ACS Chemical Neuroscience

Viewpoint

pubs.acs.org/chemneuro

Monitoring Molecules: Insights and Progress

R. Mark Wightman*

Department of Chemistry and Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States

ABSTRACT: In August, 2014, neuroscientists and physical scientists gathered together on the campus of the University of California, Los Angeles to discuss how to monitor molecules in neuroscience. This field has seen significant growth since its inception in the 1970s. Here, the advances in this field are documented, including its advance into understanding the actions that specific neurotransmitters mediate during behavior.

KEYWORDS: *dopamine, fast-scan cyclic voltammetry, microelectrode, microdialysis, brain chemistry*

During the period August 3−7, 2014, an unusual mix of neuroscientists and physical scientists gathered on the University of California, Los Angeles (UCLA) campus to discuss their research. The meeting that they attended, entitled Monitoring Molecules in Neuroscience, was the 15th gathering of this group that extends back to 1982 when the first meeting in this series was held in Nottingham, U.K. The meeting is sponsored by the International Society for Monitoring Molecules in Neuroscience (http://www.monitoringmolecules.org/). The mission of this Society is to provide a platform to facilitate the development and refinement of methods for time-resolved detection of chemicals in the living brain.

The catalyst that began this interdisciplinary area was a 1976 review in *Analytical Chemistry* written by Ralph Adams entitled "Probing Brain Chemistry with Electroanalytical Techniques."[1] Adams was a distinguished professor in the Department of Chemistry at Kansas University who had literally written the book on electrochemistry. He also held an appointment in Neurobehavioral Sciences at the Menninger School, a leading institution in psychiatry at the time. His review described what Adams termed an "intrusion" into neuroscience that he and his lab had initiated earlier in the decade. Because neuroscience was an emerging field, rarely approached by chemists, he introduced the basics of neuronal transmission as they were then understood, and then he used research examples to show that electrochemical techniques had great promise to explore several new aspects of neurochemistry and neuroscience. A major development from the Adams lab was the combination of electrochemical detection with liquid chromatography. Liquid chromatography in those days was undergoing a revolution that led to a dramatic increase in resolving power through the use of columns packed with small particles with the mobile phase delivered by high pressure pumps. The Adams lab demonstrated that thin-layer electrochemical cells were ideal detectors for oxidizable or reducible eluting analytes. The separation of catecholamines and their detection by electrooxidation allowed unprecedented trace detection of these substances extracted from brain tissue. This technique, commonly abbreviated LCEC (liquid chromatography with electrochemical detection), formed the basis for the first approaches for detection of neurochemicals sampled by microdialysis.

Another technique that Adams espoused was the use of small electrodes as implantable sensors for neurochemicals, a field known as in vivo electrochemistry. Adams suggested that electroanalytical techniques (chronoamperometry, cyclic voltammetry) were uniquely suited for monitoring neurotransmission in real time. As a post doc in the Adams laboratory, I had become fascinated with this area. At the time Adams' article appeared, I was establishing my own academic lab at Indiana University. The challenges in the area of in vivo electrochemistry were many, and so I elected to pursue it in my own academic career.

The first challenge for in vivo electrochemistry research was to develop much smaller voltammetric electrodes. Francois Gonon and co-workers used carbon fibers for this purpose and developed electrodes with diameters in the 10 μm range. Electrochemists, including ourselves, were fascinated by these microelectrodes because not only could they be used for minimally destructive measurements in the brain, they also had other unique properties such as fast time response, lack of distortion in highly resistive environments, and an altered diffusion field compared to larger electrodes.[2]

Numerous other challenges were also confronted. Among these were the belief by many electrochemists and surface scientists that electrodes would fail in brain tissue because of protein and tissue adsorption leading to electrode fouling. In spite of this concern, we found that responses from electrodes that were acutely implanted (a few hours) were only slightly distorted by implantation, and that signals that allowed for distinction of neurochemicals could be achieved, albeit with some reduction in sensitivity. However, concerns about fouling are re-emerging as investigators are now exploring the use of chronically implanted (days to weeks) electrodes to monitor neurotransmitter dynamics.[3]

