# Tools and Services for CNS Research From BAS

*Adrian W. Bott, Ph.D.* and Scott A. Glazier, Ph.D.*

*Bioanalytical Systems, Inc.*
*2701 Kent Avenue*
*West Lafayette, IN 47906*

**email:*
*awb@bioanalytical.com*

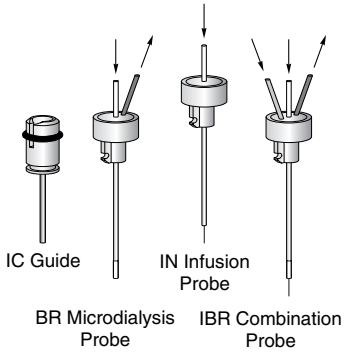
*BAS was founded nearly 30 years ago to develop commercial instrumentation for the electrochemical detection of neurotransmitters. Neurochemistry has continued to be a focus for BAS in the development of both products and services. This article discusses how BAS products and services can be used in CNS research.*

Bioanalytical Systems (BAS) has been providing products and services for neuroscience for nearly 30 years. Our tools and methodology help biologists make better chemical measurements—easier, faster, cheaper. We have organized symposia, provided hundreds of workshops, and we try to keep the state of the art moving, often in collaboration with our academic and industry partners.

CNS drugs developed in part using BAS products and services, including support for clinical trials, now have annual sales of just under $10 billion. Thousands of dedicated individuals have made this possible. Millions of people are living better lives because of their effort. Many know BAS for only a small part of what we do, whether it be liquid chromatography, contract research, toxicology support, dialysis probes, awake animal systems, and automated blood sampling. The aim of this article is to describe the full range of products and services BAS can provide for CNS research.

***F1***

*BAS brain microdialysis probes.*




## *Brain Microdialysis*

Studies of drug metabolism, neurotransmitter activity, drug delivery, or pharmacokinetics are more dynamic and informative when conducted in living animals. Microdialysis is an *in vivo* sampling technique that allows concentrations of various compounds in the extracellular space of different tissues to be determined. A probe is implanted into the appropriate tissue, and a perfusing solution is pumped slowly through the probe (μL/min). Low molecular weight analytes pass from the extracellular fluid into the perfusing solution by crossing a semi-permeable membrane. There is no net transfer of fluids across the membrane, only analytes. The collected analytes can be injected directly onto an LC system (e.g., *via* a BAS Pollen-8 On-Line injector) or collected via a microvolume fraction collector (e.g., the BAS Honeycomb refrigerated fraction collector). If collected, they are later quantitated by an appropriate analytical technique (e.g., LC/EC, LC/MS, RIA, etc.). It is even possible to infuse drugs directly into the region while the microdialysis experiment proceeds.

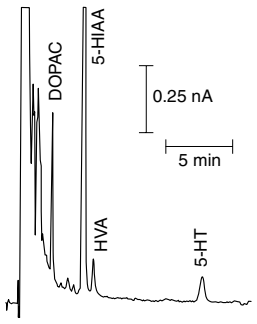
BAS brain probes are concentric probes (***F1***), which allow accurate placement and minimize tissue damage. They are generally used in combination with intercerebral (IC) guides, which allow removal and reinsertion of the probes. Membrane lengths of 2 or 4 mm are standard for BR probes. The infusion brain (IBR) probe includes an additional cannula to allow infusion of a drug simultaneously with the sampling. MBR probes are smaller probes that have been specifically designed for use in mice, or for experiments in which multiple probes are required. The standard membrane lengths are 1, 2 or 4 mm. Custom probes are also available.

## *Liquid Chromatography / Electrochemistry*

The activity of many CNS drugs is based on their modulation of the concentration of neurotransmitters such as catecholamines (e.g.,

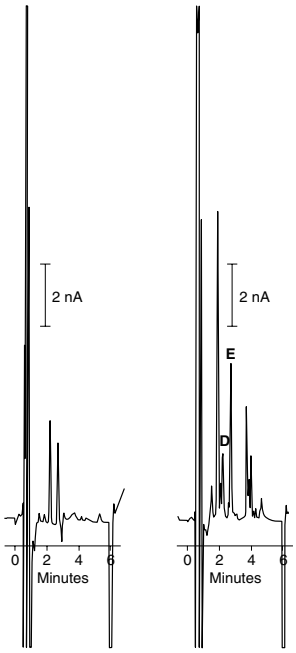
dopamine and epinephrine) and serotonin. Electrochemistry is the preferred method of detection for these compounds, as it can determine the low endogenous neurotransmitter concentrations in brain microdialysates and other *in vivo* samples. Catecholamines and serotonin (and their metabolites) are electrochemically active, and can be directly oxidized at the surface of a glassy carbon electrode (***F2***). In contrast, amino acids (i.e., glutamate, aspartate and GABA) are not electrochemically active, but can be detected following the formation of stable, electrochemically-active conjugates by reaction with *o*-phthalaldehyde/*t*-butylthiol (***F3***). Acetylcholine and choline also lack an electrophore, so an indirect method is used, based on the products of enzyme reactions.

