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Review

Targeted lipidomics: Signaling lipids and drugs of abuse

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1. Introduction to lipidomics

The workshop "Targeted lipidomics: signaling lipids and drugs of abuse" (15–17 April 2004, Washington, DC) was the first such meeting in which so many internationally recognized experts from a variety of disciplines were assembled to discuss brain lipid signaling and drug abuse. At the end of this 3-day series of presentations and discussion, a "brainstorming" session was held, to identify overall research goals and future directions for the CNS lipidomics and, more specifically, to identify NIDA's role in this area. This article is compiled from the presentations of Drs. D. Piomelli, S. Spiegel, N. Bazan and E.A. Dennis – who led the discussion – and from the insightful input from those in attendance.

As expected at this stage, much of the discussion centered on analytical issues. The general consensus was that technologies are now available to conduct thorough analyses of complex lipid mixtures in biological matrices. From the panel discussion, high pressure liquid chromatography (HPLC–MS) utilizing tandem mass spectrometry (MS/MS) and multiple reaction monitoring (MRM) approaches has emerged as the technique of choice for lipidomic profiling. In a sense, this technique is to lipidomics what 2D gel electrophoresis is to proteomics. However, state of the art MS instruments are still quite expensive. Clearly, in order to advance this area of research, it is necessary to pull together resources, to develop multi-investigator collaborations, and to share facilities. It was suggested that the NIH should continue to play a leading role in this process as exemplified by the National Institute of General Medical Sciences through the LIPID MAPS Large Scale Collaborative Grant initiative aimed at developing an integrative lipidomics approach for all lipid classes in a single cell (www.lipidmaps.org).

2. Challenges for lipid detection and analysis

The analysis of low-molecular weight lipids such as eicosanoids and endocannabinoids is relatively straightforward when compared to that of higher-molecular weight phospholipid molecules. One of the reasons for this is that eicosanoids and endocannabinoids are normally present in cells in very low amounts, but their levels can dramatically increase upon stimulation (up to 1000-fold) and often they are secreted from cells and can be detected in the media. This means that robust quantitative differences can readily be measured, which allows one to quantify the lipid compounds with acceptable statistical error. The situation is different with the phospholipids, where stimulation is not expected to lead to such enormous increases in overall quantities. As a result, a very high degree of accuracy is needed to measure significant changes in phospholipid levels. This problem is further complicated by the fact that lipids are present in all cell membranes, which makes it more challenging to quantify differential changes in individual membrane compartments.

One of the important issues is the need to look not only at biologically active products, but also at their precursors. Researchers who work in the area of eicosanoids and endocannabinoids typically look at specific active metabolites that are products of complex cascades. Yet, we may have a better chance of understanding the whole picture if the precursors for these products are also analyzed. In the area of lipid signaling this is very important, because precursors are often as bioactive as their products. In addition, these products can

serve as precursors to other signaling molecules. It is important, therefore, to explore the entire spectrum of precursors, signaling molecules and metabolites involved in lipid signaling. Techniques must be developed to simultaneously quantify bioactive molecules along with their precursors. This task is very complex and requires the development of innovative analytical approaches.

It is known that it is very difficult to accurately quantitate phospholipids. A special problem in the quantification of these biomolecules is the use of internal standards. The problem lies in the fact that there is no 'free space' in an HPLC–MS chromatogram of phospholipids, because most single mass-to-charge ratios are occupied by existing species. Because of this problem, deuterium-containing standards, which are the most effective means to do MS quantification, cannot always be used. The alternative is to use external standards. This may be a reasonable approach, but the results produced may or may not be reliable enough to answer all types of research problems. This is an issue that should be addressed in further discussions.