Another challenge that confounded progress in the early days of in vivo electrochemistry was the chemical selectivity of the measurements, a topic that was the subject of an early review.[4] Gonon and co-workers found that electrochemical pretreat-

Special Issue: Monitoring Molecules in Neuroscience 2014

Received: December 9, 2014
Published: December 16, 2014

dx.doi.org/10.1021/cn500324m | *ACS Chem. Neurosci.* 2015, 6, 5−7

ment of carbon-fiber microelectrodes dramatically altered the voltammetric characteristics of most compounds and enabled their resolution. However, dopamine and its major metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), were indistinguishable even using Gonon's treated electrodes. Additionally, the electrochemically modified electrodes were sluggish in their response to local chemical changes. Adams and Greg Gerhardt coated electrodes with Nafion, a Teflon-like cation exchange membrane. The Nafion coating restricted access of anions such as DOPAC and ascorbate to the electrode surface, while cations such as dopamine, which is protonated at physiological pH, were concentrated and readily detected. The quest for selectivity also led to the development of microdialysis, a perfusion technique that allows substances in the extracellular fluid of the brain to be extracted and analyzed by any appropriate analytical technique including LCEC. Urban Ungerstedt and Jay Justice were pioneers in this technique that has since been the subject of over 10 000 citations. Microdialysis is a superb technique for understanding drug–neuronal interactions, but the probe size (mm) and time scale of operations (minutes) preclude its use during rapid behavioral studies.

Julian Millar was interested in methods of in vivo detection that would allow real-time characterization of neurotransmission during rapid physiological events. They adopted fast-scan cyclic voltammetry for this purpose and showed that it could detect exogenous compounds transiently introduced into the brain. The method had time resolution similar to that of neurochemical processes and differentiated a variety of neurochemicals by the distinct shapes of their cyclic voltammograms. A disadvantage was the high background current that arises from the rapid sweep rates employed. While this makes it difficult to determine basal levels, the background could be removed by digital subtraction. Despite the power of this technique, as late as 1984 it had not been used to detect endogenous neurotransmitters. That year I took a sabbatical leave in Millar's laboratory in London. Jon Stamford, a graduate student at the time, and I decided to look for dopamine release with fast-scan cyclic voltammetry with a carbon-fiber microelectrode implanted in the striatum of anesthetized rats. We electrically stimulated the medial forebrain bundle, the known site of dopamine projections to the striatum, and obtained clear signals for dopamine with subsecond time resolution.[5] Our shrieks of excitement in the U.K. were probably heard around the world! This was the start of many investigations into dopamine dynamics with this technique that have guided our understanding of their various modes of neurotransmission. Notably, it took the combined efforts of neuroscientists (Millar, Stamford) and an electrochemist (myself) to demonstrate the power of fast-scan cyclic voltammetry for in vivo monitoring of neurotransmitter dynamics.

In 1992, Paul Garris was a post doc in my lab, and we attended a Catecholamine Symposium in Amsterdam, The Netherlands. Paul had demonstrated the power of fast-scan cyclic voltammetry to investigate regional differences in dopamine transmission, and also had established considerable evidence that dopamine was a volume transmitter. However, as we were listening to the ongoing presentations and were planning our future experiments, it became clear to both of us that we needed to be able to do experiments in freely moving animals. In Adams' original review, he showed implanted electrodes in awake rats. All that was missing was a technique with sufficient chemical resolution to measure neurochemical signaling during behavior. Since fast-scan cyclic voltammetry was proving to be so useful in anesthetized preparations and brain slices, Paul decided to extend its use to freely moving animals. After many trials, Paul succeeded, and this set the groundwork for the use of in vivo electrochemistry to probe brain signaling during a variety of behaviors including goal directed behavior, addiction, and aversive behaviors.[6]