***F2***



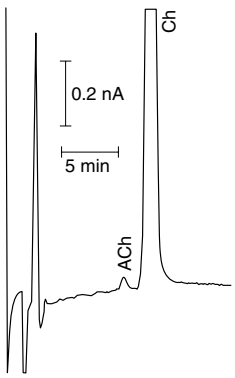

*Typical chromatogram of microdialysate from rat SCN at 30 minutes after administration of melatonin (10 mg). DOPAC = 3,4-dihydroxyphenylacetic acid, 5-HIAA = 5-hydroxyindole-3-acetic acid, HVA = homovanillic acid, and 5-HT = serotonin. Reprinted with permission from Curr. Seps. 18 (2000) 117. Copyright Bioanalytical Systems, Inc., 2000.*

***F3***




*Chromatograms of aspartate (D) and glutamate (E) in standard solution (2 pmole) (a) and rat caudate dialysate (b). Reprinted with permission from Curr. Seps. 9 (1989) 59. Copyright Bioanalytical Systems, Inc., 1989.*

***F4***




*Chromatogram of basal acetylcholine in rat prefrontal cortex microdialysate with using esterase inhibitors. (The detected ACh peak as 8 fmoles/µL.)*

Acetylcholine is first converted to choline by acetylcholinesterase, and choline reacts with choline oxidase to form hydrogen peroxide which is electrochemically active. The original working electrode of choice for the detection of hydrogen peroxide generated by the acetylcholine/choline assay was a platinum electrode. However, the detection limit obtained using this electrode was not low enough to determine the very low endogenous acetylcholine in brain microdialysates, since acetylcholine is rapidly converted to choline by acetylcholinesterase. One method that has been used to counter this problem was esterase inhibitors to increase acetylcholine concentrations. However, the addition of esterases can alter the dynamics of the acetylcholine system and hence, measurements without the addition of acetylcholinesterase inhibitors are preferred (1). Using the BAS "wired" peroxidase enzyme working electrode rather than a platinum working electrode for detection of hydrogen peroxide (2), basal acetylcholine (in the absence of inhibitors) can be observed (***F4***), as the wired electrode allows measurement at a potential (+100 mV (vs. Ag/AgCl), as opposed to + 500 mV used for platinum) where background is minimal, thereby lowering the detection limit.

BAS was the first company to commercialize LC/EC, and we have maintained our position as the leader in this field through development of the LC-4x and the epsilon multichannel electrochemical detectors and other LC accessories (e.g., the PM-80, PM-91e, and PM-92e pumps). (See *www.bioanalytical.com/products/lc.html* for a complete listing of LC products.) BAS microbore technology is particularly suited for quantitation of neurotransmitters due to the low endogeneous levels of these compounds. Our expertise in this area also allows us to offer contract services for the quantitation of many neuroactive compounds. Quantitation limits for these analyses will vary depending on sample type and the number of analytes to be determined in a single chromatographic injection. The following typical values are presented as a guide only and cannot be guaranteed for every matrix:

Acetylcholine: 20 fmoles injected
Dopamine: 100 fg injected
Serotonin: 150 fg injected
Norepinephrine: 500 fg injected
Epinephrine: 500 fg injected
Aspartate/glutamate: 0.5 µM
GABA: 50 fmoles injected

See *www.bioanalytical.com/products/support/lcanaly.html* for more details on these services.

