

# Webinar Q&A Report:

## Gold Standard Physiological Measurements and Novel Drug Delivery Methods

For Christian Schnell – Session 1:

**Q: What are the advantages of using a micro-infusion pump over doing an oral gavage?**

*[C. Schnell]:*

For our own compound, it does not cross the BBB and therefore oral gavage will not work.

For rodents, one should expect to see the same negative effect on all parameters like those shown with the marmoset during my presentation. Basically, there will be high level of stress during oral gavage in rodents too. Expect PK, metabolism and parameters not yet studied to be affected. The best situation is to get rid of stress because training is not really a solution. An implantable pump is the only way to deliver the compound without interfering at the moment of delivery.

**Q: How useful is micro-infusion pump in dosing mice against subcutaneous infections?**

*[C. Schnell]:*

Using state of the art techniques (surgery and personnel), there is no reason to have infections following surgery to implant pumps. Once pumps are implanted, they are under the skin and infections should not occur. In fact, they could be a good method for reducing infections versus other methods.

*[T. Tan]:*

### **Administration of Substances to Laboratory Animals: Equipment Considerations, Vehicle Selection, and Solute Preparation**

Patricia V Turner, Cynthia Pekow, Mary Ann Vasbinder, and Thea Brabb

J Am Assoc Lab Anim Sci. 2011 September; 50(5): 614–627. Published online 2011

September. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3189663/?tool=pmcentrez>

## Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider

Patricia V Turner, Thea Brabb, Cynthia Pekow, and Mary Ann Vasbinder

J Am Assoc Lab Anim Sci. 2011 September; 50(5): 600–613. Published online 2011

September. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3189662/?tool=pmcentrez>

**Q: In rodents, what is your opinion on adding drug PO or adding it in to the food? We can control the dose if we don't add it PO application?**

*[C. Schnell]:*

Adding to food is an interesting and straight forward method. Not all compounds can be added to get a homogenous mixture. Manufacturing of food is difficult. If feeding behavior is impacted by molecule than can be create a vicious circle. Do not exclude adding to food. In academic environment, can simplify but could be restricted to a single class of molecule.

**Q: Is there a device for measuring neural activity in unrestrained animals?**

*[T. Tan]: /Google Search <Measuring Neural Activity in Unrestrained Animals>*

*First Videos Created of Whole Brain Neural Activity in an Unrestrained Animal*

<http://www.technologyreview.com/view/534481/first-videos-created-of-whole-brain-neural-activity-in-an-unrestrained-animal/>

**A wireless transmission neural interface system for unconstrained non-human primates.**

<http://www.ncbi.nlm.nih.gov/pubmed/26269496>

**Bioluminescence as a Tool to Monitor Neural Activity in Freely Behaving Animals**

[http://www.sfn.org/~media/SfN/Documents/Short%20Courses/2010%20Short%20Course%20I/SC1\\_2010\\_3Engert.ashx](http://www.sfn.org/~media/SfN/Documents/Short%20Courses/2010%20Short%20Course%20I/SC1_2010_3Engert.ashx)

*A Wireless Multi-Channel Recording System for Freely Behaving Mice and Rats*

<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0022033>

**Q: Can the pumps be used in large animals such as swine?**

*[T. Tan]:*

Yes, they have been used in pigs, rabbits, guinea pigs, dogs, monkeys, goats and cows.

OKON E. B., LEE J. H. T., STREIJGER F., MANOUCHEHRI N. , ANDERSON L. M., KWON B. K.

**Intraparenchymal spinal cord microdialysis in unanaesthetized freely moving pigs following thoracic spinal cord injury**

Neuroscience 2013, San Diego, USA. November 9th - 13th 2013

<http://www.abstractsonline.com/Plan/ViewAbstract.aspx?sKey=26dc24fd-2afe-4922-aa98-271fb03f99fd&cKey=9bccf58f-6cc4-49d8-b1d8-25170efedd71&mKey=8d2a5bec-4825-4cd6-9439-b42bb151d1cf>

Asemu G., O'Connell K.A., Cox J.W., Dabkowski E.R., Xu W., Ribeiro R.F. Jr., Shekar K.C., Hecker P.A. Rastogi S., Sabbah H.N., Hoppel C.L., and Stanley W.C.