A major outcome of the measurement of neurochemicals and their dynamics in freely moving animals is a greatly refined understanding of the specific activity of dopamine during reward-based behaviors. As anticipated by theories of reward prediction error, in vivo voltammetry has demonstrated that dopamine is released in response to unexpected rewards and, in well trained animals, to cues that predict reward. This has been shown for both natural rewards, such as sugar pellets or sweetened solutions, as well as drugs of abuse. However, a major unanswered question concerns the actions of released dopamine on the remainder of the reward circuitry of the brain. One strategy discussed at the UCLA meeting toward answering this goal was the use of genetically altered mice with channelrhodopsin incorporated into neurons that receive dopaminergic input. A strategy we are pursuing is to develop methods to introduce pharmacological agents locally to identify nearby receptor subtypes and to modulate their activity during behavior.

Despite the progress of in vivo electrochemistry, the meeting at UCLA revealed that the whole field of monitoring molecules in neuroscience has expanded far beyond its original roots. In addition to discussions of fast-scan cyclic voltammetry, symposia were held on brain imaging that involved techniques as far reaching as positron emission tomography, functional magnetic resonance, and fluorescence histochemistry. Sensors in the form of enzyme-modified electrodes and dramatically improved microdialysis techniques were described. This diverse range of techniques was broadly used in a number of applications, with topics presented ranging from forays into fly brain neurochemistry, to neurochemical adaptations during alcoholism, cocaine abuse, and marijuana use, and the plasticity of neurotransmission. New approaches to characterize the neurochemical roles of glutamate and different neuropeptides were described using techniques ranging from chemical sensors to mass spectrometry. It was very clear that Ralph Adams' invitation to pursue neuroscience research with new analytical chemistry techniques has certainly been embraced by a broad range of scientists. The 40th anniversary of the publication of Adams' review will coincide with the next Monitoring Molecules in Neuroscience Meeting that will be held in Gothenburg, Sweden, in 2016.

AUTHOR INFORMATION

Corresponding Author

*Mailing address: Department of Chemistry and Neuroscience Center, CB #3290, Kenan Laboratories, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3290. Phone: 919-962-1472. Fax: 919-962-2388. E-mail: rmw@unc.edu.

Funding

Research in this area has been supported by NIH (DA010900).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Adams, R. N. (1976) Probing Brain Chemistry with Electroanalytical Techniques. *Anal. Chem. 48*, 1126A−1138A.
(2) Wightman, R. M. (1981) Microvoltammetric Electrodes. *Anal. Chem. 53*, 1125A−1130A.
(3) Clark, J. J., Sandberg, S. G., Wanat, M. J., Gan, J. O., Horne, E. A., Hart, A. S., Akers, C. A., Parker, J. G., Willuhn, I., Martinez, V., Evans, S. B., Stella, N., and Phillips, P. E. (2010) Chronic Microsensors for Longitudinal, Subsecond Dopamine Detection in Behaving Animals. *Nat. Methods 7*, 126−129.
(4) Wightman, R. M., May, L. J., and Michael, A. C. (1988) Detection of Dopamine Dynamics in the Brain. *Anal. Chem. 60*, 769A−779A.
(5) Millar, J., Stamford, J. A., Kruk, Z. L., and Wightman, R. M. (1985) Electrochemical, Pharmacological and Electrophysiological Evidence of Rapid Dopamine Release and Removal in the Rat Caudate Nucleus Following Electrical Stimulation of the Median Forebrain Bundle. *Eur. J. Pharmacol. 109*, 341−348.
(6) Robinson, D. L., Hermans, A., Seipel, A. T., and Wightman, R. M. (2008) Monitoring Rapid Chemical Communication in the Brain. *Chem. Rev. 108*, 2554−2584.