Another analytical challenge is posed by the great complexity of lipid signaling in a highly organized structure such as the CNS. At present no technique is available to conduct topographic lipidomic analysis in the brain, though future possibilities include the use of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI–TOF), which in the field of peptide research has been used to localize peptides in specific structures of large invertebrate neurons. A similar technique may be developed in the future for lipids, although MALDI–TOF operates at molecular weight ranges that may be too high for some lipid molecules; yet, with improvements in existing methodologies, this problem does not appear to be insurmountable. Another possible avenue, which is currently being pursued, is to couple laser capture microdissection and MALDI–TOF or HPLC–MS.

Moving from analytical to biological issues, the first topic discussed was the differences and similarities between extracellular and intracellular lipid signaling. The fact that lipids such as the eicosanoids, endocannabinoids and lysophospholipids activate G-protein coupled receptors (GPCRs) suggests an extracellular mode of action for these compounds. Yet, there is increasing evidence for intracellular targets for some lipid ligands, including nuclear receptors and ion channels. A prominent example is oleoylethanolamide (OEA), which acts as an endogenous ligand for the peroxisome proliferating activated receptor-alpha (PPAR-alpha). This raises a new analytical issue as, ideally, one should be able to determine whether lipid mediators are released inside or outside of cells and from which subcellular organelles, as we can do for cAMP and other classical intracellular signaling molecules. However, such techniques would need to be developed to achieve these ends for lipids.

A second issue raised for discussion was sequential signaling. Lipid mediators often serve as precursors for other bioactive molecules, which may display a different spectrum of biological properties from their parent compound. For example, the endocannabinoid 2-arachidonoylglycerol (2-AG) may act as substrate for cycloxygenase-2 (COX-2) and give rise to an array of active products with different activities. To fully understand such processes, we need to explain how lipid metabolism is compartmentalized in cells. A related question concerns the regulating factors involved in sequential signaling. For example, is there a constraint on 2-AG metabolism by cycloxygenase-2. And, if so, what kind of constraint?

Naturally, as the workshop was primarily directed toward CNS lipidomics, the question was raised as to whether drugs of abuse affect lipid signaling and how, in turn, changes in lipid signaling may shed light on drug abuse-related processes such as tolerance and addiction. It was pointed out that, because of the nature of the CNS, studies on all cell populations making up the brain are indispensable-neurons, astrocytes, microglia, etc. Further discussion emphasized, the need for regional brain studies focused on structures involved in drug abuse, such as the nucleus accumbens, the amygdala and other limbic areas of the brain.

3. Complexity of lipids in cellular compartments

Sphingolipids, which are highly enriched in the brain, are components of all eukaryotic membranes and their metabolism generally takes place in a membrane environment. Sphingolipid metabolism, like glycerophospholipid metabolism, or any other metabolic pathway, is a very dynamic process. It occurs in several different compartments or subsets of compartments within cells, and can generate metabolites that have very important functions in different sites within the cell. For example, initially most of the focus of research on the functions of sphingolipid metabolites was on apoptosis mediated by ceramide, the backbone of all sphingolipids, which is produced by activation of sphingomyelinases at the plasma membrane. It is now known that sphingomyelinase is highly enriched in rafts, a very mobile and dynamic environment. Ceramide plays a critical role in coordinating cellular responses to stress and caveolae respond to stress by recruiting sphingomyelinase and sphingomyelin into the lipid raft compartment, thus paving the way for ceramide formation which in turn can induce the reorganization of caveolae into active signaling compartments. However, a flurry of studies also suggests that stress stimuli can produce pro-apoptotic ceramide particularly C16-ceramide by stimulation of the de novo pathway in the endoplasmic reticulum. So, is it the ceramide that is formed in the ER that is involved in stress responses or is this C16-ceramide transported to the mitochondria where it can influence cytochrome C release and apoptosis?