**Enhanced Resistance to Permeability Transition in Interfibrillar Cardiac Mitochondria in Dogs: Effects of Aging and Long Term Aldosterone Infusion**

Am J Physiol Heart Circ Physiol ajpheart.00674.2012; published ahead of print December 15, 2012, doi:10.1152/ajpheart.00674.2012

<http://ajpheart.physiology.org/content/early/2012/12/10/ajpheart.00674.2012.abstract?sid=1c8187a4-b1a5-41e2-9e88-20610af15128>

**Q: Has the iPRECIO pump been used in mice?**

*[C. Schnell]:*

Yes, they can be with the SMP-300 iPRECIO Micro Infusion Pumps. They are much smaller than the SMP-200 pump and designed for mice in mind.

**Q: I was wondering if you could speak to the ability of these pumps to intermittently deliver drugs instead of continuous delivery?**

*[C. Schnell]:*

The programmability of the pumps allows any speed and scheduling to be programmed. ON/OFF, ON/OFF. The only thing is that you need to know what you want to program in advance or up front.

For new pump (SMP-300 instead of SMP-200 used at Novartis), it will allow pre-programming and in-vivo programming. This capability opens tremendous opportunity to be reactive to observations seen during dosing with the pump.

*[T. Tan]:*

Selected iPRECIO intermittent publications. For example, from second webinar session (Kelly et al.)

Kelly E. Henry, Clinton T. Elfers, Rachael M. Burke, Oleg G. Chepurny, George G. Holz, James E. Blevins, Christian L. Roth, Robert P. Doyle

**Vitamin B<sub>12</sub> Conjugation of Peptide-YY<sub>3-36</sub> Decreases Food Intake Compared to Native Peptide-YY<sub>3-36</sub> Upon Subcutaneous Administration in Male Rats**

Endocrinology February 6, 2015 doi: 10.1210/en.2014-1825

<http://press.endocrine.org/doi/abs/10.1210/en.2014-1825>

T. Kroon, J. Gabrielsson, N.D. Oakes

**The abruptness of terminating nicotinic acid delivery has a profound effect on free fatty acid and insulin rebound in rats**

51st EASD Annual Meeting, Stockholm 2015

<http://www.easdvirtualmeeting.org/resources/the-abruptness-of-terminating-nicotinic-acid-delivery-has-a-profound-effect-on-free-fatty-acid-and-insulin-rebound-in-rats--2>

Tobias Kroon, Ann Kjellstedt, Pia Thalén, Johan Gabrielsson, Nicholas D. Oakes

**Dosing Profile Profoundly Influences Nicotinic Acid's Ability to Improve Metabolic Control in Rats**

The Journal of Lipid Research, doi: 10.1194/jlr.M058149 , July 13, 2015

<http://www.jlr.org/content/early/2015/07/13/jlr.M058149.abstract>

**Q: What is the maximum size of compound that can be infused? (Molecular weight)**

*[C. Schnell]:*

Do not expect size of compound/molecular to be rate-determining factor for pumps. Most likely solubility of the compound will determine factor. If molecule crashes of solution/formulation, then not really good for pumps. Usually test at room temperature that concentration after infusion is as expected. Formulation collected with Eppendorf tube and then tested. Making sure that molecules stay in solution and does not crash out. Usually tested over 24 hour period at RT.

**Q: Are you aware of these pumps being used simultaneously with microdialysis experiments and can you comment on the challenges and success associated with this set-up?**

*[C. Schnell]:*

Not aware of publication where these pumps are used with simultaneously with micro-dialysis but predict that it could be a good combination. Used Pumps with radio telemetry and there was no crosstalk and worked fine. Predict that should work fine but remains to be seen.

*[T. Tan]:*

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[Dmitry Zaretsky, Indiana University School of Medicine, [dzaretsk@iu.edu](mailto:dzaretsk@iu.edu)]:

General thought #1: For microdialysis a researcher needs to pump the liquid through the probe and then collect the perfusate. Replacement of syringe pump with the implantable pump removes the need of the inlet tubing, but what to do with the outlet tubing is still an issue. In big animals attaching the collection Eppendorf tube and manual replacement to collect samples is probably doable without major stress for an animal. However, in small animals, while it is doable (using the saddle, for example), the repeated manipulation of replacing the collection tube could outweigh the advantages of formally free-moving status.

