

Topic: SBFI Con10

Keywords: SBFI, chick embryo, spinal cord

Summary: With previous retrograde label experiments, we have always had difficulty with loss of signal over the course of the experiment. We do not know if this is due to post-slice leakage, bleaching, irreversible effects of different solutions (e.g. drugs, [Na]). Soooooo, we're going to test how signal changes over time and after adding drugs only using the low-Cl solution with Na

Through Ventral White Matter

intensifier = 1.01ish?

video gain = 0.808

Real Time	Video Time	[Na+]	pH	temp (°C)	details
??	0:12-0:28	Tyrode's	?	26.5	

Transverse Slices

We looked at two transverse slices. One have three faintly labeled cells.

The other had a totally bomber cell with processes and all. We decided to do the experiment on the slice w/ three cells, but take before & after images of the pretty one.

These were all conducted using the 155mM calibration solution.

Single Cell Slice

Real Time	Video Time	pH	temp (°C)	details
12:??	0:37-0:52	????	????	intensifier @ 1.01ish (probably too high... we re- did this)
12:??	0:52-1:10	????	????	focusing in at out at 340, gain at ~1.01ish
12:??	1:10-2:04	????	????	intensifier @ .997 (just barely reddish @ 380)
15:55	3:49-4:01	7.16	????	intensifier @ .997 this time it is way less bright... weird!

16:15	4:01-4:18	7.12	27.1	0.5mM OUABAIN, xx uM MONENSIN, xx uM GRAMICIDIN at 16:05
17:52	5:44-6:00	7.17	27.1	this is 113min after adding drugs, but
17:54	--			started perfusing in 0Na
17:55	--			reaches bath
17:58	--	6.87	24.5	due to low pH, we are adding 1KOH
18:02	--	7.09	24.7	
18:11	10:45-10:54	7.06	25.7	

Three-Cell Slice

Real Time	Video Time	pH	temp (°C)	details
12:55	2:07-2:16	7.18	????	0min intensifier @ 1.068
13:25	2:17-2:29	7.27	????	30min
13:55	2:29-2:46	7.29	27.1	60min,
14:25	2:46-3:05	7.14	26.9	90min, ++++++++before this recording, added some more saline to the perfusion bath.
14:55	3:05-3:20	7.15	27.2	120 min
15:25	3:20-3:35	7.16	26.8	150 min
15:55	3:35-3:49	7.16	27.2	180 min
16:19	4:18-4:31	7.12	27.1	ADDED 0.5mM OUABAIN, 10uM MONENSIN, 3uM GRAMICIDIN at 16:05 this is 14min after adding drugs
16:49	4:50-5:04	7.14	27.2	this is 44min after adding drugs
17:19	5:10-5:25	7.15	27.1	this is 74min after adding drugs

				(5:04-5:10 are accidentally at 380nm)
17:49	5:25-5:44	7.17	27.1	this is 104min after adding drugs
18:17	10:55-11:05	7.06	26.5	regular reading, 0Na
18:18	11:05-14:05	7.06	26.5	bleaching at 340nm
18:21	14:05-17:05	7.06	26.5	bleaching at 380nm
18:24	17:15-17:25	7.06	26.6	

Chronological Log

Real Time	Video Time	details

drugs at 4:05

white matter... right LS2. 340/380. 00:12-00:28... intensifier ~1.01ish?

super good cell

00:37-00:52... 340/380... intensifier gain was adjusted here at 340 so probably saturating out at 380... we can verify this later but we'll repeat this again (intensifier at ~1.01ish?)

00:52-1:10.... focusing in an out at 340; ~1.01ish?

intensifier changed to .997 (just barely reddish @ 380)

1:10-2:04... 340/380

three pseudo-good cells

intensifier changed to 1.0--

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2:07-2:16 ...
12:55
pH=7.18