Ceramide can also be deacylated by ceramidases to sphingosine, another sphingolipid metabolite that has been implicated in cell growth arrest and apoptosis. Moreover, sphingosine can be phosphorylated by sphingosine kinases to form sphingosine-1-phosphate (S1P), a bioactive sphingolipid mediator that has been implicated in the regulation of vital biological processes, including growth, survival, cytoskeletal rearrangements, cell motility, and more recently in regulation of neuritogenesis. Abundant evidence indicates that S1P acts as both an intracellular messenger and an extracellular ligand for a family of five specific G protein-coupled S1P receptors. Hence, compartmentalization of S1P formation can dictate its important functions. Cellular levels of S1P are tightly regulated in a spatiotemporal manner through its synthesis catalyzed by sphingosine kinases and degradation by S1P lyase and specific S1P phosphohydrolases. Surprisingly, the latter enzymes are located in the ER whereas the former are cytosolic enzymes that can be translocated to different membranes. In PC12 cells and dorsal root ganglion neurons, NGF translocates sphingosine kinase type 1 to the plasma membrane where its substrate sphingosine resides. Differential transactivation of S1P receptors and consequent antagonistic signaling pathways modulate

NGF-induced neurite extension. These are just a few examples that highlight the importance of developing methods to isolate different subcellular compartments and quantitate the complete spectrum of sphingolipid species residing in these compartments. These are difficult issues to address. It is still not clear whether all lipid species present in membranes are retained during isolation procedures but new developments in ESI–MS/MS technology can now be used to answer this question.

Because of the interconvertibility of ceramide, sphingosine, and S1P and the opposing effects on cell fate of ceramide and sphingosine (anti-growth, pro-apoptotic) compared to S1P (pro-growth, anti-apoptotic), the dynamic balance between S1P and ceramide/sphingosine has been proposed to form a "cellular rheostat" that determines cell survival or death. As there is accumulating evidence that the sphingolipid rheostat is ultimately regulated by the relative activities of enzymes controlling the turnover of these sphingolipid metabolites, it will be important to correlate lipid levels with regulation of the relevant metabolic enzymes.

4. Lipidomics challenges and complex interactions

Another important issue is whether all sphingolipid species can in fact be identified and quantified by a general lipidomics approach. The number of different species of glycosphingolipids, neutral glycosphingolipids, gangliosides with different fatty acids, etc., is mind-boggling and there may be thousands of previously unidentified molecules, some of which may have important biological activities. This picture could be further complicated if all the potential enantiomeric, stereoisomeric, and regioisomeric molecules are also be identified, resolved, isolated and evaluated. For example, resolving enantiomeric, regioisomeric, and stereoisomeric bioactive lipids are particularly important for the numerous bioactive cyclooxygenase, and lipoxygenase-derived molecules as well as those arising non-enzymatically from reactive oxygen species on membrane phospholipids.

Another very interesting and complex aspect that can only be studied by a global lipidomics approach is lipid-to-lipid communication and lipid networks. For example, sphingosine-1-phosphate is an upstream activator of COX-2 induction and PGE2 production in response to TNF-alpha. In addition, ceramide phosphate, which is formed by a unique kinase that phosphorylates ceramide, has been suggested to be an upstream direct activator of cytosolic PLA_2 that might act in concert with other lipids, such as phosphatidylinositol-bisphosphate (PIP2), to activate this enzyme resulting in the formation of a host of arachidonate metabolites. Since one lipid mediator can influence a whole class of different mediators, technology that can simultaneously evaluate all of these metabolites is necessary to reveal critical connections.

Enzyme interaction at the lipid bilayer does not appear to be a simple and routine interaction. For example, in the case of cPLA2, the surface that enzyme sees when interacting on the bilayer has potentially 50 lipids under it. Therefore, there could be an enormous number of binding sites with specificity for different lipids. One should consider the components associating the soluble enzymes with the membrane lipids. Under certain conditions, this interaction may have multiple sites of binding and association, and therefore, multiple activation systems. Thus, it is clear that a great deal of data will potentially be generated by

lipidomics and there is a pressing need to also develop a bioinformatics approach to analyze these data as a reliable means to evaluate changes in lipids and their metabolites, potentially in real time.

