CHAPTER 4.5

An Atlas-Based Database of Neurochemical Data

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Abstract

The aim of this chapter is to outline the approaches and tools used in our laboratory for building a useful neurochemical database. The data currently collected by a typical neurochemical laboratory are very diverse and heterogeneous and include molecular, neurochemical, and anatomical information. We have therefore selected a few prototype studies in the area of excitatory synaptic neurotransmission and learning models to illustrate the architecture of the object-oriented database being used to construct the neurochemical database within the framework of the NeuroCore database schema for neuroscience data of the USC Brain Project (USCBP).

4.5.1 Synaptic Neurotransmission: Molecular and Functional Aspects

Inter-neuronal communication in the central as well as peripheral nervous systems depends on a complex sequence of neurochemical events taking place at the synaptic level. The molecules mediating synaptic transmission consist of about nine small specialized molecules, amino acids, derivatives of amino acids, or other chemical structures (such as amines) and a large number (50 to 70) of neuroactive peptides. In addition, receptors, ion channels, G-proteins, transporters (carriers), cytoskeletal proteins, and various enzymes play a major role in this process. A major goal of modern neurochemistry is to provide a detailed mapping and a complete understanding of all these elements and to integrate them into synaptic models that not only can account for experimental observations but can also direct research to test new hypotheses. Furthermore, such detailed mapping could then be used to generate an atlas-based database of neurochemical features for all neuronal cell types and pathways. Another unique feature of the brain is the fact that many more genes are expressed in brain cells than in any other cell types, and the identification and localization of brain-specific genes is of primary importance for understanding brain function. This task will become more important as the genomes of complex organisms will become fully available in the near future.

A question that has fascinated neuroscientists for decades concerns the understanding of the effects of past experience on the characteristics of synaptic transmission, as it is widely believed that information in the central nervous system (CNS) is stored as distributed modifications of synaptic efficacy in complex neuronal networks. It is now well established that the underlying mechanisms for such modifications involve alterations in the expression of various genes, as well as structural reorganization. Two simple forms of synaptic modifications—long-term potentiation (LTP) and long-term depression (LTD), representing a long-lasting increase or decrease in synaptic efficacy resulting from the

activation of monosynaptic excitatory connections by specific pattern of electrical activity, have been extensively studied, as it is widely assumed that they represent cellular mechanisms of information storage (Baudry and Davis, 1991, 1994, 1996). Although very few studies have addressed the issues of the duration of these types of synaptic modifications in intact animals, LTP has been shown to last for weeks and possibly longer in hippocampus (Staubli and Lynch, 1987). In turn, these types of synaptic modifications have been incorporated as learning rules in neural networks and the behavior of these networks in various learning tasks investigated (Lynch and Baudry, 1988). Thus not only do these neurochemical databases need to incorporate widely disparate types of information, but they should also be easily linked with brain atlases, as well as with time-series databases and models of neuronal networks.

4.5.2 Roles of Glutamatergic Synapses in LTP and LTD

Excitatory synapses use mainly glutamate as a neurotransmitter, and both LTP and LTD have been shown to occur at glutamatergic synapses and to involve glutamate receptors. There are four families of glutamate receptors: AMPA (α-amino-3–hydroxy-5–methyl-4–isoxazole proprionic acid), NMDA (*N*-methyl-D-aspartate), kainate, and metabotropic glutamate receptors (Hollman and Heinemann, 1994). AMPA and kainate receptors are relatively simple transmitter-gated channels, while NMDA receptors are both voltage-and glutamate-gated ion channels. Furthermore, the NMDA receptor/channel is a calcium channel. The glutamate metabotropic receptors belong to the family of G-protein coupled receptors and their activation leads to changes in second messenger systems (Conn and Pin, 1997). A number of studies have shown that LTP and some forms of LTD (but not all) at glutamatergic synapses depend on NMDA receptor activation and are associated with alterations in the properties of AMPA receptors (Baudry and Davis, 1991, 1994, 1996). In contrast, LTD in cerebellum has been shown to involve both the AMPA and the glutamate metabotropic receptors (Linden and Connor, 1993). It is thus important to have detailed knowledge concerning the relative location and numbers of AMPA, NMDA, and metabotropic receptors at different synapses. Furthermore, experimental evidence has accumulated indicating that different synapses exhibit various forms of plasticity with different time-courses, mechanisms, and functional relevance for behavioral learning. An important challenge for the future will be to understand the relationship between the cellular features of synaptic modifications and the behavioral features of learning and memory processes. Of particular interest are the links between the time courses of the establishment of different forms of plasticity and those of the behavioral phenomena to which they could be related. As pointed out by several authors (Goelet *et al.*, 1986; McGaugh, 1989), there are more than two time courses for memory, and the distinction between short-term and long-term memory does not seem to capture all the temporal features of memory processes. At a more theoretical level, it remains to be demonstrated that time courses or other features of synaptic modifications are necessarily isomorphic to features of memory processes. It is our assumption that the further development of an integrated NeuroCore database environment in which neurochemistry, neurophysiology, and behavioral studies are brought together in conjunction with network models will provide critical answers to these questions (see Chapter 2.3 and others in this book).

In particular, two subfields within the hippocampus have very peculiar features: the mossy fiber terminal zone in CA3 and the stratum lacunosum moleculare in CA1. The first one exhibits an NMDA receptor-independent form of LTP, and there is considerable discrepancy as to the nature of this form of LTP. The latter receives a distinct projection from the entorhinal cortex via the perforant path, and little is known concerning the mechanisms and functions of synaptic plasticity at the synapses generated by these fibers. Furthermore, considerable evidence indicates that LTP at the mossy fiber/CA3 synapses exhibit features different from those found at other glutamatergic synapses. Such peculiar features should provide unique opportunities to apply an integrated approach of the type developed by the USCBP.

