# The mechanism of action of dimethylfumarate, a treatment option in Multiple Sclerosis


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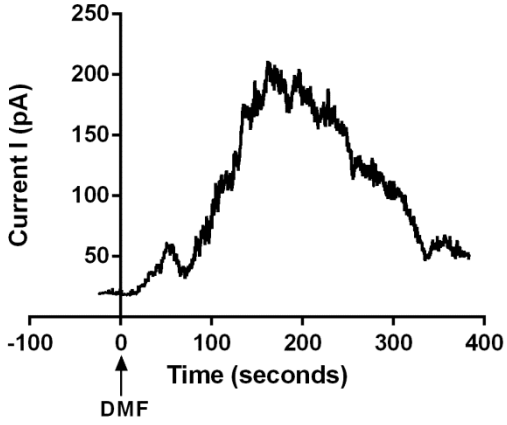
Home Institution: Lab of Prof. Dr. Methner, Department of Neurology at the University Medical Center of Johannes Gutenberg University Mainz, Germany

Host Institution: Lab of Nicola Hamilton-Whitaker, PhD, Institute of Psychiatry, Psychology & Neuroscience, Wolfson Centre for Age-Related Diseases, King´s College London, UK


1) During my training stay at the Institute of Psychiatry, Psychology & Neuroscience in the Wolfson Centre for Age-Related Diseases at King´s College London I had the unique opportunity to learn whole cell patch clamping. In order to do this I had to isolate mouse splenocytes. I learned how to set up the patching equipment and software, how to use them and how to analyze the results. I also obtained experience in troubleshooting to get the whole patch clamp system running. I learned a lot of the basics about electrophysiological topics and gained experience in doing it. As the lab was international I improved not only in laboratory techniques, but also in communicational skills and critical thinking in lab meetings.

For my project I tried to prove whether Dimethyl fumarate (DMF), a novel oral therapeutic agent used in multiple sclerosis (MS), increases TRPA1-mediated $Ca^{2+}$ currents. TRPA1 is a member of the transient receptor potential (TRP) cation channel family that can be activated by oxidative cysteine modification through reactive oxygen species [1]. DMF causes short-lived oxidative stress and thereby induces the antioxidant response including glutathione synthesis [2] but the immunosuppressant mechanism of action of DMF is still unclear. I found that DMF significantly increases the $[Ca^{2+}]_i$ and alters redox homeostasis in mouse and human immune cells. The effect on $[Ca^{2+}]_i$ can be blocked with the TRPA1 inhibitor HC030031 suggesting an involvement of this receptor. In the lab of Nicola Hamilton-Whitaker I indeed saw a current induced by application of 10 µM DMF (A) that has a T current-voltage relationship resembling a TRP channel (B), but after adding the TRPA1 inhibitors A967079 or HC 0030031 there was no significant decrease in current measureable (C). In order to find a clear answer as to whether the DMF is activating TRPA1 more experiments are needed.

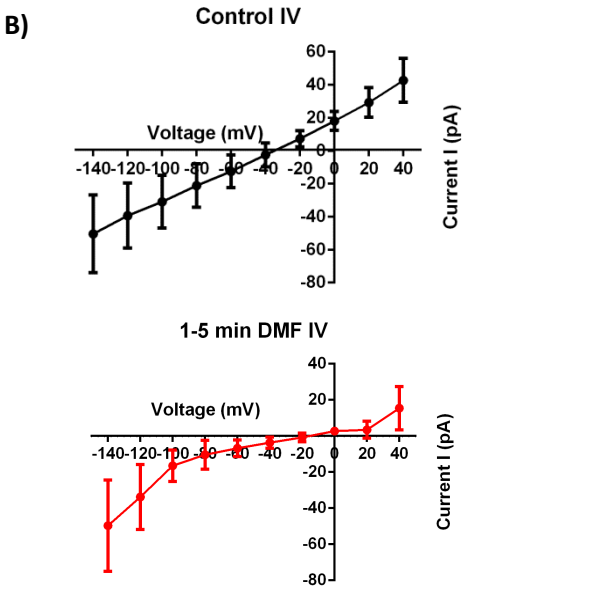
**A)**

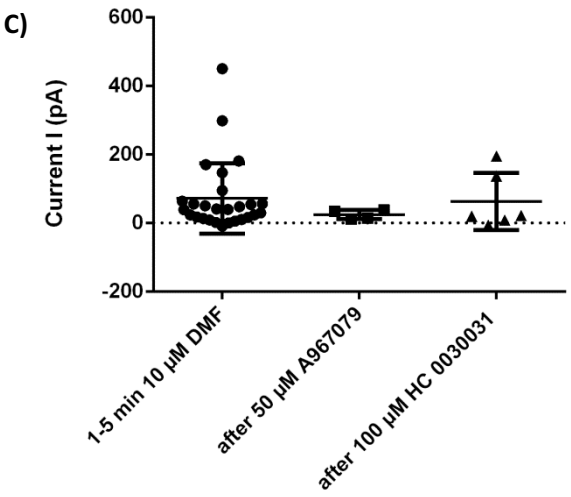



**A) Example current at +36 mV of a cell after 10 µM DMF was added**

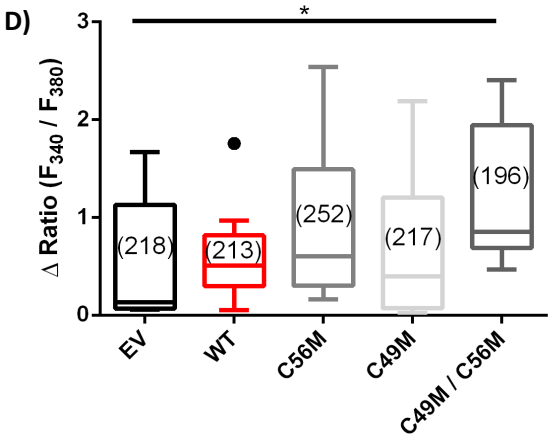
**B) Control IV and DMF IV of splenocytes measured at -80mV**

**C) Current of cells after 10 µM DMF or 50 µM A967079 or 100 µM HC0030031 was added**




2) During my training stay I also had the opportunity to study MEF cell cultures and stain different STIM1 cell lines, including STIM1 wildtype (WT) and different STIM1 mutants (STIM1-/- + STIM1 WT, STIM1-/- + empty vector (EV), STIM1-/- + STIM1 C49M, STIM1-/- + STIM1C56M and STIM1-/- + STIM1C49M C56M) for $Ca^{2+}$ and analyzed the living cells by fluorescence microscope. I detected significant differences in $Ca^{2+}$ entry between the double mutant STIM1 C49M, C56M and STIM1 EV after the treatment with 100 µM $H_2O_2$ for 20 min before adding 2 mM $Ca^{2+}$ to the cells to trigger SOCE (D).




**D) Store operated Calcium entry (SOCE) of different STIM1 MEF cell lines after treatment with 100 µM H2O2 for 20 min**

The patch clamping as well as the $Ca^{2+}$ concentration measurement skills I obtained during my stay in the Hamilton-Lab will allow me to set up new experiments at my home institution and will be invaluable for my future scientific career. Further experiment in these fields can now be established in my home lab and I can help to pass my skills to my fellow students. Thank you Network of European Neuroscience Schools for having granted me this training stay to advance my skills.

**References:** [1] Takahashi et al., Channels 2008; [2] Albrecht et al, J Neuroinflamm 2012