The future will emphasize knowledge of lipid networks, connections between lipid pathways, as well as their regulation and interaction with non-lipid signaling cascades (e.g., protein–protein interactions, protein phosphorylation/dephosphorylation) so that the biology of these lipids is better understood. New connections are emerging with COX-2, sphingosine-1-phosphate, docosanoids (e.g., neuroprotectin D1), etc. PAF is an example of a lipid that is connected to multiple signaling pathways, because for reasons that are not well understood, the precursor of PAF in the central nervous system, is enriched either in arachidonic acid or docosahexaenoic acid. Therefore, as soon as you activate that pathway, PAF is subsequently formed. The released arachidonic and docosahexaenoic acids lead to different cascades, but interestingly those cascades very likely interact with other lipids.

Another example of lipid networks in the area of drugs of abuse is the demonstration that the CB1 receptor is coupled to the generation of ceramide, and ceramide is produced via two pathways, sphingomyelin hydrolysis and ceramide synthesis de novo. Marijuana abuse is associated with schizophrenia, and the psychotomimetic ingredient of marijuana, tetrahydrocannabinol (THC), is an agonist at the endocannabinoid receptor.

There is a considerable difference between the monoamine, amino acid, and peptide neurotransmitters and lipid mediators, in terms of release, storage, and transport. It is becoming very clear that these lipid modulators differ significantly, for example, from other classes of biomolecules, such as biogenic amines. Here we have a lot of neuronal circuitry and highly organized storage mechansims, but nevertheless, their number is specified and limited. But for lipids, we have a situation where there is first of all, combinatorial biosynthesis, with the opportunity to produce thousands of modulators. So, in terms of organization now, one may look at them in two different dimensions, maybe separating them into microlipidomics and macrolipidomics. Initially one may examine infrastructure there, where we have the big picture of lipidomics, and then go into finer detail, because if one goes entirely into microlipidomics, the picture could be too complex.

Specificity of protein–protein interaction may be maintained by phosphorylation and other covalent modifications. Likewise, for lipid–lipid signaling, the specificity of lipid signaling (in terms of the intercellular signaling or within a particular compartment of the cell) should be determined. One needs to think about what is actually regulating the signaling mechanisms. In this regard, it is also important to understand that phosphorylation/dephosphorylation acts as a "switch on/off" mechanism for signaling cascades that involve mainly proteins. However, with the advent of lipidomics, and the discovery of more biologically relevant lipids, covalent modification may also play a vital role in lipid signal transduction.

In order to examine the various pathways and mechanisms in detail, it may be necessary first to enhance local lipid concentrations. One way that may be possible to increase local concentration of the lipid is using caged lipids. Then, if a particular lipid can be selectively increased within a certain compartment of the cell, for example, by using lasers, it will be easier to visualize with what molecules the ligand is interacting and the signaling mechanism within a particular compartment of the cell can be better understood. The development of new techniques to study lipid–lipid or lipid–protein interactions within a particular intra-

cellular compartment is very important. Use of laser-based technology, and caged lipids to modulate local concentration within cells, and studying subsequent cellular and biological responses, will be particularly fruitful to understand the cascades of lipid signaling.

When a specific compartment is involved, it should be remembered that all compartments are made of structural lipids and when signaling and compartmentalization are discussed, it should be emphasized that the compartment itself plays a role in signaling, as seen with rafts. Therefore, it is important that we understand the significance and specificity of lipids during the life span of the cell.

One advantage with proteins is that many of them can be visualized through use of GFP and can be examined within a cell. At present this cannot be applied to lipids. One can't put a big fluorescent label on a tiny lipid and not change its localization dramatically. This may also be a problem with proteins, but it works in many cases due to the overall properties of large proteins. But when you put a fluorescent label on a lipid, that label changes the delicate balance of its lipid properties, thereby easily changing its location. Adding a labeled lipid to a cell also may not work for the same reason. Second, what one really wants to do is generate a labeled lipid in situ, just as one generates GFPs. The notion of generating a labeled lipid, and generating it biosynthetically, is not straightforward, if it could be done at all. Therefore, application of confocal microscopy of proteins to confocal microscopy of lipids is not expected to constitute a straightforward approach. New technologies for lipids are needed.

