20 Jan 2010

Objectives
- sodium imaging experiment !

Notes: Pete took notes today. Below are photocopies from his notebook

SBFI con 3

Date 1-20-10

beautiful overnight fill

starting by going through ventral WM at room temperature. 2 cephalotomy tape after WM of SBFI con 2 (1"40 sec). Then transverse SBFI con 2 (7"40)

rostral @ bottom of image

use unlabeled MNS (rostral) @ bottom

from 0 - 27 sec. (10:12 AM) thru WM at LS2 20x at Room temp WIND 50 on 380 Rltn (340 then 380)

warm up & repeat (started warm up at 10:16 AM
{ 10:25 AM (27-1"04)

one more time 10:33 (1"04-1"34)

then moving more caudal pt of LS2 (1"34-2"26)

Now transverse cut

1st piece not main one but int 993, hide 826
at 11:48 AM (2"26-2"49)

Now other/main piece → starting at a ~~Ro~~ deep focal plane (2"49-3"07)

then more superficial - but see density all ~~use for calibration~~ (3"07-3"26)

Now for real at deep plane (3"26-3"46)
{ will use R>r calibration (12:00 PM)

@ 12:12 same (3"46-4"17)

@ 12:27 same (tape started at 0 → 22 sec)

@ 12:41 same (22-40 sec)

@ 1:15 same (40 sec - 1"10)

Page

get dendrite of saturated cell is dated from cell body

Date

@ 1:18PM 0 Na + ionophores stated
@ 1:22 PM at bath.
@ 1:27PM 5 min after 0 Na/ionoph (1"10 - 1"57)
@ 1:32 10 min " " (1"57 - 2"21)
@ 1:37 15 min " " (2"21 - 2"48)
generate ROI * → @ 1:47 25 min " " (2"48 - 3"07)
@ 1:52 start perfusing in 10 Na ionoph
@ 1:54 10 mM Na reaches bath
@ 1:59 5 min after 10 Na (3"07 - 3"31)
@ 2:04 10 min " " (3"31 - 3"53)
@ 2:09 15 min " " (3"53 - 4"07)
start 30 Na + ionophores at 2:13 PM
at 2:15 PM 30 mM Na at bath
@ 2:20 PM 30 Na + ionophores (4"07 - 4"29)
@ 2:25 PM 10 min " (4"29 - 4"45)
@ 2:30 PM 15 min " (4"45 - 4"59)
start perfusing 60 mM Na at 2:34 gets to bath at 2:
@ 2:41 5 min 60 mM Na (4"59 - 5"21) low PH 1
↳ I think solution wrong (because 380 brighter)
Perfusing in 155 Na at 2:57 in bath
@ 3"02, 5 min after 155 Na+ (5"21 - 6"02)
@ 3"17, 20 min after 155 Na+ after clearly fluorescent gunk. 6:02 - 6:30
@ 3"25 again (after PH in from 7.13 - 7.21) 6:42 - 7:11
at 7:32 PM start 60 mM Na sol again (w/o gunk) at bath by 3:34 extra look
PH 5.9 → @ 3:39 60 mM 5 min Na + ionoph (7"14 - 7"33)
@ 3:56 again 10 min after 60 mM Na } (? ?)
after PH in correctly

Page

Start 90 mM perfusion ~4:00 PM
at bath ~4:02
@ 4:07 5 min after 90 mM Na (7"54 - 8"11)
@ 4:12 10 min " " (8"11 - 7) 8"35

at 4:15 start 0 Na again, at bath by 4:18
@ 4:23 5 min 0 Na (8"51 - 8"51)
@ 4:30 10 min 0 Na 8"35 → (8"51 - 9"05)

26 Jan 2010

ectives
ollect optical data from lidocaine cord

rding Log

left LS2
rough white matter ↙ start: 0 end: 21'
eld of view adjacent to nub)
bg @ 340nm: $\bar{I}$ = 28 on cell @ 340 nm: $\bar{I}$ = ~~151~~ 129
@ 380nm: $\bar{I}$ = 51 @ 380 nm: $\bar{I}$ = 131

↘ ↙

[cell - bg] @ 340: 129 - 28 = 101
@ 380: 131 - 51 = 80

#2
ield of view a bit caudal) start: 21' end: 44'
start 44' end: 1" 03 (20 min later)

ices
ut three slices. after examining, there are labeled cells in irst and third. for baseline we'll image from both, but hen do [Na] changes in just the first one.

perfusion just re-started:

* third slice: 340 @ 103 - 1:15 dendrites look great
(11:44a) 380 @ 1:15 - 1:24 in this one!