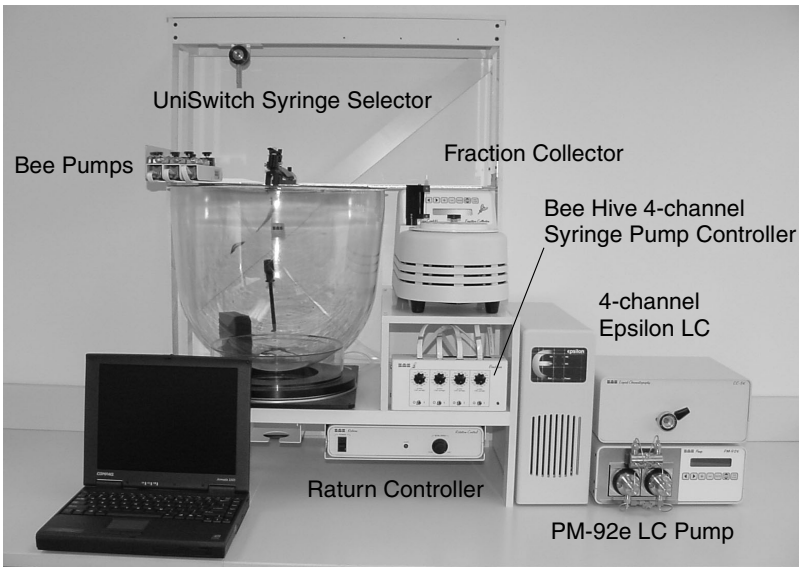
### BAS Systems for Awake Animals

*In vivo* sampling experiments work best in an environment that allows the subject some freedom of movement, comfortable bedding, and free access to food and water. Samples collected under these conditions will better represent normal physiology than those collected from stressed or anesthetized animals. This consideration is particularly important for neurotransmitters. BAS has a number of systems designed with the above considerations in mind.

One such system is the BAS Bee-Keeper System. Key components of this system are the counter-balanced arm, which gives the animal freedom to move about the bowl, and the dual-channel liquid swivel, which prevents twisting of the fluid lines. However, there are problems associ-

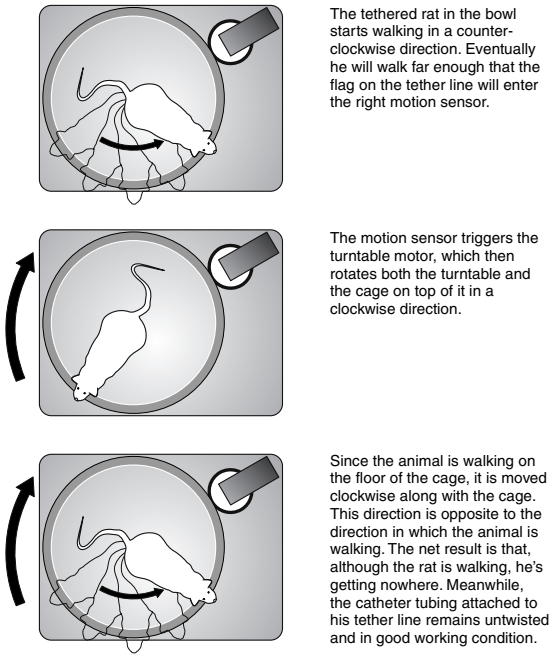
**F5**

*Benchtop workstation for* in vivo *studies of neurotransmitters, including the Raturn, 4-channel epsilon electrochemical detector, Bee Pumps, Bee Hive 4-channel syringe pump controller, and Honeycomb refrigerated fraction collector.*




**F6**

*Illustration of the Raturn mechanism.*




ated with liquid swivels, including clotting in the lines, leaking between channels, high maintenance, difficulty with sterilization resulting in likely bacterial contamination on internal parts, and substantial dead volumes. These problems are eliminated in the BAS Raturn® which replaces the liquid swivel with optical switches (***F5***). As the animal moves in the bowl, one of the optical switches is activated, causing the bowl to rotate interactively to counter movement of the rat. This allows the rat to move freely while preventing the fluid lines from twisting (***F6***). Multiple lines are possible when using the Raturn. Another version of the Raturn includes the Metabolic Chamber (***F7***), which allows collection of urine and feces during, for example, ADME studies. The Rodent Workstation (***F8***) provides up to four Raturn systems on one cart.

An additional feature of the Raturn is the optional Activity Monitoring software, which records signals from the optical switches, including direction, frequency and duration of the signals. This can provide initial information about the animal's response to an administered drug.

A Raturn metabolic chamber specifically designed for mice is being used in the BAS drug metabolism research laboratory and will be released for sale in the summer of 2002.

