Culex Automated Blood Sampler Part II: Managing Freely-Moving Animals and Monitoring their Activity

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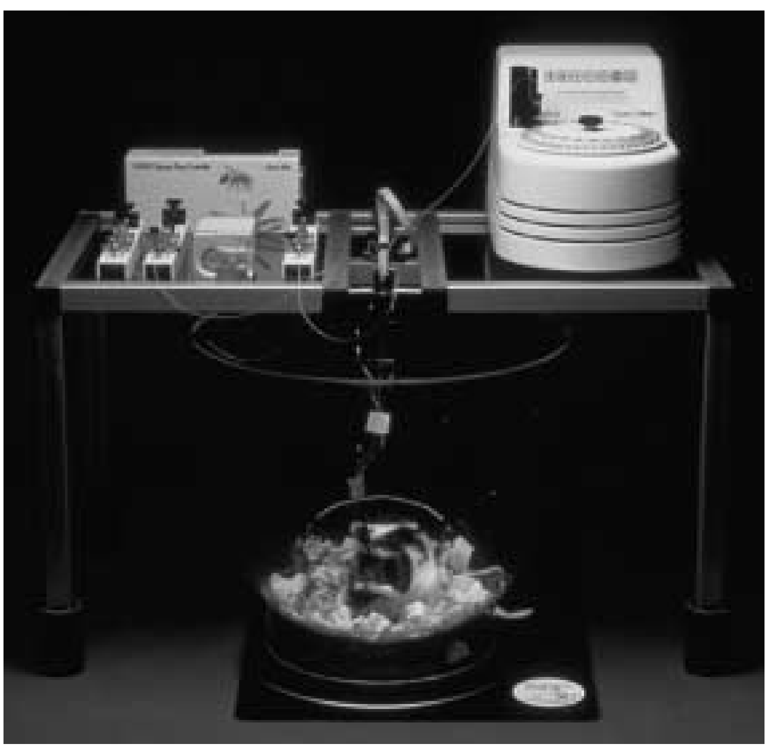
As a company involved with biomedical analysis for more than 25 years, BAS has processed all of the common body fluids including whole blood, plasma, serum, urine, bile, and CSF. It is more common for researchers in biomedical research to collect these biofluids than to conduct microdialysis, an alternate in vivo sampling method that we have offered since 1985. Yet, it was microdialysis, and one of the products we developed to support the technique, that lead us to design the Culex™ Automated Blood Sampler. The discussion offered here provides some background on our approach to managing the natural movement and activity of animals housed in the Culex. This approach derives from the BAS Raturn™ System for Awake Animals, which was originally developed to protect the integrity of inlet and outlet tubing lines used in microdialysis.

In the Spring of 1997, BAS introduced the Raturn[1] (pronounced *rat turn*), an instrument system designed to house a single rodent (rat, mouse, hamster, guinea pig) during a microdialysis, drug infusion, or in vivo biosensor experiment (***F1***). A microdialysis experiment requires that a probe containing a semi-permeable membrane be implanted into tissue (brain, liver, kidney, dermis, heart, adipose tissue, eye, bile duct, blood vessel, etc.) in a living animal and then flushed continuously with a physiological saline solution. Molecules in the tissue such as drugs, drug metabolites, or endogenous chemicals, are swept away by this fluid as they diffuse through the semi-permeable membrane.