4.5.3 Glutamate Receptor Regulation and Synaptic Plasticity

Much has been learned over the last 15 years concerning the characteristics and regulation of ionotropic receptors. At the molecular level, molecular biologists have cloned families of genes coding for subunits of the three families of ionotropic glutamate receptors, the NMDA receptors, the AMPA receptors, and the kainate receptors, as well as for members of the glutamate metabotropic family (Hollman and Heinemann, 1994). Changes in the expression of these genes under different experimental conditions have been documented (Dingeldine *et al.*, 1999). Increased attention has been devoted to the study of post-translational regulation of glutamate receptors, as this level of regulation is well fitted to produce rapid modifications of synaptic efficacy at glutamatergic synapses. In particular, phosphorylation of AMPA and NMDA receptors by several protein kinases, including protein kinase A, protein kinase C, calcium/calmodulin protein kinase, and tyrosine kinases has been shown to produce functional alterations of the receptors (Bi *et al.*, 1998). Partial proteolysis of both AMPA and NMDA receptor subunits as a result of the activation of the calcium-dependent protease, calpain,

has been shown to generate new species of the receptors lacking a small fragment in the C-terminal domains of several subunits of the receptors (Bi *et al.*, 2000). As the C-terminal domain exhibits multiple consensus sites for phosphorylation, it is likely that phosphorylation and proteolysis interact to regulate the properties of AMPA and NMDA receptors. In addition, the phospholipid environment of the AMPA receptors appears to be critical for regulating the binding and functional properties of the receptors (Massicotte and Baudry, 1991). The C-terminal domain of receptor subunits has also recently been shown to be critically involved in the targeting of receptors to the appropriate neuronal domains, and proteins interacting with the C-terminal domains of NMDA and AMPA receptors have been identified (Ziff, 1997). However, little is known concerning the mechanisms regulating the insertion and the internalization of the receptors. Likewise, very little information is currently available regarding receptor turnover rates and their modifications under various experimental conditions (Luscher *et al.*, 1999; Shi *et al.*, 1999). Both NMDA and AMPA receptors are involved in various pathological conditions. In particular, epilepsy is characterized by increased neuronal excitability that could be due to modifications of the properties of AMPA and/or NMDA receptors (Mody and Staley, 1994). Finally, both types of receptors have been associated with the phenomenon of excitotoxicity (Choi and Rothman, 1990). Thus, although a rich literature is available concerning the characteristics and regulation of the properties of glutamate receptors and their participation in synaptic plasticity processes, much more needs to be understood in order to integrate these data in neurochemical databases and in models of synaptic plasticity in neural networks.

4.5.4 How To Build a Useful Neurochemical Database

The exceptional diversity and heterogeneity in neurochemical information being collected nowadays, in the same or different laboratories, require development of unique tools for storage, manipulation, and comparative analysis of the data. Neurochemical studies involve anatomical mapping of various proteins and receptors and of the activity of various types of enzymes; they also involve collecting information about the functions and neurochemical properties of different ion channels and data about the effect of drugs, neurotoxins, hormones, nutrients, etc. on the brain. In the framework of the USC Brain Project, we have developed an object-oriented database in which a wide variety of neurochemical data can be easily stored, retrieved, and analyzed.

The database design process is represented in Fig. 1. The first step of the database design consisted in

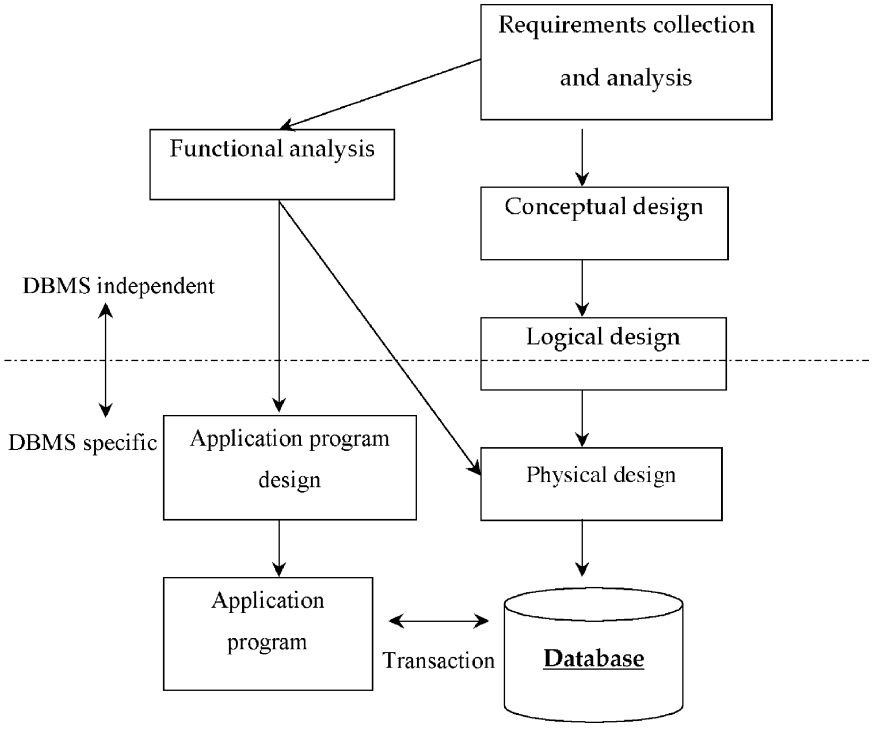

Figure 1 Database design process. In order to design an effective high-level conceptual data model, several steps need to be followed. The step in which the collaboration between neuroscientists and computer scientists is the tightest is requirement collection and analysis. The result of this step is a concise definition of users' requirements. The conceptual design allows a more abstract description of the data types, relationships, and constraints. The next step, the logical design, is the actual implementation of the database using a database management system (DBMS). The last step characterizes the specification of internal storage structures and file organization.