### *Culex® Automated Blood Sampling (ABS) (3-5)*

Although microdialysis has considerable potential for pharmacokinetic studies, current drug development protocols require pharmacokinetic information based on the variation of drug concentration in the blood with time. The technology developed for the Raturn has been used by BAS (with input from some major pharmaceutical companies) to develop the Culex Automated Blood Sampler (ABS). Key components of the Culex ABS are as follows:

#### Metabolic Chamber

As discussed above, this allows the animal to exist in a stress-free, contained environment while remaining connected to peripheral devices (e.g., for blood collection, drug infusion, etc.). Urine and feces can be collected which allows measurement of drug and metabolites in the urine in addition to in the blood. The Activity Monitoring software also allows correlation of pharmacokinetic data with behavioral changes.

#### Honeycomb Fraction Collector

This allows collection of blood samples to be automated. Once collected, the samples are maintained at 3°C.

#### Blood Sampling Protocol

The blood sampling protocol (i.e., collection times and blood sample volumes) is defined by the user. Protocols for four different animal stations can be run simultaneously and asynchronously. Blood clotting in tubing is eliminated by moving the blood through the sampling system using sterile saline containing a small amount of heparin. Heparin can also be added to the collection vials if plasma sampling is required.

After the specified volume of blood has been drawn, an equal volume of saline is returned to the animal in order to maintain fluid balance. Movement of fluids through the collection system is achieved by pre-programmed syringe pumps

Since the Metabolic Chamber can accommodate multiple lines, microdialysis experiments can be run in parallel with blood sampling. Therefore, a drug can be administered and the variation of the drug and its metabolites can be monitored as a function of time, both in the blood and at the site of action.

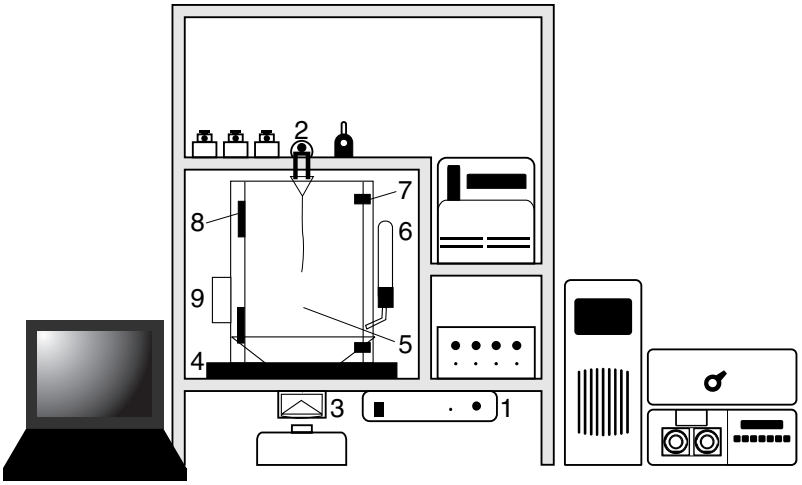
One major advantage of the Culex ABS is elimination of the stress associated with manual blood sampling. This was recently illustrated in a study at BAS (6), which compared plasma stress hormones (epinephrine and norepinephrine) using the Culex vs. a manual sampling method. As can be seen in ***T1***, plasma stress hormones are significantly higher when the manual blood sampling method was used.

More information on the Culex ABS is available at *www.culex.net.*

***F7***

*Schematic illustration of the Raturn Metabolic Chamber for awake animal studies.*

*1) Raturn controller, 2) counter-balanced arm with movement sensor, 3) urine/feces separator/ collector, 4) turntable base, 5) hinged and removable door, 6) water tube, 7) door lock (1 of 2), 8) hinge (1 of 2), 9) food bin for pellets or granules.*




***F8***

*Rodent Workstation with multiple Raturn systems.*

***T1***

*Culex vs. Manual Sampling Method*

| | RAT A pg/mL PLASMA | | RAT B pg/mL PLASMA | |
|---|---|---|---|---|
| | Epinephrine | Norepinephrine | Epinephrine | Norepinephrine |
| Culex | 65.9 | 251.9 | 57.5 | 483.6 |
| Manual | 1302.2 | 1365.6 | 3003.8 | 1200.1 |

## *Contract Analytical Services*

In addition to the neurochemical assays discussed above, GLP and GMP contract services are available through the Analytics division of BAS. In particular, our bioanalytical services division has extensive experience monitoring CNS drugs using LC/MS for preclinical pharmacokinetics and in all four phases of clinical trials. A list of validated assays is available at *www.bioanalytical.com/info/assays.html*.

This article was intended to be a brief description of the capabilities of BAS relevant to those involved in research and development of CNS drugs. For more information, please contact *bas@bioanalytical.com*.

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