(11:54a) 340 @ 1:24
380 - 1:42

* first slice: @380, intensifier saturates at 1030
~~std dev~~ (std dev)
tune down to 1002
to stay in range
(for 0Na condition)

(12:01) 340 @ 1:42
380 @ - 1:59

(12:31) 1:59 - 2:15

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*first slice (cont.) std. soln

(13:01) pH = 7.25
2:15 - 2:31 ← use this to calibrate!

(13:05) put 10 μM (350μL into 350mL) bum

(13:20) 2:31 - 2:55

(13:35) 2:55 - 3:20

NOTE: times w/o qualifiers indicate time tape recorder was on during a 340→380nm excitation.

NOTE: All sclutions have gramicidin + monesin right in beginning

(13:36) begin perfusing 0Na
reaches bath @ 13:38

(13:43) 5 minutes after 0Na in bath
3:20 - 3:44
pH = 7.21

(13:55) 17 minutes after 0Na
3:44 - 4:08

(14:08) 30 minutes after 0Na
4:08 - 4:28

(14:09) begin perfusing 30 Na
reaches bath @ 14:11

NOTE: perfusion appears slightly quicker than previous...

(14:16) 5 minutes after 30Na
4:28 - 4:49
pH = 7.17

(14:21) 10 minutes after 30Na
4:49 - 5:12

(14:22) begin perfusing 60 Na
reaches bath @ 14:24

(14:29) 5 minutes after 60Na
5:12 - 5:51
pH = 6.1 → way to low... adding KOH to bring back

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~~(14:34)~~ ~~10 min after~~

(14:38) pH = 7.2

(14:39) 15 min after 60 Na
5:51 - 6:15

(14:40) begin perfusing 90 Na
reaches bath @ 14:42

(14:47) 5 min after 90 Na
6:15 - 6:44
pH = 6.0 ⟶ need to adjust

(14:54) pH = 7.2

(14:55) 13 min after 90 Na
6:44 - 7:07

NOTE: Pete made soln for 0Na, 10 Na, 20Na, 30Na and 155 Na; I made the 60Na and 90 Na soln and didn't (probably) add enough choline bicarb so that's probably why those two soln have lower initial pH

(14:57) begin perfusing 155 Na
reaches bath @ 14:58

(15:03) 5 minutes after 155 Na
7:07 - 7:33
pH = 7.1

(15:12) minutes after 155 Na
7:33 - 7:58

add 100µL of gram + man to solutions

(15:11) begin perfusing 0Na (again)
reaches bath at 15:13

(15:18) 5 minutes after 0 Na
7:58 - 8:40 (accidentally left recorder on)
~~pH =~~ we used same stock as beginning of experiment

(15:24) 11 minutes after 0 Na
8:40 - 9:05

during this time I made a bit of 122.5 mM Na soln (12.5mL of

(15:32) 19 minutes after 0 Na
9:05 - 9:30

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122.5 Na begins @ 15:37

(15:36) begin perfusing 122.5 Na
reaches bath @ 15:37

(15:42) 5 minutes after 122.5Na
9:30 - 9:47
pH = 6.8 → added 0.1 M KOH is
until pH = 7.15 (at 9:49)

(15:50) 13 minutes after 122.5Na
9:47 - 10:02

(15:55) 18 minutes after 122.5Na
10:02 - 10:21

I extracted all videos to MINGSTON for ImageJ analysis.

09 Feb 2016

Objectives
- control SBFI experiment

Notes
9:45 Pete looked at fill... one bubble in pipette, but dye below looks good. Pulled off tip and removed pia on ventral side. Started running sol'n through op. chamber. Warmed up lamp.
~~10:00~~
10:15 transferring cord to op. chamber.
be sure to place cord near exit of perfusion chamber due to position over inverted microscope.

camera gain @ 8.22 (NOTE: lower gain → higher intensity contrast)
intensifier @ 1,000

10:55 Warmed to 30.00°
(fire alarm in Whitehead)

11:10 Recording
- through white matter @ focal plane #1 (four cells)
  0:00 - 0:27
- focal plane #2 (four cells)
  ~~0:43~~ 0:27 - 0:43
- focal plane #3 (two cells)
  0:43 - 0:59
- focal plane #4 (one cell)
  0:59 - 1:15

11:23 Prepare to slice...
cut just above (rostral to) root of interest → since Pete labeled LS2, we cut between LS1 and LS2
afterwards, remove membrane completely → be sure to keep track of rostral + caudal sides (caudal will taper off)
pin to vibratome

11:30 Slicing
parameters: to approach + check: speed = 5; freq = 0
to slice: speed = 2; freq = 8.5

16 Feb 2010

Objectives
- SBFI control experiment

Experimental Notes

* through white matter
  intensifier @ 1.002
  video gain @ 0.861

  0:00 - 19:21 superficial plane (brighter cells)
  19:21 - 39:11 deep plane (dimmer cells)
  39:21 - 59:29 " " but slightly shifted stage

* slicing
  speed : 2
  freq : 8-9
  } vib. settings

  4 slices → only labeled cells in last (very thin) slice but they were nice! so I took two additional slices and tried to also make them thin.