One approach worth exploring is to use the concept of total internal reflectance microscopy, which is being used for single-molecule visualization with fluorescent proteins, but one can also explore its use it with fluorescent lipids. Another approach is to utilize two-headed lipids, These lipids can be monitored as they bounce around inside their compartments and then bounce to another field and wobble around in another area. One can begin to approach doing single-molecule lipid dynamics with admittedly modified materials, so two lipid sites are available for modification

Another approach is to attempt to make a fluorescent arachidonate mimic. If one makes something that *looks* like an arachidonate, an anandamide, or an eicosanoid precursor, which is not an eicosanoid precursor, this could result in a arachidonyl analog that is not converted into prostaglandins, but might exhibit all the other activities. Another approach is "caging" and certainly anandamide can be caged and is fairly straightforward. The approaches described above might also work for sphingolipids and especially for studying sphingolipid metabolism: for example, to determine where ceramide, is formed, or sphingosine is formed. Another interesting approach would be the generation of antibodies against lipids to examine ceramides, ceramide phosphate, sphingosine-1 phosphate, to see where they are generated.

It is likely that designing compounds with extra fluorescent methylene groups to be monitored by fluorescent techniques will raise problems. An alternate approach to visualize and study lipids is to design a spin probe, and monitor it with an appropriate technique. This is something that one could follow with other suitable techniques, not necessarily fluorescence.

It is true that with proteins, one has the advantage that they can be expressed, and one can design them the way one wants, and the cell will synthesize them. This cannot be done with lipids. But lipids also have a special feature that can be used to advantage: lipids are very

plastic, pass through barriers effectively, and when the precursor is added from outside, it enters the cell in the same manner that the endogenous compounds do, if one knows how to do it.

5. CNS lipidomics

Many classes of lipids, in general, and glycolipids, in particular, are extremely complicated and difficult molecules to study with all of those sugar structures. The lipids are comprised of so many different classes and are important as signalers, more so than sugars, except in a certain restrictive way. So logically, perhaps, lipidomics, this newest era of metabolomics, is the next exciting "omics," especially in the areas of biochemistry and cell biology.

For CNS lipidomics, it will be very important to establish a causal role of lipids in behavior. There have been some examples where specific lipids are linked to behavior, addiction disorders, schizophrenia, Parkinson's, Alzheimer's, etc. Endocannabinoids exhibit a wide array of behavioral activity and play a role in regulating sedation, euphoria, appetite, memory, etc., and interact with a number of physiological systems, opioid, stimulant, reproductory, analgesic, etc. This is under the umbrella of multidisciplinary approaches: for example, the relation between LTP and lipids whereby specific lipids involved in LTP have brought the work from a cellular model of LTP into memory formation. Clearly, the linkage between lipid signaling and various forms of behavior, including memory formation and other forms of behavior, will be of major importance to study drug addiction. And future research will continue to define those types of roles for lipids. Sleep and sleep impairments are very important from a neurological and drug addiction point of view, including the role of endocannabinoids in the hippocampus, the correlation between deprivation and signaling mediators such as the endoconnabinoids.

There are many, many other examples of correlation between specific actions of lipid mediators and specific forms of behavior. Another important task is to define the role of lipid mediators in developmental neurosciences, such as: (1) correlating the developmental aspects of the brain with cell migration mechanisms whereby the cerebral cortex is formed and (2) mechanisms by which the areas of the brain more specifically linked to the limbic system are formed with the neurocortex. The Miller-Dieker syndrome is another example. About 10 years ago, the *Lys –1* gene was discovered, which turned out to be a PAF acetylhydrolase subunit. And now, the involvement of lysophospholipids and lysophosphatadic acid is going to be a very important area. There are some studies of fetal alcohol syndrome that imply disturbances in lipid signaling, and these might consequently affect behavior. This is going to be a very important area in the near future. A multidisciplinary approach to developmental neurosciences will serve not only to establish correlations, but also to establish cause and effect.

