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Topic: SBFI Con9
Keywords: SBFI AM, chick embryo, spinal cord

Summary: We dissected out an E10 spinal cord, sliced rostral half, incubated with 15mM SBFI AM for three hours. Moved to optical rig and washed out excess dye for one hour. Measured fluorescence of several cells in ventral horn, then perfused in calibration solutions with sodium concentrations ranging from 155mM (physiological extracellular concentration) to 0mM. Membrane was perforated using ionophores and active sodium pumps inactivated prior to calibration. Assessment of "normal" sodium concentrations will be conducted by comparing fluorescence ratio to calibrated ratios & concentrations. This analysis will be performed post-hoc using ImageJ to define motoneuronal regions-of-interest and measure average fluorescent, and Matlab to compute ratios.

intensifier gain: 0.977
video gain: 0.808

Real Time	**Video Time**	**[Na+]**	**pH**	**temp (°C)**	**details**
14:17	0:00-0:15	Tyrode's	7.11?	23.1?	bath not on
14:32	0:15-0:32	"	7.15	29.8	bath turned on!
14:37	0:32-0:47	"	7.15	30.0	

Perfuse in **155mM Na+** w/o drugs at 14:43; reaches bath at 14:45.

14:50	0:47-1:06	155mM	7.06	29.1	no drugs, 5min
15:00	1:06-1:21	"	?	?	no drugs, 15min

Add **0.5mM ouabain**, **3uM gramicidin**, **10uM monesin** at 15:04.

15:14	1:21-1:37	155mM	7.03	29.1	10min
15:39	1:37-1:54	"	7.04	29.0	25min
15:54	1:54-2:08	"	7.04	29.4	40min
16:09	2:08-2:27	"	7.06	29.4	55min
16:24	0:00-0:12	"	?	?	70min

Perfuse in **120mM Na+** solution at 16:26; reaches bath 16:28.

16:33	0:12-0:26	120mM	7.09	30.1	5min

16:38	0:26-0:41	"	7.10	30.9	10min
16:43	0:41-1:00		7.11	30.8	15min

Perfuse in **90mM Na+** solution at 16:45; reaches bath at 16:50.

16:55	1:00-1:16	90mM	7.25	30.6	5min
17:00	1:16-1:32	"	7.29	30.6	10min
17:05	1:32-1:47	"	?	?	15min

Perfuse in **60mM Na+** solution at 17:07; reaches bath at 17:09.

17:14	1:47-2:05	60mM	7.13	30.6	5min
17:19	2:05-2:22	"	7.13	30.7	10min
17:24	2:22-2:35	"	7.13	30.7	15min

Perfuse in **30mM Na+** solution at 17:29; reaches bath at 17:31.

17:46	2:35-2:57	30mM	7.13	30.6	15min
17:51	2:57-3:19	"	7.15	?	20min

Perfuse in **0mM Na+** solution at 17:58; reaches bath at 18:00.

18:05	3:19-3:32	0mM	7.40	30.3	5min
18:10	3:32-3:49	"	7.30	29.9	10min
18:15	3:49-4:08	"	7.32	30.1	15min
18:20	4:08-4:25	"	?	?	20min

NOTE: The 155mM Na+ solution had dropped to a pH of 6.88 even without bubbling (it was bubbling in the other room during perfusion of other calibration solutions). I added 10 drops of 0.1M KOH to increase pH. (In the mean time, I kept perfusing the bubbled 0Na solution and took an extra reading at 20min).

Perfuse in **155mM Na+** solution at 18:22; reaches bath at 18:24.

18:29	4:25-4:37	"	7.13	30.9	5min
18:39	4:37-4:50	"	7.20	31.0	15min
18:49	4:50-5:06	"	7.21	31.0	25min

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