  Slices 4, 5, and 6 all look promising.

  One problem w/ thin slices is weight cannot hold them down very well... some difficulties adjusting a fluorescent portion of mesh while still holding down prep. Letting it sit for 30 min (~1:30-2:00)

* baseline
  ← [14:10]
  59:29 - 1:20:17 approximately 6 cells (using 4th slice)
  metal bar in lower right view, but iris closed down as much as possible
  video gain: 0.856
  intensifier 1.096 ⤵ warming

  1:20:17 - [14:38]
  abort → slice has moved...gah.
  Pete is re-positioning it.

  1:20:17 - 1:38:25 [14:55]
  imaged new position / warm

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[14:57] 155 Na reaches bath, switch return to new sol'n
turning down perfusion rate (seems faster than previous experiments)

[15:04] 1:38 - 1:56
155 Na - 7 min

[15:07] 1:56 - 2:10
155Na - 10 min

[15:10] adding gram + monesin
we're using 100 mL solution rather than 250mL ... previously we used 100μL of each, so now we use 40μL.

[15:23] moving focal plane and stage

[15:25] 2:10 - 2:25
155Na - ionophores - 15 min

[15:33] 2:25 - 2:47
NOTE: shutter had been left open since previous recording
155Na - ionophores - 23 min

[15:49] 2:47 - 3:14
155 Na - ionophores - 39 min

[15:59] 3:14 - 3:35 155 - Na - ionophores - 49 min

[16:10] adding 29 mg of ouabain (0.5 mM)
NOTE: took about 5-10 minutes to dissolve before adding into solution

[16:25] 3:35 - 3:58 ← shutter left open @ 380, don't use
155 Na - ouabain - 15 min ← don't use .... 380 to saturated light

[16:28] 3:58 - 4:23
155Na - ouabain - 18 min

[16:47] 4:23 - 4:45
155Na - ouabain - 37 min

[16:55] 4:45 - 5:03
155Na - ouabain - 45 min

[16:56] start perfusing 122.5 Na calibration solution w/ 20mg ouabain (0.348mM)

ouabain
580 g/mol
for 0.5 mM
in 100mL sol'n

$\frac{580\,g}{1\,mol} \times \frac{0.5 \times 10^{-3}\,mol}{1\,L} = \frac{0.290\,g}{1\,L}$

$\frac{290\,mg}{L}$

$\frac{29\,mg}{100\,mL}$

$58 \overline{)200.00}$ → 3.448

174
260
232
280
232
480
464
16

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:04] 5:03 - 5:23
122.5Na - 5min

pH = 7.00 ⟶ no additional KOH

09] 5:23 - 5:45
122.5 Na - 10min

14] 5:45 - 6:06
122.5 Na - 15min

5.8mg in 100mL

17] begin perfusing 90Na calibration sol'n + 100μM ouabain + ionophores

21] 90Na calibration sol'n reaches bath

:26] 6:06 - 6:23
90Na - 5min

pH = 6.9
↓
added a drop of 1 M KOH and shook up to 7.5... then slowly drop

:33] 6:23 - 6:40
90Na - 12min

:36] 6:40 - 7:02
90Na - 15min

:40] perfusing in 60Na cal sol'n + 100μM ouabain + ionophores

:42] 60Na cal sol'n reaches bath

:47] 7:02 - 7:21
60Na - 5min

pH = 6.3 ⟶ added 2 drops of 1 M KOH

:52] 7:21 - 7:38
60Na - 10 N'a

:57] 7:38 - 7:55
60Na - 15 Na

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[18:00] 30Na cal sol'n reaches bath

[18:07] 7:55 - 8:16
30Na - 7 min

[18:10] 8:16 - 8:34
30Na - 10min

[18:15] 8:34 - 8:58
30Na - 15min

[18:17] begin perfusing 0Na cal sol'n + 100μM ouabain + ionophores

[18:19] 0Na cal sol'n reaches bath
NOTE: switched return later than usual (like at 18:21) to ensure there is no lingering Na in bath