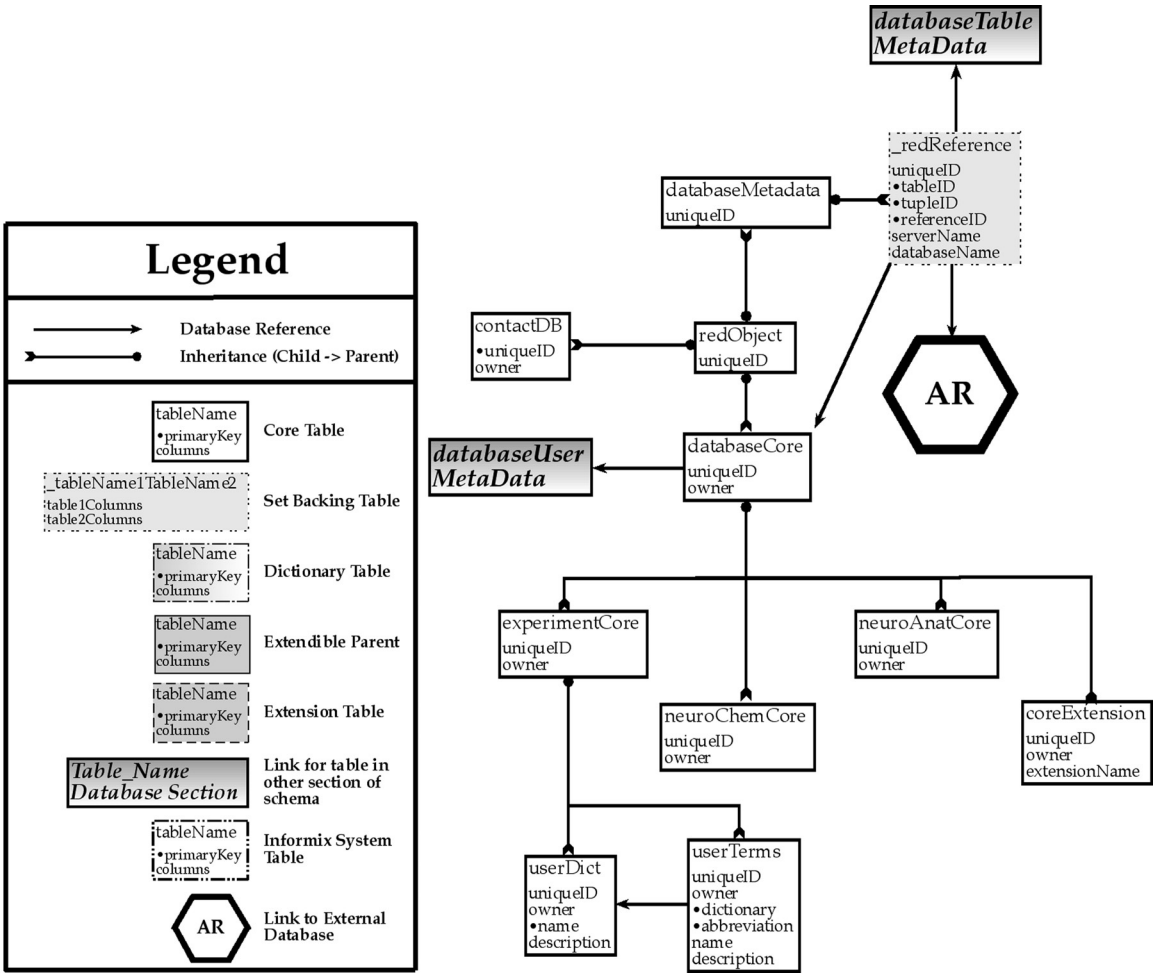

Figure 2 Top-level schema of the Core database, NeuroCore, and NeuroChem. This figure represents the top-level schema of the Core database tables and the parent tables of all children tables created within NeuroCore and NeuroChem. It shows the different modules of NeuroCore (databaseMetadata: necessary information regarding tables, columns, and relations within the database; contactDB: information regarding researchers and laboratories; databaseCore: parent for all experiment related tables in the database, its direct children are experimentCore, neuroChemCore, neuroAnatCore and coreExtension). This figure illustrates the tight interaction that exists at the higher hierarchy level between NeuroCore and NeuroChem.

analyzing the requirements for data collection and analysis. During this step, the database designers interviewed prospective database users (in our case, neurobiologists) to understand and document their requirements. The result of this step was a concisely written set of users' requirements. These requirements should be specified in as detailed and complete a form as possible. In parallel with specifying the data requirements, it was useful to specify the known functional requirements of the application. These consist of user-defined operations (or transactions) that will be applied to the database, and they include both retrievals and updates (functional requirements).

Once all the requirements were collected and analyzed, the next step consisted of creating a conceptual schema for the database, using a high-level conceptual data model. The NeuroCore database architecture, discussed in other chapters of this book, has been used as a framework in which NeuroChem has been developed in order to store neurochemical data. This step is called *conceptual database design*. The conceptual schema is a concise description of the data requirements of the users and includes detailed descriptions of the data types, relationships, and constraints related to the data. Because these concepts did not include any implementation details, they were usually easier to understand and could be used to communicate with non-technical users. The high-level conceptual schema could also be used as a reference to ensure that all users' data requirements were met and that the requirements did not include any conflicts.