Something important from the point of view of CNS lipidomics is to ask, what is unique about the brain from the point of view of lipids? One unique feature of brain lipids is not arachidonic acid, but docosahexenoic acid. Docosahexaenoic acid, for reasons that are barely understood so far, is heavily concentrated in the central nervous system. It should be emphasized that the presence of docosahexaenoic acid (DHA) and the omega-3 fatty acids,

from the lipid point of view, is one of the most unique features of the central nervous system. For reasons that are not understood well, the brain retains docosahexaenoic acid avidly and tenaciously. A year or two of essential fatty acid deprivation in the diet is needed to deplete docosahexaenoic acid from the brain. In fact, work in recent years, clearly demonstrate the role of docosahexaenoic acid in memory formation, and researchers have compared this compound with arachidonic acid. Lipids unique to the CNS must be characterized and this area needs further exploration.

There are molecular mechanisms that we are just beginning to understand by which the brain and the retina retain these fatty acids. The essentiality of arachidonic acid is different. Arachidonic acid is present in the same quantity all over. And now it is beginning to be understood that DHA is the precursor for several bioactive molecules. So, in the future, omega-3 fatty acids and docosahexaenoic acid should be investigated further. For example, it was discovered a couple of years ago that ethanolamides of docosahexaenoic acid are present in the brain This is a lipid whose significance is waiting to be defined, and a number of new research findings will emerge in this area.

Other important lipids in the brain including the glycosphingolipids and the gangliosides, are highly abundant in the brain and their structures are very complex. Most of the lipid-storage diseases are brain diseases involving the breakdown of gangliosides. And so, we know that they are vitally important. It is questionable whether adequate technology is presently available to characterize all these molecules, and these need to be made amenable to current LC/MS and MS/MS technology. The abundance of glycosphingolipids and the gangliosides in the brain suggests that they have numerous yet-to-be discovered functions for several of these lipids. The development of different experimental strategies to determine the exact nature of these functions is critical.

Sterols are super-important molecules in the brain. They modulate several receptors and ion channels, and there is an extensive literature in this area, Cholesterol is an important sterol that has very important implications for drug-abuse studies. The role of cholesterol in lipid rafts are critical areas for further investigations. A point that was prominently discussed is AIDS and lipid rafts. Perhaps it is related to the glycosphingolipid and the ganglioside story, and the sterol story. Cholesterol, for example, is related to lipid rafts and their dynamics. And maybe that is something that can be explored by lipidomics: to separate the different domains of membranes, and determine which lipids are associated with certain domains. Lipid rafts, to the extent they exist, exist in thermodynamic equilibrium as collections and aggregates of lipids, and when they are removed to identify them, it is likely that their “true nature” is changed. Therefore, all the techniques for precipitating them, Triton extractions, etc., may result in obtaining some modified material, and there is no assurance that they exist in the cell in the form isolated. It is very important to keep in mind that it’s not going to be easy to isolate lipid rafts and then make realistic conclusions. Rafts exist, almost like micelles, depending for their existence on the solvent in the membrane that defines them. And so, techniques to visualize them in the intact cell should be developed if they are to be examined and their roles understood.

There is emerging evidence that cholesterol is involved in the early stages of Alzheimer’s disease. There has been an exponential interest in the last few years from several laboratories regarding the significance of cholesterol in specific regions of the brain, the subiculum, and also in the hippocampus, and how cholesterol might play a role in early cognitive changes

in Alzheimer's disease. The last category of compounds of interest in the brain are the isoprenoids, the fat-soluble vitamins, the quinines, etc. Special attention should be given to the retinoic acids. Of course, these are quite important in general, not only for the eye, but also for CNS lipidomics research.