[18:24] 8:58 - 9:24
0Na - 5min

[18:29] 9:24 - 9:45
0Na - 10min

[18:34] 9:45 - 10:05
0Na - 15min

[18:35] begin (re) perfusing 155 cal sol'n + 500μM ouabain + ionophores
↑ same as original sol'n

[18:37] 155Na cal sol'n reaches bath

[18:42] 10:05 - 10:23
155Na - end - 5min

[18:47] 10:23 - 10:42
155Na - end - 10min

[18:52] 10:42 - 10:59
155Na - end - 15min

[18:57] 10:59 - 11:18

23 Feb 2010

Objectives
- SBFI experiment

Experiment Notes

* through white matter
  → beautiful label. brightest cells near root
  ⇒ intensifier: 0.876
    video gain: 0.820
  ⇒ plane 1: ~~0.820~~
    00:00 - 00:18
  ⇒ plane 2:
    ~~00:00~~ 00:18 - 00:40
    int: ~~0.820~~ 0.900 — caudal
  ⇒ plane 3: (shifted ~~from 1+2~~ stage)
    00:40 - 01:01
  ⇒ plane 4: 01:01 - 01:26
    (stage shifted rostral from ~~2+3~~ 1+2)
  ⇒ plane 5: 01:26 - 1:46
    same as 4 but with iris turned down
    and w/ int → 0.956

} same stage pos.
} near nub.
} same (rostral) pos

* cutting: make initial cut right in optical chamber.

* 0.22 g glucose
  10 mL 10x stock (plus H_2O to dilute)
  0.5 mL divalent
} recipe for 100mL of cal. soln

* BASELINE (warmed) time: 13:07
  int: 1.003 rec: 1:46 - 2:02
  v. gain: 0.820
  ↓ brought iris down to get rid of crap on edge
  five cells visible rec: 2:02 - 2:17

* 155 Na cal soln (no ionophores, no ~~0.820~~ ouabain)
  reaches bath @ ~~13:14~~ 13:14

  155Na_begin_noIO_5min : 2:17 - 2:33 [13:19]
  " " " 10 min : 2:33 - 2:50 [13:24]

NOTE: at 380nm, brightest cell had tinge of yellow.

10μM monensin
3μM gramicidin

155 Na + ionophores + ouabain

↳ 0.5 mM (290 mg in 100 mL)

↳ 10μM gramicidin
3μM monensin
} based on C. Rose Na+ imaging experiments

added ionophores (40μL monesin, 40 μL gramicidin) + ouabain (290 mg) at ~~Bl20~~ 13:26; added/dissolved ouabain at 13:29

155Na - begin - 30 min: 2:50 - 3:06 [13:59]

" " 40 min: 3:06 - 3:24 [14:09]

" " 50 min: 3:24 - 3:46 [14:19]

" " 60 min: 3:46 - 4:03 [14:29]

" " 70 min: 4:03 - 4:23 [14:39]

↑ NOTE: yellow tinge @ 380nm gone!

122.5 Na + ionophores + ouabain

↑ 10μM / 3μM ↑ 0.1 mM

reaches bath at: 14:46

122.5 Na - 5 min: 4:23 - 4:41 [14:51]

" 10 min: 4:41 - 5:01 [14:56]

pH = 6.92 → added 2 drops 1M KOH
pH = 7.04

122.5Na - 18 min: 5:01 - 5:23 ~~[15:04]~~

90Na + ionophores + ouabain (10μM / 3μM, 0.1 mM)

reaches bath at: 15:12

90 Na - 5 min: ~~5:23~~ - 5:40 [15:17]

~~90Na - 10 min:~~ pH = 6.20 → added ~~4~~ 10 drops 1M KOH ~~[15:29]~~

* 90 Na + ionophores + ouabain (cont.)

90 Na – 10 min : 5:40 – 5:58 [15:22]

" 15 min : [15:27]

* 60 Na + ionophores + ouabain

reaches bath at : 15:32

60 Na – 5 min : 5:58 – 6:40 [15:37]

pH = 6.34 → added 10 drops 1 M KOH
pH = 7.3

60 Na – 10 min : 6:40 – 7:05 [15:42]

" – 15 min : 7:05 – 7:26 [15:47]

* 30 Na + ionophores + ouabain

reaches bath at : 15:53

30 Na – 5 min : 7:26 – 7:49 [15:58]

30 Na – 10 min : 7:49 – ~~8~~ 8:16 [16:03]

" 17 min : 8:16 – 8:36 [16:~~08~~ 10]

* 0 Na + ionophores + ouabain

reaches bath at : 16:17

0 Na – 5 min : 8:36 – 8:55 [16:22]

" – 10 min : ran out of tape in middle [16:27]
↓ changed to new one.