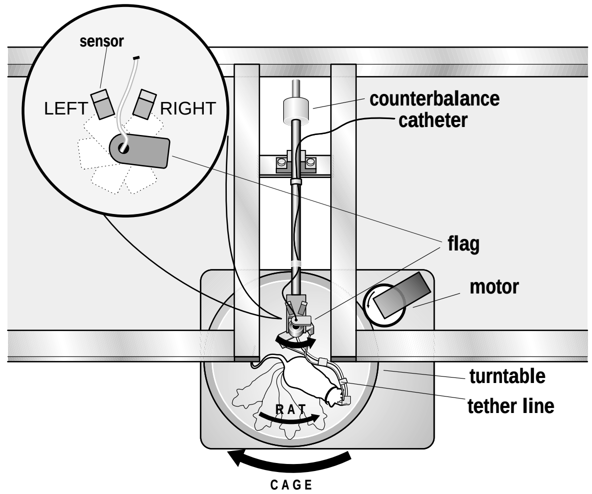
Microdialysis enables a researcher to monitor the concentration flux of such compounds in a specific living tissue. It is a valuable tool for pharmacokinetics[2,3]. The technique requires that lines of tubing are attached between a syringe pump and a probe in the animal, and between the probe outlet and a collection vessel at some distance from the animal. Rodents are normally allowed to roam free during such experiments to minimize stress which might otherwise influence metabolism. This freedom of movement requires management of the inlet and outlet tubing lines which run from the peripheral devices (syringe pump, fraction collector) to the ani-

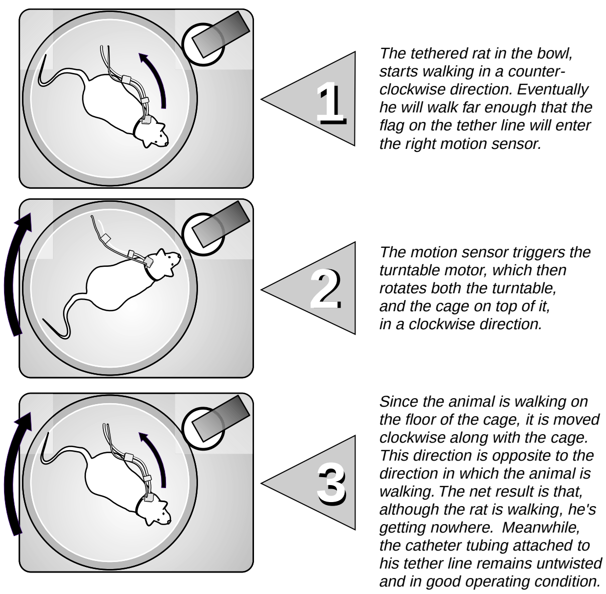


F1

The BAS Raturn system was developed to handle the multiple lines of tubing common to a in vivo microdialysis experiment with an awake and freely-moving animal. This concept worked so well that it was also applied to manual blood sampling. The logical extension of these developments was an automated blood sampling system which became the robotic system known as the Culex ABS.

F2





An animal housed in either the Culex or Raturn system is free to move up and down, or left and right, at will. The catheter line(s) are attached to a tether wire which is in turn attached to a counterbalanced arm. The arm responds to the animal's vertical movements by pivoting up when the animal rears up, or down when he returns to a resting position. The arm also holds the sensor assembly which registers rotational behavior within the round cage. Clockwise, or counterclockwise rotations beyond 270° will trigger a sensor, which in turn starts a turntable under the cage rotating in an opposite direction. Because of these systems, the catheter tubing remains unstretched, untwisted, unchewed, and intact.

mal. Otherwise, these lines would be chewed by the animal, or twisted by the animal's movements.

The traditional solution to such a problem had been the use of a liquid swivel device. Swivels provided rotational movement but also were limited by the number of fluid channels, a large swept volume, a tendency to leak (including internal leaks between inlet and outlet channels which were not visible to the user), and internal parts which were not chemically compatible with saline solutions or reactive to certain analytes flowing through them. Some people also did studies connecting wires from a potentiostat to implanted electrodes, or connecting fiber optic cables between an external light source and an implanted probe. These experiments would require a swivel commutator, for transfer of the electrical or optical signal from one end of a swivel connection to the other. Serious loss of signal and "commutator noise" was an expected component of such a setup.

No Swivels

The Raturn system offered a distinct alternative to the liquid swivel or swivel commutator. It jettisoned such devices entirely. Instead of routing fluids or signals through an intermediate device, the tubing lines, or wires, or cables remained intact from the animal to the external controlling or collecting device. Fluids were no longer exposed to any surfaces other than the inside of the conducting tubing. Swept volumes were reduced to the volume of the tubing itself, which was considerably less than the swept volume of the swivel, thus shortening the collection time. There was no commutator noise because there was no commutator, resulting in better signal quality.