The next step in our database design was the actual implementation of the database, using a commercial DBMS (this step is called *data model mapping*). Finally, the last step was the physical database design phase, during which the internal storage structures and file organization for the database were specified. In parallel with these activities, application programs had to be

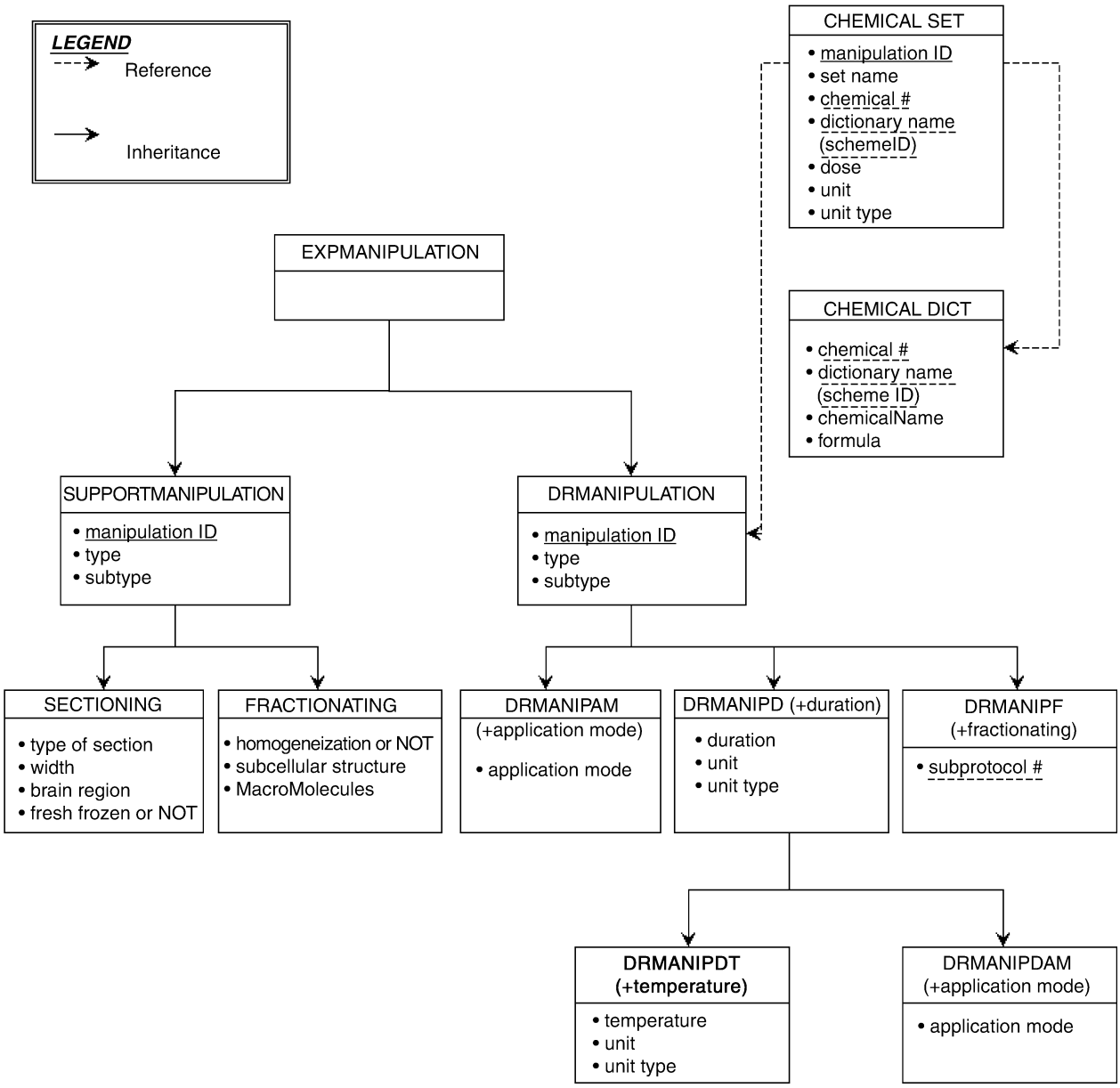

Figure 3 NeuroChem architecture. NeuroChem is built using Informix, a database management system (DBMS) that allows direct reference between tables and inheritance. Attributes that refer to other objects located in other tables are called *references*. They represent relationships among tables. The *Sectioning* and *Fractioning* tables are subclasses from the superclass *supportManipulation*. They inherit the same attributes as their superclass, as well as having attributes of their own. The architecture described here takes full advantage of the inheritance principle. The tables *DrManipAM*, *DrManipD,* and *DrManipF,* for example, have the same attributes as their parent table, *DrManipulation,* but add attributes related to their specificity (i.e., respectively, application mode, duration, and fractioning).

designed and implemented as database transactions-corresponding to the high-level transaction specifications.

The first architecture of the database is represented in Figs. 2 and 3. The database management system that was chosen is Informix. The Informix Universal Server is an object-relational database management system (ORDBMS) that combines both relational database and object-oriented programming technologies. Informix's relational database system supports standard SQL data types, functions, and queries. Additional features extend the standard relational database model with object-oriented features and include the ability to add new user-defined data types and functions, built-in support for large objects as datatypes (e.g., images, audio, video, etc.), and support of object-oriented concepts such as inheritance and function overloading.

4.5.5 Incorporating Neurochemical Data into the NeuroCore Repository of Empirical Data

A close collaboration between neuroscientists and computer scientists has been necessary. In order to define the data that populate the database, neurochemical publications are used. Parameters of experiments and the corresponding results are entered into the database. This primary set of data will allow us to test, validate, and improve the quality of the database design and architecture. Three sets of experiments included in the coming two sections will be presented to illustrate the effort in this area. These experiments are representative of the types of studies conducted in our laboratories.