6. Importance of lipidomics study for disease

It's worth considering that by dry weight, the brain is mostly lipid. Moreover, it is also clear that understanding lipid composition and signaling mechanisms will provide insights into the mechanisms of drug abuse itself an will have implications for pharmacotherapy. So, knowing about brain's lipids and related components is potentially very useful for NIDA research and drug development.

More studies are needed, from a variety of specific cell types in the nervous system. With that information at hand, it will be possible to attempt to synthesize the information to characterize fully the role of lipid functioning in brain. Multidisciplinary research approaches will be required. That is, insofar as lipidomics research is in its early stages, it is important to bring together teams of multidisciplinary scientists (such as neuroscientists, chemists, immunologists, cell biologists, biophysicists, histologists, analytical and metabolic chemists, AIDS scientists) frequently to discuss ways to further the science of lipidomics and to move the field forward in terms of funding and organization. Frequent national and international meetings might help to stimulate the field.

To promote lipidomics, it must become technically transparent, so that one can focus on the important questions. At this stage, the challenge is really not to amplify complexity, but to bring it down to something that is understandable. One way do it may be to identify biological targets and then to focus on lipid pathways, metabolism, enzymology and "druggable" targets.

At present, there is adequate knowledge to do this, to go to the cellular level, and there are enough tools to bring a multidisciplinary approach to get specific answers. At present, some of the enzymes and proteins involved in lipid metabolism are not only drug targets, but targets for drugs that are blockbusters, for example, the cyclooxygensases and HMGCoA reductase.

Progress in lipidomics can start with enhanced chemistry. The research community needs reagents, analytical standards and deuterated internal standards, etc. that are not readily available, and they must be made available to researchers to promote research. All the "endolipids" in the CNS should be characterized as a first step. It is a simple fact that there are thousands of lipids and many, likely, will have important biological functions. Some of them may have very low abundance, but they are bound to be involved in basic behavioral motives such as approach versus avoidance, which may underlie drug abuse. It should be emphasized that it will not be possible to understand how the brain works until one knows what are all the endogenous constituents, precursors, end products, etc. It should be followed by studies on the signaling molecules.

Support is needed for research looking at novel signaling mechanisms that could influence some component of drug abuse. It has been shown that several drugs of abuse play a part in development and this must be a focus area, along with enhanced research in the

maternal-fetal pharmacokinetics area. This will serve a useful purpose in understanding not only the fetal development, but also developmental and other effects that may appear in later stages of life, such as at the adolescent and adult period. Another suggestion for research support would be "lipid modulators of addiction" or "molecular analysis of spatiotemporal changes in lipid signals," similar to what NCI does with the IMAT program.

Another aspect to consider in terms of lipidomics and drug abuse is the understanding of the nervous systems in terms of reward pathways and the role of lipids. The lipidomics technology is already underway, the tools are available, and if the critical signaling molecules to study are identified, one will have a good rationale why one would like to do certain kinds of experiments, especially on mechanisms of reward and addiction.

At present, a lot of emphasis is placed on developing technology, but not so much on using the technology that is already available to answer many questions regarding lipids and signaling in the brain. Application of existing technology should be encouraged. There is a critical need in this area for bioinformatics, whereby one can start inputting many signaling molecules, and establishing correlations between vast amounts of data with complex behaviors. Computational neurosciences area, an emerging area in the neurosciences, also will have an impact.

Lipidomics has already helped to promote progress in several areas: the role of PLA2 and PLA2 products in various forms of plasticity, LTP, LTD, eicosanoids in neuroprotection, roles of fatty acid anandamides in the regulation of pain, understanding the mechanisms of inflammation, etc. However, the lipidomics approach holds considerable promise for contributing to several areas of neurosciences such as signaling and pathways.