Counter-Rotation

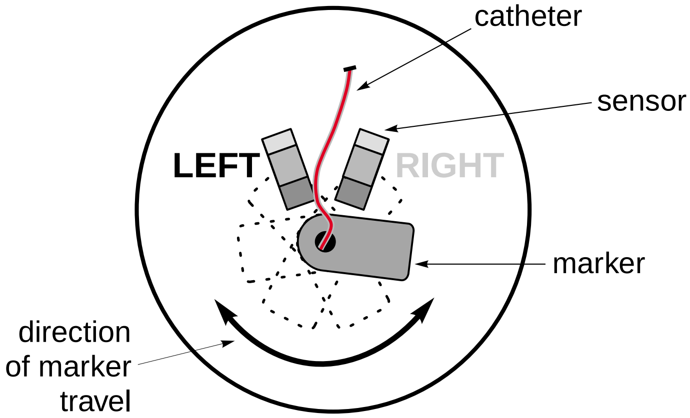
With the Raturn, all lines are connected to a tether wire attached to a collar or harness on the rat. When the tether moves, so do the lines attached to it. As shown in ***F2***, the tether wire is in turn attached to a sensor assembly mounted on a counterbalanced arm above the cage. The counterbalanced arm pivots up and down to accommodate rearing behavior. The tubing and cables pass intact through the center of the sensor assembly and then out to their respective controlling devices. The sensor assembly has two sensors, one monitoring clockwise rotation of an animal in the round cage below, and another monitoring counter-clockwise rotation of the animal. When a sensor is triggered by the animal's movement, a motor is also triggered. The motor rotates a turntable under the animal's cage, so that the cage (and the animal within) is turned in a direction ***opposite*** to the animal's direction of rotation. To put it simply, if the rat moves one way, the Raturn moves the cage (and the rat) the other way. Lines don't become twisted because the system opposes the animal's movement with an equal and opposite movement.

Taking a Stroll in a Plastic Bowl

From the animal's point of view, a Raturn is probably like a a hamster wheel. If the animal wants to run, it can. Sometimes an animal will run, sometimes it will walk, sometimes it sleeps or is sedentary. The cage moves only when it must counter the animal's movement. The overall level of animal activity in a Raturn

F3

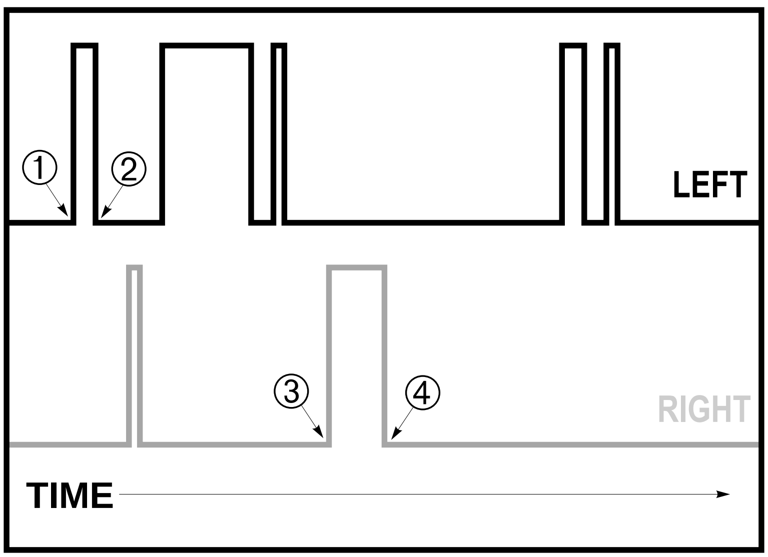
When the marker on the Culex arm (or Raturn) enters either the left (or right) sensor, it's like turning on a switch. As long as the marker is in the sensor, the "switch" remains on. Only one sensor can be active at one time since there is only one marker turning around a central axis. LEFT sensor counts indicate clockwise rotation by the animal, while RIGHT sensor events represent counterclockwise rotation.