Role of Proteases of the Calpain Family in LTP

We recently showed that *in situ* activation of calpain, a calcium-activated neuronal protease, produced marked alterations in the immunoreactivity of several subunits of AMPA receptors, in particular, GluR1 subunits, as well as in NR2 subunits of NMDA receptors (Bi *et al.*, 1996, 1997). Calpain was found to have a selective proteolytic activity on the C-terminal domains of GluR1, GluR2/3, and NR2. To further characterize calpain-mediated GluR1 proteolysis, we studied the effect of incubating frozen-thawed rat brain in the presence or absence of calcium on GluR1 immunoreactivity, examined with antibodies directed towards the N- or the C-terminal domains of the subunits (Fig. 4). Analysis with C-terminal antibodies (Figure 4D–F) showed that incubation with calcium (Figure 4D) decreased GluR1 immunoreactivity in several hippocampal regions, including the stratum lacunosum-moleculare of the CA1 region, stratum moleculare of the dentate gyrus, and dendritic fields in the cortex. No such effect was observed when GluR1 was detected with the N-terminal antibodies (Figure 4A,B). These effects were blocked by calpain inhibitors, indicating that calpain activation produces partial proteolysis of GluR1 subunits (Bi *et al.*, 1996, 1997). These results are of particular interest considering the evidence implicating calpains, calcium and glutamate receptors in mechanisms of synaptic plasticity and neurodegenerative processes (Lynch *et al.*, 1986; Bi *et al.*, 2000).

To illustrate the population of the database by experimental data, Table 1 shows the overall architecture of the database, illustrated with reference to a specific experiment.

Glutamate Transporters Modulate Glutamate Neurotransmission: Regulation by Kainic Acid in Rat Brain and Hippocampal Organotypic Cultures

Upon its release and interaction with pre- and postsynaptic receptors, glutamate is removed by an active

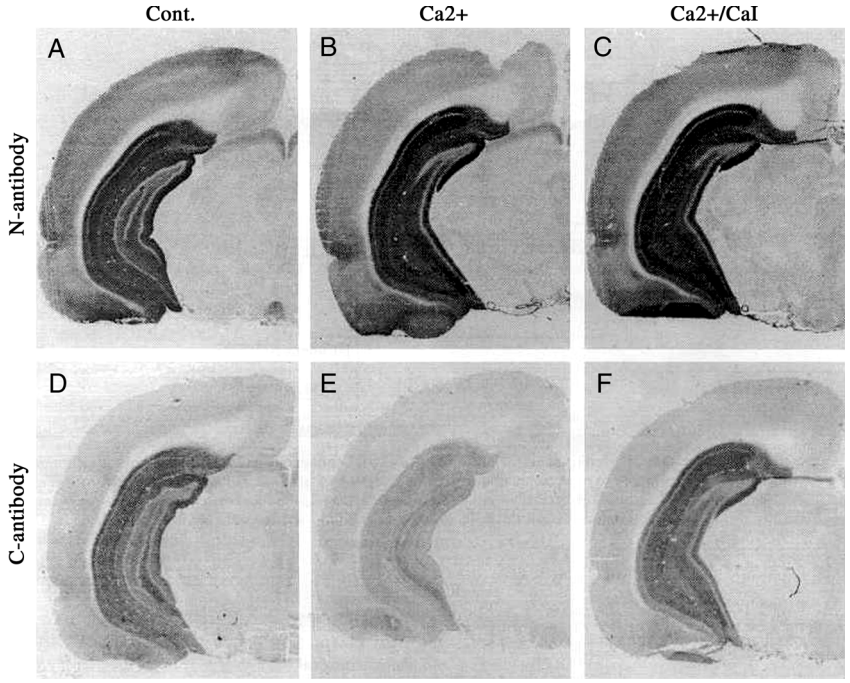

Figure 4 Effect of calcium on GluR1 immunoreactivity. Frozen-thawed rat brain sections were incubated in the absence or presence of calcium at 37°C. After incubation sections were processed for immunohistochemistry with antibodies directed to the N- or the C-terminal of the GluR1 subunits of AMPA receptors. Analysis with the C-terminal antibodies (C and D) showed that incubation with calcium (D) decreased GluR1 immunoreactivity in several hippocampal regions. No such effect was observed when GluR1 was detected with the N-terminal antibodies (A and B). These effects were blocked by calpain inhibitors, indicating that calpain produces partial proteolysis of GluR1 subunits.

Table 1 Study: Effect of Calcium Treatment of Frozen-Thawed Rat Brain Sections on GluR1 Subunits of AMPA Receptors

Antibodies directed towards N-terminal domain	Antibodies directed towards C-terminal domain
No effect	Incubation with calcium induces a decrease in GluR1 immunoreactivity in several hippocampal regions Effect blocked by calpain inhibitors ⇒ Effect produces partial proteolysis of GluR1 sub-units

uptake system made up of specific glutamate transporters. During the last few years it has been found that neurons as well as glial cells possess glutamate transporters, indicating therefore that both cell types are involved in controlling the extracellular level of this major excitatory neurotransmitter. Five glutamate transporters have been cloned so far, and they have been named *excitatory amino acid transporters 1–5* (EAAT1–5) (Gegelashvili and Schousboe, 1997). Of this group, EAAT1 and EAAT2 were found mainly in astrocytes, EAAT3 in neurons distributed in many brain regions, EAAT4 in cerebellar Purkinje cells, and EAAT5 in the retina. Keeping in mind that the rate and efficiency of glutamate reuptake have various implications, we analyzed the expression of glutamate transporters upon neuronal activation, using kainic acid as a prototype inducer. Two experimental conditions have been used: (1) following systemic injection of kainic acid, and (2) following *in vitro* treatment of hippocampal slices maintained in cultures.

