

Topic: SBFI Con11

Keywords: SBFI, chick embryo, spinal cord

Description: This is the first experiment with new Warner perfusion system for quickly switching between calibration solutions. Because of the low number of cells, we decided to conduct the experiment through the white matter. The general experimental plan is as follows:

- A. Tyrode's: check at two times points 10min apart
- B. low Cl, 155mM Na, w/ drugs: check at 15min and 30min after solution reaches bath
- C. low Cl, 90mM Na, w/ drugs: check at 15min after solution reaches bath
- D. low Cl, 30mM Na, w/ drugs: check at 15min after solution reaches bath
- E. low Cl, 0mM Na, w/ drugs: check at 15min after solution reaches bath
- F. low Cl, 155mM Na, w/ drugs: check at 10min after solution reaches bath
- G. low Cl, 90mM Na, w/ drugs: check at 10min after solution reaches bath
- H. low Cl, 30mM Na, w/ drugs: check at 10min after solution reaches bath
- I. low Cl, 0mM Na, w/ drugs: check at 10min after solution reaches bath

intensifier gain: 1.000

video gain: 0.808

Three cells visible (upper right quadrant in chosen field-of-view).

Real Time	Video Time	[Na+]	pH	temp (°C)	details
11:45	0:00-0:15	Tyrode's	--	27.2	no drugs
11:48	--	Tyrode's	7.2	--	pH check: Tyrode's
11:48	--	155mM	7.08	--	pH check: 155Na
11:55	0:26-0:36	Tyrode's	--	26.9	no drugs
12:16	0:57-1:16	155mM	--	27.4	155Na_15min 0.1mM ouabain, 3uM gramicidin, 10uM monensin
12:26	--	155mM	6.98	--	pH check: 155Na
12:26	--	90mM	7.1	--	pH check: 90Na
12:31	1:26-1:36	155mM	--	26.7	155Na_30min 0.1mM ouabain, 3uM gramicidin, 10uM monensin
12:47	--	90mM	7.01	--	pH check: 90Na
12:47	--	30mM	7.13	--	pH check: 30Na
12:49	1:45-1:59	90mM	--	27.0	90Na_15min 0.1mM ouabain, 3uM gramicidin, 10uM monensin
13:08	2:06-2:16	30mM	7.08	26.9	30Na_15min 0.1mM ouabain, 3uM gramicidin, 10uM monensin + pH check

					note: there was a fairly slow flow rate on this one
13:27	2:28-2:42	0mM	--	--	0Na_15min 0.1mM ouabain, 3uM gramicidin, 10uM monensin
13:33	--	0mM	7.04	--	pH check: 0mM
13:33	--	155mM	7.05	--	pH check: 155mM
13:40	2:57-3:13	155mM	--	--	155Na_10min 0.1mM ouabain, 3uM gramicidin, 10uM monensin
13:52	--	90mM	7.02	--	pH check: 90mM
13:53	3:30-3:42	90mM	--	27.2	90Na_10min 0.1mM ouabain, 3uM gramicidin, 10uM monensin
14:05	--	30mM	7.08	--	pH check: 30mM
14:06	3:50-4:01	30mM	--	27.2	30Na_10min 0.1mM ouabain, 3uM gramicidin, 10uM monensin
14:18	4:10-4:22	0mM	--	27.3	0Na_10min 0.1mM ouabain, 3uM gramicidin, 10uM monensin
14:31	4:30-4:50	155mM	--	27.0	155Na_10min_end 0.1mM ouabain, 3uM gramicidin, 10uM monensin

Additional Notes

- be sure to run some solution through lines BEFORE experiment so that when switching there is not too large of an air pocket... this will cause flow to stall otherwise
- there were bubbles that entered the influx line in switching between Tyrode's & 155Na... so flow rate went down significantly
- the experiment did not seem to work
- pH test at end...
 - in 90Na bath: 7.04
 - in chamber: 7.68-7.77