F4

When a sensor is activated by the marker, a pulse is generated. The height of the pulse is constant, while the width represents the time the sensor remains engaged (duration). Sensor counts measure the frequency (incidents per unit time) of movements in one direction. Duration indicates intent - how determined was the animal to continue moving in that direction? The signal starting at ① and ending at ② represents a single activation of the left sensor. The signal ③-④ is a right sensor activation. The time between points ①-② or ③-④ is the duration, in seconds.

The period between the pulses represents a time in which the animal was either not moving, or not moving enough to engage either sensor.



F5

The Animal Activity Report lists sensor events. Each event is described in terms of the time it occurred relative to the start of the experiment (e.g. 0:42:59 means 42 minutes and 59 seconds after start, and 2579 is that time converted to seconds); the sensor identity (e.g. Left); and the duration (e.g. 0:0:1 and 1.00 both represent one second) This raw data can be exported into a spreadsheet such as Excel, for manipulation into graphs such as the one in F6.

```
Data file name is C791100.rtd
(Start) Time = 11/2/99 7:42:06 AM
Data Sampling Interval = 1 (Sec)

Page 21, Event #1: (@ 11/2/99 8:23:06 AM), 0:41:0, 2460,
Oral dose administration 2mg
Page 11496, Event #2: End of RUN
Total Run Time = 392:17:39 (1412259)
(END) Time     = 11/18/99 3:59:45 PM

==== Interval Report ====
Page 22, 0:42:59, 2579.00,  Left,  0:0:1, 1.00
Page 22, 0:43:3, 2583.00,  Left,  0:0:1, 1.00
Page 22, 0:43:12, 2592.00,  Left,  0:0:1, 1.00
Page 22, 0:43:16, 2596.00
```

appears similar to that of an animal not in a Raturn. Rats tend to rest during the day and become more active at night, both in normal cages and in the Raturn.

Animal Activity Measurements

The measurement of animal activity is a feature of the Raturn that was a byproduct of its development. Every time a sensor is activated by the animal's movements, a signal is recorded by a computer. This logs the identity of the sensor (left sensor = clockwise, right sensor = counterclockwise), the time of the event and the number of seconds that the sensor remained engaged (duration). The sensor will be active as long as the animal keeps moving in that direction. As illustrated in ***F4***, the right sensor event labeled ③④ would indicate that the animal moved in the counterclockwise direction for a longer period than it had in the prior left sensor event ①②. The consideration of duration, as illustrated in ***F6***, provides more information about the animal's behavior than sensor counts alone.

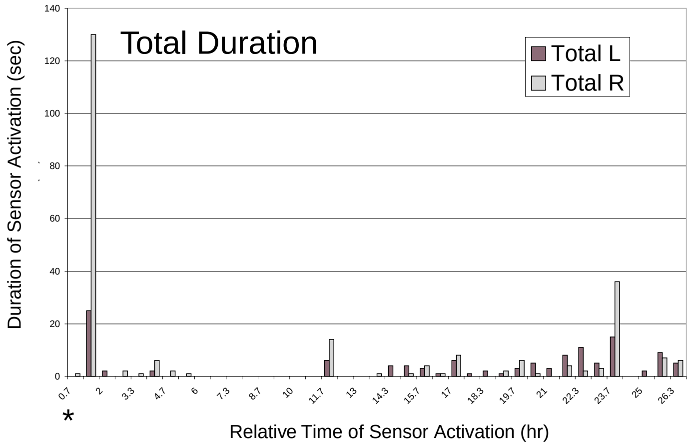
From Raturn to Culex

Although the Raturn was invented to address the needs of a microdialysis experiment, it was apparent that it could be used for other applications. These included drug infusion, biosensor or in vivo electrode monitoring, light transmission through fiber optic cables, and blood sampling from an intravenous catheter. This last application stimulated more investigation. It was logical to add automation to the blood withdrawal process already made possible by the Raturn and the resulting robot became the Culex Automated Blood Sampler.