7. General approach to lipidomics

It is expected that the "LIPDS MAPS" initiative will pave the way to a number of discoveries and substantially advance lipid research in the discovery and quantification of new endogenous lipids and in the elucidation of lipid pathways.

The ongoing LIPID MAPS initiative includes six Cores, which are set up to use LC/MS, to explore specific lipids with each Core focused on a particular category of lipid. These include the: eicosanoids/fatty acids, neutral lipids, phospholipids/lysophospholipids, sphingolipids, isoprenoids, and sterols.

Table 1 shows the multitude of lipids discussed by speakers at the NIDA Workshop. Interestingly, the LIPID MAPS categories of fatty acids/eicosanoids, phospholipids/lysophospholipids and sphingolipids were extensively discussed by participants at the workshop with a great deal of focus on the canabinoids which LIPID MAPS considers under the fatty acids/eicsoanoids category. At this point in time, the investigators present did not emphasize neutral lipids, sterols, or isoprenoids as brain signaling molecules, although clearly some of them are.

The first category includes the eicosanoids and fatty acids. In addition to HETES and HODES, arachidonic acid itself is considered to be a signaling molecule as well as a substrate for other enzymes, DHA, and the many oxygenated forms of DHA are other important molecules for consideration. This brings up a fundamental nomenclature problem. One needs to include under the eicosanoids, DHA and other n-3 fatty acids that are

Table 1
The different classes of lipids discussed at the NIDA workshop

Eicosanoids/fatty acids	Prostaglandins HETES, HODES Leukotrienes AA, DHA and Oxygenated Forms
Phospholipids/lysophospholipids	PA, PC, PIP2, PIPs, Isoprostanes PAF LPA, LPC, Lyso Alkyl/Vinyl Ethers
Cannabinoids/related compounds	Anandamide, 2-AG, Fatty Amides (sulfates, amino acids) Fatty Esters, Oxidized Forms
Sphingolipids	Sphingosine-1-phosphate Ceramide

especially prevalent in brain, These oxygenated forms in some cases are analogues of the prostaglandins and leukotrienes, just with an extra double bound, or an extra couple of carbons on them. So, the question is whether a new name is needed for this whole collection of related molecules, or whether we should just stick with eicosanoids.

Important molecules discussed in the Workshop include: phospholipids and lysophospholipids, PIPs, isoprostanes (not clear if these should be classified as phospholipids or as eicosanoids, because they are prostaglandins made on a phospholipid backbone), lyso, alkyl and vinyl ethers, as well as the now well established signaling molecules LPA and LPC, cannabinoids (anandamide, 2AG, fatty amides, and sulfated ones, amino acid conjugates to the amides, fatty esters, etc.). Then there are oxidized forms of all of these molecules. An important question is: are these oxidized forms more like the prostaglandins and leukotrienes and HETES? Or are they distinct, and what should we call them? Sphingolipids were extensively discussed.. Sterols are another important category, and especially one should include cholesterol as it appears to be is involved in metabolic pathways in a major way.

Finally, there really are only four kinds of biological molecules—nucleic acids, amino acids, sugars, and lipids. Nucleic acids and proteins have been well recognized and explored extensively. The majority of the neuroscience and biochemistry community does not yet recognize that lipid research is “mainstream” research, and regard the lipid molecules as the “taken for granted molecules”. Hence, lipidomics is not now in the mainstream. So, it has to be integrated into the other concepts – drug addiction, behavior, or development – before it gets accepted. Lipid research in all areas of science has yet to fully emerge and be recognized for its central role. Hopefully in this decade, the systems biology approach to lipidomics will give the lipid area a greater visibility in the scientific community like that enjoyed by both genomics and proteomics. This was probably the first major scientific conference that was held with the title “lipidomics”, and it succeeded very well in bringing together all of the related areas of lipidomics with a focus on the brain. It is sure to have stimulated an exciting new direction in lipid research.