Expression of the glial (EAAT2) and neuronal (EAAT3) transporters was determined at the protein and mRNA levels, using specific antibodies and oligonucleotide probes, respectively (Simantov *et al.*, 1999a). The immunocytochemical analysis indicated that treatment of young adult rats with kainic acid decreased EAAT3–immunoreactivity in stratum lacunosum moleculare of the hippocampus within 4 hours, prior to any evidence of neuronal death. Upon pyramidal cell death (5 days after kainate treatment) EAAT3 expression in CA1, CA2, and stratum lacunosum moleculare was practically abolished. The fast effect of kainic acid on the expression of EAAT3 was confirmed by *in situ* hybridization; kainic acid treatment decreased EAAT3–mRNA levels in CA1 and CA3 regions of the hippocampus within 4– to 8 hours. Kainate had an opposite and more widespread effect on EAAT2 expression; both the immunocytochemistry and *in situ* hybridization showed a modest but significant increase in the level of EAAT2 in several hippocampal and dendate regions. Developmental analysis of EAAT2 and EAAT3 expression by *in situ* hybridization indicated that the fast regulation of the transporters upon kainic acid application was absent in rats younger than 21 days (Simantov *et al.*, 1999a). This result is in line with previous studies indicating the resistance of young rats to kainic acid neurotoxicity (Tremblay *et al.*, 1984). Hippocampal organotypic cultures, which lack a major input of fibers from the entorhinal cortex through the perforant path, were used to further analyze the kainate effect. Treatment with kainic acid slowly decreased ^{3}H-D-aspartate uptake, an effect reaching a maximum decrease at 17 to 48 hours (Fig. 5). These studies indicated that one of the earliest effects of kainic acid in hippocampus consists of a down-regulation of the neuronal transporter EAAT3 in restricted regions, concurrent with a modest increase in the glial transporter EAAT2. These and additional studies suggest that glutamate transporters play an important role in kainate-induced neurotoxicity (Simantov *et al.*, 1999). The implications of these observations regarding the role of glutamate transporters in modulating neuronal plasticity, seizure, and LTP/LTD are yet to be determined.

In an attempt to further elucidate the role of different glutamate transporters in hippocampal subfields involved in LTP and LTD, expression of the neuronal or glial transporters in hippocampal slice cultures was inhibited with selective antisense oligonucleotides, and kainate toxicity was determined. These experiments confirmed earlier studies (Bruce *et al.*, 1995) that kainate is more toxic in CA3 than in CA1. More interestingly,

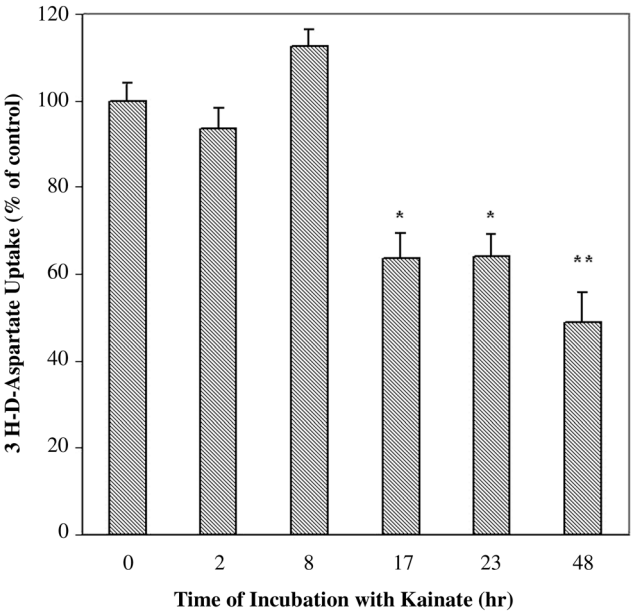

Figure 5 Kainate effect on ^{3}H-D-aspartate uptake by rat hippocampal slice culture. Hippocampal slices were prepared and maintained in culture as described in Simantov *et al.* (1999a). Uptake of ^{3}[H]-D-aspartate (200 n*M* final concentration) was determined at 0– to 48 hours after treatment with 10 (μM kainate. Data are means SD from 4– to 6 experiments, each assayed in quadruplicates. * and ** indicate statistically different from control, $p < 0.01$ and 0.001, respectively.

treatment with an antisense oligonucleotide to EAAT3 increased kainate toxicity in CA3, but had an opposite effect on the CA1 layer (Simantov *et al.*, 1999b). Antisense to EAAT2 increased kainate toxicity in both CA1 and CA3. NMDA was more cytotoxic in the CA1 than CA3 cell layer, and the antisense oligonucleotides to EAAT3 and EAAT2 increased NMDA toxicity in both CA1 and CA3 subfields. These and additional experiments (Simantov *et al.*, 1999b) indicate that glutamate transporters play a key role in regulating glutamate concentration in the vicinity of the pyramidal cell layers. EAAT3, the neuronal transporter, has a unique role in CA3.

The set of experiments described above is far more complex than the first one we examined; however, the flexibility of the architecture adopted still allows the storage of these experiments. Table 2 shows the overall architecture of the experiment as stored in the database. In the next section are the details of one of the protocols used for the experiment described above. Furthermore, the tables populated with data specific to the protocol used here illustrate the database population.

Overall architecture of the experiment as stored in the database:

EXPERIMENT: EFFECT OF KAINIC ACID AND PENTOBARBITAL TREATMENTS ON THE IMMUNOHISTOCHEMICAL DISTRIBUTION OF GLUTAMATE TRANSPORTERS IN RAT HIPPOCAMPUS

Subject (hypothesis): Short- and long-term treatments with kainic acid alter the expression of glutamate transporters.

EXP ID: 0001

Date: 5.5.1997

Treatments: 15 Sprague-Dawley rats (males, 3 months old) were divided into three groups of five rats each and treated intraperitoneally (ip) as follows (1–3):

1. Control group
2. 4–hour treatment
3. 5–day treatment

The control group received 1 ml 0.15 *M* NaCl (saline). Groups 2 and 3 were treated with 10 mg/Kg kainic acid, prepared in saline.

Tissue Preparation (4–10):

4. Rats were anesthesized with sodium pentobarbital (150 mg/kg).
5. They were then perfused intracardially with phosphate buffered saline (PBS).
6. Perfusion was followed by 300–400 ml of 4% paraformaldehyde in 0.1 *M* phosphate buffer (PB, pH 7.4).
7. After perfusion, the brain was removed
8. The brain was then washed with PB.
9. The brain was transferred to PB containing 30% sucrose.
10. Sections (40 μm thick) of the brain were cut, using a microtome.

