Validation and optimization of automated patch clamp voltage-gated Ca2+ channel

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WEBINAR Q&A

Q: Can the cells on the hole in the plate be washed off and plate reused? or do you have to use a new plate for every new experiment?

A: We do not recommend re-using the chips, as cell debris can remain on the patch clamp aperture thus dramatically reducing the success rate in subsequent experiments. The NPC chips, NPC-16 on the Patchliner, are equivalent to a patch clamp pipette which can also only be used once. One study has shown that Port-a-Patch chips could be cleaned, but since the aperture is less accessible in Patchliner chips with their fluidic channels on both sides of the hole, we would not expect that this can be done to the Patchliner chips and would not recommend it. We can only guarantee good success rates using new chips for every experimental run, similar to manual patch clamp where the glass pipette is also changed after every experiment.

Q: How long normally can the cells remain healthy in these conditions? (how long can the recording be)

A: We would say generally 30 mins are the lifetime of the experiments. Of course it is not written in stone, it can also last much longer or less, depending on the channel or cell type. Compared to manual patch clamp, recordings tend to last longer, presumably because the cells on a planar chip are much less affected by vibration/movement.

Marc: In the Patchliner Cav2.2 experiments we aimed for stable recordings and drug testing over a 20 minute whole-cell duration, which was sufficient for double applications (2x2 min) of 4 increasing concentrations of test compounds after a 4 minute period of baseline stabilization.

Q: Is there sensors detecting when the cells is attached on the hole in the plate? In other words, the -ve pressure would not be on all the time right?

A: Yes, the Patchliner software instantly reduces the suction once the seal resistance crosses a customizable value. A customizable amount of pressure can be maintained during the recording to maintain the whole cell configuration. The negative pressure can also be completely removed after the whole cell configuration is reached, this is up to the user and is simply programmed into the experimental protocol.

Q: How does the solution exchange do not affect patched cell

A: Once a good seal is formed between the cell and the glass of the chip, this connection is very stable and will not be affected by exchanging the external solution. In addition, the speed and the amount of solution exchanged can be adjusted to suit the experiment. If your cells are particularly fragile you can lower the speed of application, if you are

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recording fast desensitizing ligand-gated ion channels on the other hand, you can increase the speed of application to minimize ligand exposure.

Marc: We could optimize the speed, volume and timing of liquid applications onto each cell/well to ensure we did not 'knock-off' the cell from the hole during the IC_{50} experiment. Such failures were generally quite low, especially if the seal was of high quality.

Q: Do you have an opportunity to make single channels recordings using your Patchliner system?

A: Yes, it is possible to record single channels. It was successfully applied on Erythrocytes in cell-attached mode:

https://www.nanion.de/en/products/patchliner/137-articles/141-erythrocytes-single-channel-recordings.html

Alternatively, single channel recordings from ion channels reconstituted into bilayers can also be performed:

https://www.nanion.de/en/137-articles/306-gramicidin-bilayer-recordings.html

Q: Single-channel recordings (cell attached) with Patchliner - are they possible? Noise level? Examples? Alternatively, single channel recordings from ion channels reconstituted into bilayers can also be performed:

A: Yes, it is possible to record single channels. It was successfully applied on Erythrocytes in cell-attached mode, with low noise level see the following link:

https://www.nanion.de/en/products/patchliner/137-articles/141-erythrocytes-single-channel-recordings.html

Alternatively, single channel recordings from ion channels reconstituted into bilayers can also be performed:

https://www.nanion.de/en/137-articles/306-gramicidin-bilayer-recordings.html

Q: Is Dynamite an essential add on for all applications or specific studies?

A: The Dynamite[8] was specifically designed for improved recordings of cardiac action potentials and is not needed for any other application.

Q: Some customers felt that seal enhancer may lead to a different outcome on the results. Can we run experiments without using seal enhancer with our machines?

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A: We have replaced the classical seal enhancer by an external recording solution containing 10 mM $CaCl_2$, which is completely replaced by physiological external solution after brief exposure. The IC_{50}s recorded with this improved approach perfectly matched the literature values (see CiPA study), proving that with this approach, the Patchliner delivers accurate pharmacology recordings.

In rare cases where calcium cannot be used, we have alternative recipes that we share with our customers upon request.

Q: Did you set up an assay for mouse $Ca_V2.2$? As you said no effect of lead series compounds in $Ca_V2.2$ KO mice...

A: We tested compounds against native $Ca_V2.2$ in mouse DRG (on manual patch clamp), but
a) did not have a mouse $Ca_V2.2$ cell line, nor **b)** ran DRG cells on Patchliner. We are aware that some groups have tested native DRG neurons on PL in the past, but with low success.

Q: Have you tried running your Ca_V channels at physiological temp (either automated or manual)? What was your final success rate here?

A: No, we did not use temperature control features available on the Patchliner (or on our MP rigs) to test $Ca_V2.2$ compounds at anything other than room temperature. We are not aware of anyone else doing this, at least from competitor Ca_V programs run at big pharma and biotech companies.

Q: Why Ca assays only for pain drug assays when other ion channel assays are there?

A: The Patchliner is not limited to studying Ca_V channels as pain targets. Metrion has set-up successful assays for several pain-related TRP channels on the Patchliner, as examples of using the ligand-gated features of this platform. We also ran major $Na_V1.7$ and $Na_V1.8$ screening campaigns for clients using the Patchliner, which are major pain drug discovery targets. Some of this data is available on a poster hosted on our website, here:

https://www.metrionbiosciences.com/wp-content/uploads/2017/03/Metrion-Biosciences-poster-Ion-Channel-Retreat_Vancouver-July-2015-v4.pdf