General thought #2: there are two aspects of using the pump for microdialysis – first, the technical ability of the pump to perform the job; second, the validity of the results.

Let's start with the first question. We have used reversed microdialysis to deliver bicuculline to the hypothalamus through the commercial microdialysis probe (CMA11 microdialysis probe, Harvard Apparatus, MA). The flexible elastic outlet catheter of the pump was cut and was connected to thick-wall Teflon microdialysis tubing to reduce dead volume. The pump was set at the highest rate, and we got reproducible responses to the bicuculline which were very similar to what we usually observe in our microinjection experiments.

The researcher needs to consider that the maximal flow rate is 30 ul/h, which is 0.5 ul/min. For comparison, microdialysis is usually performed at 1-2 ul/min. The reason for that is to collect sufficient volume within reasonably small amount of time, for example 10 min. The volumes could be a problem for some applications.

To conclude, the pump is able to do the job of perfusing the probe, considering the flows and resistance of microdialysis probe.

Second aspect. Microdialysis experts are insisting on the need of flow stability. The manufacturers of commercial microdialysis pumps stress that their pumps are pulsation-free within requirements of the technique. That is quite reasonable: the exchange in the CMA11 probe occurs in the semipermeable tubing with outer diameter of 0.24 mm. The volume of 1 mm of this tubing is less than 0.05 ul even before considering thickness of the wall and presence of another tubing inside. It is obvious that even at 1 ul/min the liquid is exchanged virtually every second. Due to their mechanism of action, peristaltic pumps don't provide that stability.

Nevertheless, the probe which is perfused by a peristaltic pump will provide some results. Obviously, if time of sample collection will significantly exceed the length of one cycle (there are four pumping cycles per each revolution of the motor), any effects of pulsations will be averaged. It cannot be directly compared to microdialysis performed by syringe pump, but I personally would not object comparisons within the same experiment. The estimations of absolute concentrations outside the probe will be questionable, but still defensible if appropriate techniques are used. So, the results could be valid within limitations.

Major conclusion: the researcher needs to assess the advantages and disadvantages of minipump for the microdialysis. In many scenarios disadvantages will outweigh the difficulty to defend the results in peer-reviewed manuscripts.

ZARETSKY D.V., ZARETSKAIA M.V., DURANT P.J., RUSYNIAK D.E.

**The use of microinfusion pump to perform intrahypothalamic injections in conscious rats**

Neuroscience 2012, New Orleans, LA, USA. October 13-17, 2012.

<http://www.abstractsonline.com/Plan/ViewAbstract.aspx?mID=2964&sKey=87d8b951-316f-466a-9eb7-4b154d0bbd2c&cKey=b4b8338f-9bd2-44e2-bcf4-6e05a36cbbcb&mKey=%7b70007181-01C9-4DE9-A0A2-EEBFA14CD9F1%7d>

**For Robert Doyle – Session 2:**

**Q: How much of your PYY effects is due to changes in leptin sensitivity?**

*[R. Doyle]:*

Have not looked at Leptin Level changes and is on the to-do-list. Expect that Leptin level changes are playing havoc and compensation tied to Leptin Levels. Would like to co-infuse conjugate with an antagonist (oxytocin).

**Q: What were the best resources for learning how to program the iPRECIO mini pumps? Was it relatively simple?**

*[R. Doyle]:*

Highly skilled technician used iPRECIO for the first time and did not have any difficulties and expect that similar skilled technicians to be fine. Medicinal chemists with only basic husbandry have been trained by technician (Monday – Friday week), to program and implant (surgery). Never lost a pump and never had a student make any mistakes.

*[T. Tan]:*

Resources are available to iPRECIO users and customers. These include:

- Surgery training videos,
- Step by step programming guides
- Telephone and online assistance.

Contact Primetech to have a copy of receive of copy of these resources.

If you have additional questions for Christian Schnell or Dr. Robert P. Doyle regarding content from their presentations or wish to receive additional information on the iPRECIO micro-infusion pump, please contact Mr. Schnell, Dr. Doyle or Mr. Tsung Tan from Primetech Corporation by email:

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