Immunostaining (11–24):

11. Three brain sections from the hippocampal region of each rat were preincubated for 1 hour in 10 m*M* Tris-buffered saline (TBS, pH 7.4) containing 0.1% Triton X-100 and 4% normal goat serum.
12. The sections were then rinsed for 30 minutes in TBS.

The sections were then incubated for 48 hour in TBS containing 2% normal goat serum, 0.1% Triton X-100, and one of the following three antibodies:

13. Anti-EAAT1 (0.2 μg/ml)
14. Anti-EAAT2 (0.17 μg/ml)
15. Anti-EAAT3 (0.06 μg/ml)

Sections were then:

16. Rinsed (30 minutes, TBS)

Table 2 Study: Analysis of Expression of Glutamate Transporters Following Kainic Acid-Induced Seizure Activity

Two experimental conditions	Treatment with kainic acid	Earlier effects of kainic acid on hippocampus are
1 Following systematic injections of kainic acid	4 hours: Decrease in EAAT3 immunoreactivity in stratum lacunosum moleculare of hippocampus	Down-regulation of EAAT3
2 Following *in vitro* treatment of hippocampal slices maintained in culture	5 days: EAAT3 expression in CA1, CA2, and stratum lacunosum moleculare practically abolished	Modest increase in glial transporter EAAT2
	In situ hybridization: Same effect on EAAT3 mRNA levels in CA1 and CA3 within 4 to 8 hours	Decrease in-H-D-aspartate uptake
	In situ hybridization + immunocytochemistry: Increase in EAAT2 levels	

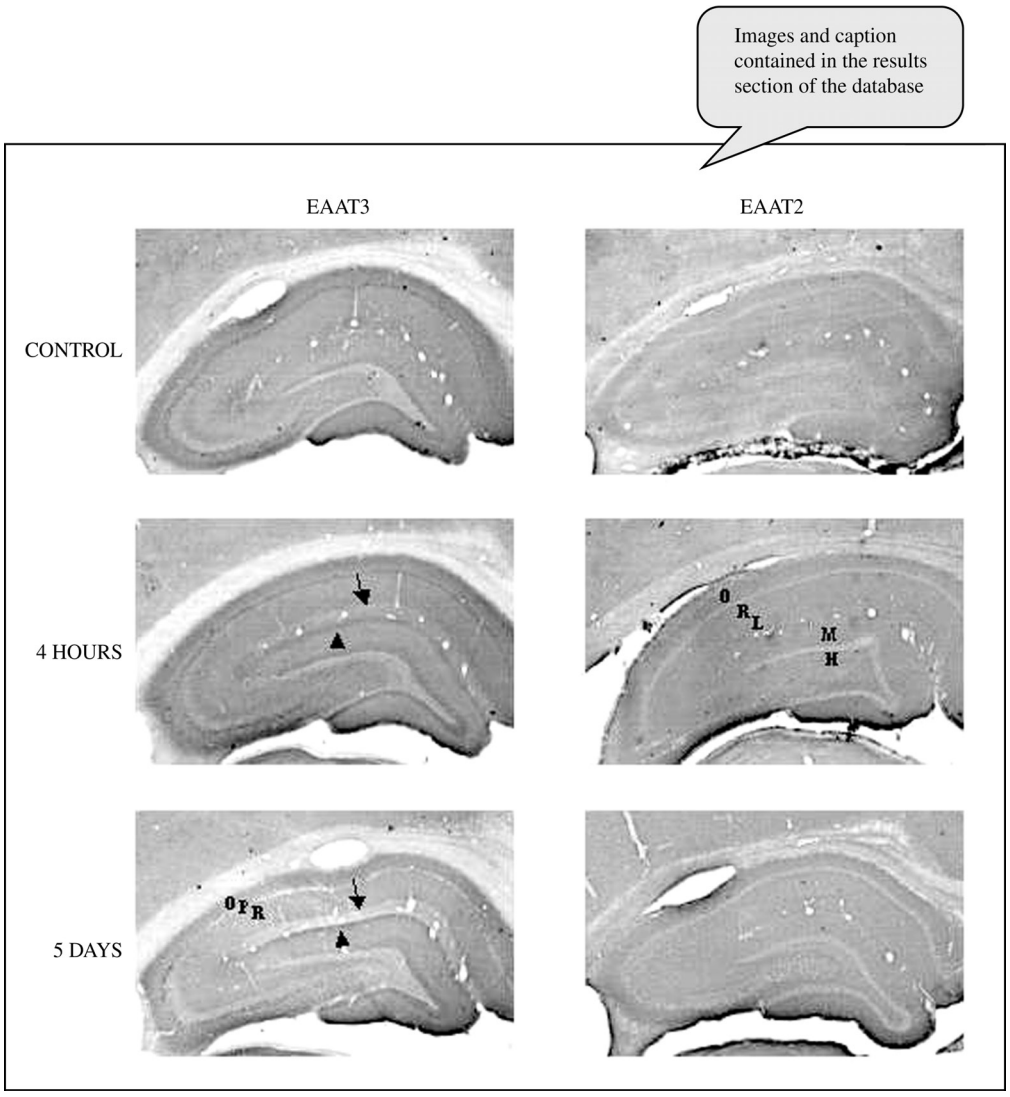

Figure 6 Immunocytochemical analysis of the neuronal (EAAT3) and glial (EAAT2) transporters in kainite-treated rats. Sections from control and kainite-treated rats (4 hours and 5 days) were analyzed. O: Stratum oriens, P: pyramidal cell layer, R: stratum radiatum, L: stratum lacunosum moleculare of the hippocampus, M: molecular layer of the dentate gyrus, H: hilus. Arrow and arrowhead point to significant changes in immunoreactivity in stratum lacumosum moleculare of the hippocampus and outer molecular layer of the dentate gyrus, respectively.