Just as microdialysis tubing is handled by the Raturn, a catheter line can be attached to the tether wire and extended outside of the cage in the Culex system. The Culex uses the same sensor assembly as the Raturn, and also monitors animal activity by recording the direction of rotation, time of sensor activation and duration. Like the Raturn, the Culex allows the use of multiple lines to be connected to the animal. These are

F6

*Animal activity data may reflect drug-induced changes in behavior. In this example, an oral dose of a CNS active drug was administered at the time point marked * (41 min. after starting the experiment). A review of sensor counts (not shown) and duration data revealed an increase in activity shortly after dosing, followed by a long period of inactivity. The animal was observed to be awake but heavily sedated during the inactive period. After 12 hours,it resumed activity. Sensor counts indicated that it moved clockwise (left sensor) more frequently than counterclockwise (right sensor). However, duration data showed that although the animal moved counterclockwise less frequently, it clearly spent more total time moving that way. The operator observed the rat running for a long period.*



F7

Part of the metabolic cage for the Culex is a collection system which separates and stores urine and feces. The urine is collected in a glass scintillation vial and maintained at a temperature less than 4°C for periods up to 16 hours. The feces are separated from the urine immediately and remain out of the direct path of urine to prvent cross-contaminatiion.

most likely to include the intravenous catheter and a drug infusion catheter. In the Culex, the IV catheter is connected to a "tubing set" which is external to the animal cage, and mounted on the Culex controller.

A syringe drive attached to this tubing set diverts a blood sample to the fraction collector and returns blood and sterile saline to the animal back through the catheter[5].

Less Handling, More Success

The catheter line is filled ("locked") with heparinized saline between blood sampling events, just as it would be in manual blood sampling. However, unlike manual sampling, an animal installed in the Culex system is not disturbed or handled during the blood sampling process, or afterwards. This lack of handling protects the catheter. Catheters are often implanted a few days prior to blood sampling, which doesn't allow much time for healing. When an animal is picked up and restrained for a blood draw, the ensuing struggle may displace the line. Since the Raturn eliminates handling, it improves the chances of maintaining a well-positioned implant and enabling subsequent blood draws. Since the blood does not have to pass through swivels, valves, or other disparate materials, the probability of initiating a clot is reduced.

Metabolic Studies Involve More than Blood Alone

We anticipated that the primary users of the Culex system would be researchers involved in pharmacokinetics and drug metabolism research. Since this group studies disposition and excretion of drug, we added a feature to the Culex system which enables the collection and separation of urine and feces while an animal is caged for the blood sampling experiment. The concept of being able to compare metabolite levels in the urine with drug concentrations in the blood *collected simultaneously from the same animal* seemed like an improvement both in time management and quality of data.

Cage Concepts

A new cage was designed for the Culex that used the original Raturn line management concept. The cage includes a removable food hopper, that could be weighed to monitor food intake, and a water tube that was easy to refill/replace. The animal resides on a stainless steel grid with free access to both food and water. Wastes pass through the grid and are channeled by a stainless steel funnel underneath the grid towards a separator. The urine passes through the stainless steel mesh in the separator and is collected in a glass vial maintained at a temperature of less than 4°C. The feces roll down the steep incline of the separator to be

F8

The standard cage provided with the Culex is the metabolic cage (left) which includes the means to collect and separate urine and feces, and has removable food and water dispensers.

An optional cage for the Culex is the original bowl developed for microdialysis (right). This round bottomed bowl is used with bedding material and a water tube can be hung from the inside wall of the cage. There is no provision for collecting metabolic wastes with this bowl.

stored outside the drip path of the urine, and prevent contamination.

Conclusion

The Culex ABS evolved from the Raturn System for Awake Animals and uses the same principles to allow the animal to move freely without damaging the catheter, or other test lines. The addition of animal activity measurements, and the collection of urine and feces, extends the research value of the Culex beyond blood sampling alone.

References

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