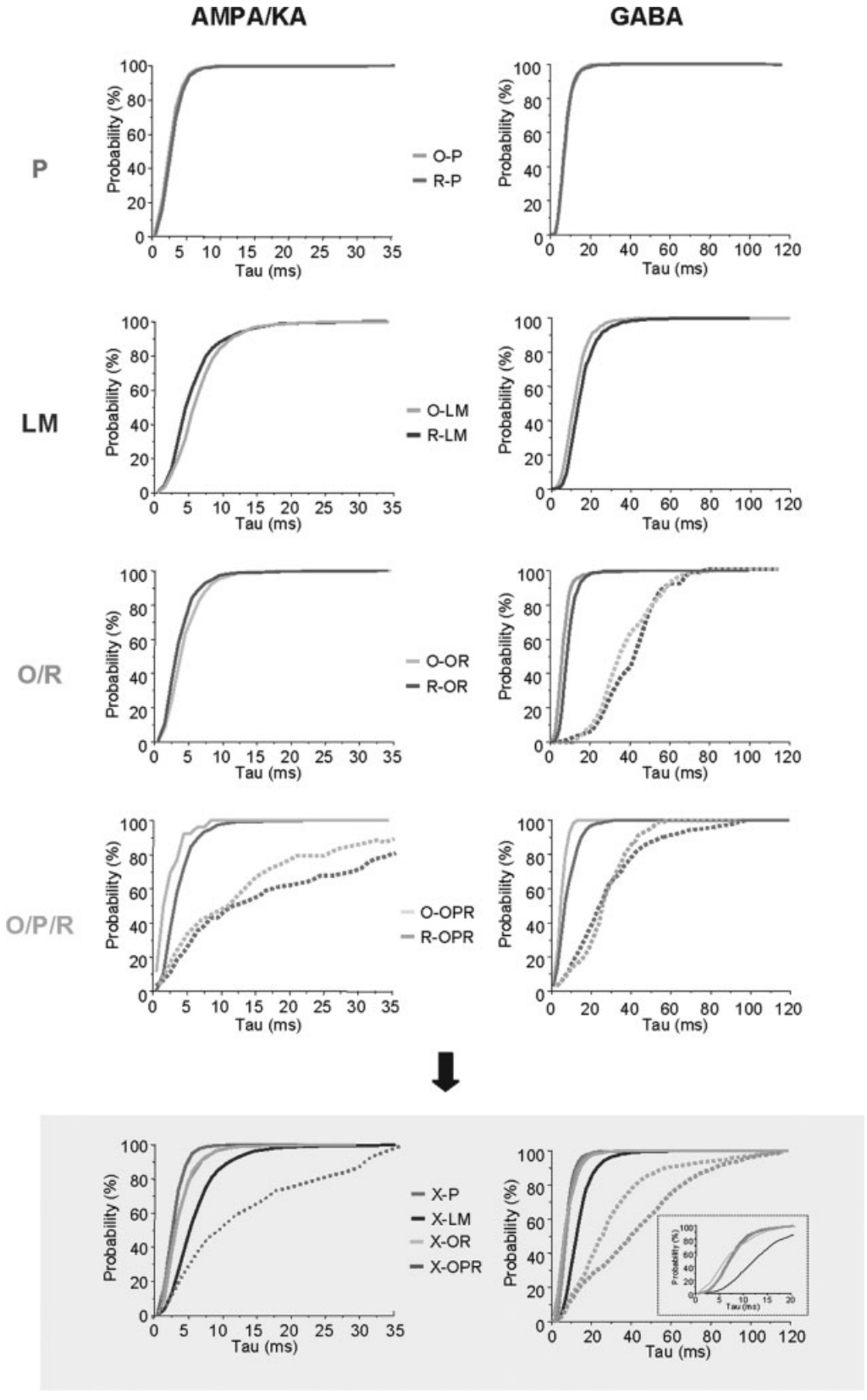

FIGURE 6. Cumulative distributions of decay times overlap for cells belonging to the same family. Comparison of the cumulative distributions of decay time constants of single mEPSCs (AMPA/KA-R-mediated, left) and mIPSCs ($GABA_A$-R-mediated, right) for each family defined by the laminar distribution of its axon (P, pyramidale; O, oriens; R, radiatum; LM, lacunosum moleculare). Each cumulative distribution plot represents the pooled decay time values for 200 consecutive single events from all cells in our dataset in a given morphological type. tau1, plain line; tau2, dotted line. The distributions of decay times do not depend on the cell body location for interneurons targeting the same layers of the CA1 region, but are different between interneuron innervating different areas (see comparison bottom gray, chi-test, $P < 0.01$).

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resistance), although affecting measurements (i.e., the absolute values of decay time constants), do not bring an artificial bias to the morpho-physiological correlation itself (the error is the same across the population of recorded cells). (ii) Dendritic morphology might induce an electrotonic filtering specific to a given family. Inconsistent with this hypothesis is the fact that cluster analysis performed on dendritic morphometry and kinetics parameters failed to produce coherent groups. Finally, it can be argued that interneurons within a given family might receive inputs from identical origins. This argument does not hold at least for the X-LM family: morphological studies have demonstrated that O-LM and R-LM interneurons receive excitatory afferents from distinct origins, e.g., CA1 pyramidal cells, CA3 pyramidal cells, and the entorhinal cortex (EC), respectively (Freund and Buzsáki, 1996; Vida et al., 1998). Nevertheless, the repertoires for mEPSCs received by these different types of interneurons overlapped (Fig. 6).