17. Incubated for 60 min in TBS containing 2% normal goat serum, 0.1% Triton X-100, and goat anti-rabbit antibodies (1:200 dilution)
18/19. Rinsed *twice* with TBS
20. Stained with Vectastain ABC Kit (Vector Laboratories)
21. Dried
22. Washed in H_2O
23. Dehydrated with alcohol
24. Mounted.

Imaging and Storing the Data: Sections were scanned and data stored in PC 307 C:/Windows/RS/GlutTr/May 5 as Neuro1-5.tif, glt1a-e.tif, or glast1-5.tif.

4.5.6 Available Resources

NeuroChem: Current Status

The first version of NeuroChem is implemented as an extension of NeuroCore. This version has been implemented based on the needs expressed for the proper storage and retrieval of information regarding the experiments described above and other experiments currently being run in Dr. Baudry's laboratory (University of Southern California). It is in actuality being populated. So far, only protocol-related and chemicals-related data have been entered. The data obtained from the experiments (brain slices, arrays of data, etc.) have not been stored yet. Even though NeuroChem has been really

Table 3 DRUGMANIPULATION

uniqueid	owner	extensionname	manipulationid	type	subtype
			18	ICC	Rinsing
			19	ICC	Rinsing
			20	ICC	Staining
			21	ICC	Dry
			22	ICC	Washing
			23	ICC	Dehydration

Table 4 DRMANIPD

uniqueid	owner	extensionname	manipulationid	type	subtype	duration	durationUnit	dUnitType
			11	ICC	Preincubation	1	HR	Duration
			12	ICC	Rinsing	30	Min	Duration
			13	ICC	Incubation	48	HR	Duration
			14	ICC	Incubation	48	HR	Duration
			15	ICC	Incubation	48	HR	Duration
			16	ICC	Rinsing	30	Min	Duration
			17	ICC	Incubation	60	Min	Duration

Table 5 CHEMICALSET

uniqueid	owner	extensionname	manipulationid	setname	schemeid	chemicalid	dose	doseunit	dunittype
			11	TBS 7.4	IND	7	10	mM	conc.
			11	Triton X100	IND	8	0.1	%	conc.
			11	Normal goat Serum	IND	9	4	%	conc.
			13	N G S	IND	9	2	%	conc.
			13	TBS	IND	7	–	–	–
			13	Triton X100	IND	8	0.1	%	conc.
			13	Anti EAAT1	IND	10	0.2	microg	conc.
			14	NGS	IND	9	2	%	conc.
			14	TBS	IND	7	–	–	–
			14	Triton X100	IND	8	0.1	%	conc.
			14	Anti EAAT2	IND	11	0.17	microg	conc.
			15	NGS	IND	9	2	%	conc.
			15	TBS	IND	7	–	–	–
			15	Triton X100	IND	8	0.1	%	conc.
			15	AntiEAAT3	IND	12	0.06	microg	conc.
			12	TBS	IND	7	–	–	–
			16	TBS	IND	7	–	–	–
			17	TBS	IND	7	–	–	–
			17	NGS	IND	9	2	%	conc.
			17	Triton X100	IND	8	0.1	%	conc.
			17	Goat anti-rabit serum	IND	13	1/200	–	dilution
			18	TBS	IND	7	–	–	–
			19	TBS	IND	7	–	–	–
			20	Vectastain ABC kit	IND	14	–	–	–
			22	H_2O	IND	15	–	–	–
			23	Alcohol	IND	16	–	–	–

stable so far and responded in a reliable manner to the queries that have been run throughout its development, the plan for the near future is to test the reliability and consistency of the links within the tables of NeuroChem and between the NeuroCore schema and NeuroChem. For this purpose, collaborations will be established between Dr. Baudry's group and Jade developers. These collaborations should also be of benefit to

Jade as far as testing its ability to deal with data it was not originally designed for.

For the latest release information and further documentation of the NeuroChem database, please visit the University of Southern California Brain Project Website at http://www-hbp.usc.edu/Projects/neurochem.htm.

Integration with Three-Dimensional Registration and NeuArt

Many neuroanatomical studies of brain tissue sections depend on the qualitative evaluation and quantitative analysis of radiolabeled samples obtained from experimental animals. Such a tight interaction between neuroanatomy and neurochemistry illustrates the need of integrated computational tools that help neuroscientists with regards to each aspect of their work. The integration of computational sets of software tools and databases has been an increasing field of study (Peterson, 1995; Dashti *et al*., 1997; Bloom, 1996) in the past few years to provide a means for conveying complete descriptions of experiments, a platform for virtual experiments; and an interface where modeling and experimental results could be exchanged.

The integration of NeuroChem with three-dimensional registration and NeuArt is possible via the NeuroCore framework (Diallo *et al.*, 1999) and represents a real opportunity for neuroscientists to retrieve data related to neurochemistry, neuroanatomy, and electrophysiology. For example, it is possible for a neuroscientist to query NeuroChem on the various experiments realized on Sprague-Dawley rats treated with kainic acid, to retrieve these experiments, to observe images of brain sections, to compare them with other brain sections issued from an atlas, and to be able to determine in a precise manner the location and angle of the actual experimental brain sections as compared to the sections present in the atlas. Such integration should create a means for conveying complete descriptions of experiments and a set of computational tools for complete analysis.

4.5.7 Conclusion

Considering the immense amount and heterogeneity of the neurochemical information being collected at the present time, it is becoming necessary to develop new methods for accumulating, handling, and querying the data. Indeed, effort is being made by several research centers to construct databases and repositories to address this issue (Sheperd *et al.*, 1998). In this chapter, we have summarized our effort within the framework of the USC Brain Project and have provided step-by-step examples of two prototypes of neurochemical studies. The architecture of the object-oriented database presented herein provides the groundwork for the construction of a global neurochemical database, in which a wide variety of neurochemical data from different laboratories can be easily stored, available to the entire scientific community, and productively analyzed. The object-oriented architecture and the use of a core database such as NeuroCore also provide a solid basis for the creation of an integrated computer-aided neuroscience.

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