Although the physiological results reflect both recording conditions and biological properties, the cluster analysis allowed interneuron classification. Although different types of interneurons are known to receive slow or rapid synaptic currents (Geiger et al., 1997; Hajos and Mody, 1997; Bartos et al., 2001; Carter and Regehr, 2002; Galarreta and Hestrin, 2002), the surprising finding of this study lies in the fact that different cell types express similar current kinetics if their axon projects to the same regions. The variability of decay time constants within a given cell type can have different origins, including different afferent inputs (Hajos and Mody, 1997; Mody, 2001; Walker et al., 2002). One possibility is signal filtering from the postsynaptic site to the recording site, making distal inputs slower than proximal ones, as shown by paired recordings (Biro et al., 2005). However, other results suggest that a "kinetics normalization" between synaptic inputs at the dendrite and the soma may occur (Mori et al., 2004). Since interneurons can express different molecular types of glutamate and GABA receptors (van Hooft et al., 2000; Cossart et al., 2002; Walker et al., 2002), the most conservative hypothesis is that interneurons within a family may express similar combinations of receptor subunits, which should result in different kinetics. The answer is not, however, as straightforward as parvalbumin- and CCK-containing basket cells, which target the perisomatic region of pyramidal cells, give rise to IPSCs with identical kinetics (Wilson et al., 2001), despite the fact the underlying $GABA_A$ receptors have a different subunit composition (Klausberger et al., 2002). Conversely, cerebellar interneurons that appear to express a single combination of $GABA_A$ receptor subunit ($\alpha1\beta2\gamma2$) (Nusser et al., 1999a,b) display large decay variability (Nusser et al., 2001). Also, the decay times measured at unitary connections between CA1 pyramidal and O-LM cells, by themselves, display a remarkable variability (Biro et al., 2005). The extensive repertoire of kinetics measured at the soma may also stem from variation in neurotransmitter concentration (Nusser et al., 2001) or the clustering of receptors (Chen et al., 2000). Together, the previous results suggest that kinetics variability reflects a general principle of organization at the synapse. Since variance in the synaptic signals received by neurons plays an important role in information processing (Santhakumar and Soltesz, 2004), kinetics variability may constitute another mechanism to generate variance in the network.

Different developmental mechanisms could lead to an organization of interneuronal networks as reported here. We could first envision that axon development and kinetics are both genetically encoded. This has been established in small and simple nervous systems such as *C. elegans*, in which the homeodomain protein UNC-42 regulates both axon outgrowth and glutamate receptor subunit expression (Brockie et al., 2001). Second, the target of the axon might determine the subunit expression or receptor trafficking of the presynaptic interneuron through a retrograde signaling process (Markus et al., 2002). This is consistent with the fact that the axon of interneurons develops before their dendrites (Hennou et al., 2002). Finally, the subunit composition of the synaptic receptors expressed by a given neuron could provide the molecular basis for axonal guidance towards the target. Such mechanism has been reported in the olfactory system, where different odorant receptors are determinant for precise axonal convergence (Bozza et al., 2002). These hypotheses remain to be tested.

One can only speculate on the raison d'être of such a morpho-physiological organization. One intuitive link is that it provides a spatio-temporal continuum between the synaptic inputs on a given interneuron and its postsynaptic targets, i.e., a kind of long-range target specificity. For example, X-P cells receive fast synaptic inputs and produce fast output signals on their postsynaptic targets (the perisomatic region of principal cells), thus insuring fast-in, fast-out information processing. This does not necessarily imply that the two different cell types, parvalbumin- and CCK-containing basket cells, perform the same computation. Differences between these two cell types are found at all levels: neurochemical (parvalbumin vs. CCK), physiological (fast vs. regular spiking cells; expression of Ca^{2+} channels and metabotropic receptors on the presynaptic terminals established on pyramidal cell somata) and functional (oscillations vs. general physiological and emotional state of the animal) (Freund and Buzsáki, 1996; Wilson and Nicoll, 2002; Freund, 2003). In contrast, X-LM cells receive slower synaptic inputs and generate slow, summating signals on the distal dendrites of pyramidal cells (Pouille and Scanziani, 2001, 2004; Jonas et al., 2004). Similar axonal wiring may represent an economic solution to insure convergence of different information onto the same target zone (Freund, 2003), i.e., enhancing network performance without having to split the population of pyramidal cells into information-specific dedicated subnetworks (Buzsaki et al., 2004). It has been proposed that this pathway-specific expression of postsynaptic receptors endows a given cell with different computational properties (e.g. oscillation at different frequencies). The computational power of a given cell may thus be linked to a given repertoire of kinetics (Foldy et al., 2004). Since the frequency of network oscillations should depend upon the synaptic current kinetics (Traub et al., 1996; Wang and Buzsaki, 1996; Fuchs et al., 2001), the kinetics signature we describe could be part of the mechanisms enabling the brain-state and cell-type-specific firing of inter-

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neurons observed in vivo during oscillations (Klausberger et al., 2003; 2004). Modeling studies aiming at understanding the principles for the generation of network oscillations should benefit from including this property in their simulations. Finally, such morpho-physiological organization seems to constitute a general feature of cortical GABAergic networks in different animal species, since a similar correlation between synaptic input kinetics and axonal morphology was found in mouse primary visual cortex (R. Yuste, personal communication).

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