0 Na – 15 min : 0:00 – 21:05 [16:32]

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~~0.5mM~~ 0.5mM

155Na_end + ionophores + ouabain

reaches bath at: 16:42

155Na_end_5min: ~~2:45 - 0:02~~
0:21 - 0:44 [16:47]

155Na_end_10min: 0:44 - 1:07 [16:52]

155Na_end_20min: 1:07 - 1:25 [17:02]

155Na_end_30min: 1:25 - 1:40 [17:12]

0.1mM

45Na + ionophores + ouabain

for this I just poured the previous 30Na and 60Na sol'n together

reaches bath at: 17:17

45Na_5min: 1:40 - 1:59 [17:22]

45Na_10min: 1:59 - 2:17 [17:27]

45Na_15min: 2:17 - [17:32]

16 Mar 2010

Objectives
- lid2 SBFI recording

Notes
- cut off pia one segment above and below LS2 on left side
- turned off bath in dissection chamber; running soln through optical chamber
- positioned cord in optical chamber, turned on arc lamp

Optical Recordings

1. Through White Matter

view 1 (focused up):
- 0:00 - 0:21
- 11:42
- intensifier: 0.964
- v. gain: 0.820

view 2 (same as #1 but focused down)
- 0:21 - 0:45
- 11:47

2. Transverse: Baseline

a. warm, 14:25
0:45 ~~0:47~~ - ?:??

b. warm, 14:34
?:?? - 1:19

3. Transverse: 155Na (reaches bath at 14:38)

a. 5 min (14:43)
1:19 - 1:39
pH = 7.01

b. 10 min (14:48)
1:39 - 1:58

Slice Preparation
⇒ used razor blade on dorsal and ventral sides to make incision just rostral of LS2
→ first slice came out ~3x too thick... all cells appear to be in that slice.

Drug Dilutions
- ouabain: for 0.5mM, use 29 mg in 100mL solution
- use 40µL of bath gram and man for 100ml solution

4. Transverse: 155Na + 0.5mM ouabain + 10μM gramacidin + 3μM manensin

added at 14:55

a. 30 min (15:25)
1:58 - 2:15

b. 40 min (15:35)
2:15 - 2:32

c. 50 min (15:45)
2:32 - 2:52 → seems a bit dimmer at 380nm than previous

d. 60 min (15:55)
2:52 - 3:13

5. Transverse: 120 155Na + 0.1mM OUA, 10μM gram, 3μM man

reaches bath at 16:01

a. 5 min (16:06)
3:13 - 3:38

b. 10 min (16:11)
3:38 - 4:05
pH = 7.07

c. 15 min (16:16)
4:05 - 4:28

6. Transverse: 90Na + 0.1mM OUA, 10μM gram, 3μM man

reaches bath at 16:21

a. 5 min (16:26)
4:28 - 4:49
pH = 7.02

b. 10 min (16:31)
4:49 - 5:08

6. Transverse: 90 Na (cont.)

c. 15 min (16:36)
5:08 - 5:29

7. Transverse: 60 Na + 0.1 mM OuA, 10 μM gram, 3 μM man

reaches bath at 16:41

a. 5 min (16:46)
5:29 - 5:52
pH = 7.03

b. 10 min (16:51)
5:52 - 6:10

c. 15 min (16:56)
6:10 - 6:31

8. Transverse: 30 Na + 0.1 mM OuA, 10 μM gram, 3 μM man

reaches bath at 17:00

a. 5 min (17:05)
6:31 - 6:52 ← I couldn't make out a single cell here, either at 340 or 380 nm
pH = 7.06

b. 10 min (17:10)
6:52 - 7:15

c. 15 min (17:15)
7:15 - 7:40

9. Transverse: 0 Na + 0.1 mM OuA, 10 μM gram, 3 μM man

reaches bath at 17:20

a. 5 min (17:25)
7:40 - 8:03
pH = 7.07

9. Transverse: 0Na (cont.)

b. 10 min (17:30)
8:03 - 8:26

c. 15 min (17:35)
8:26 - 8:54

10. Transverse: 155Na + 0.5mM OUA, 10 μM gram, 3 μM man (again)

~~x 5~~ reaches bath at 17:39

a. 5 min (17:44)
8:54 - 9:24

b. 10 min (17:49)
9:24 - 9:48

c. 20 min (17:59)
9:48 - 10:09

d. Since I haven't seen cells since 30Na, I decided to move stage and focus to see if I could relocate them... I was unsuccessful at finding the prior-seen cells, but I did see a couple others and figured I'd image them. 10:09 - 10:27

21 May 2018

TX
1 mg (GT limit is 100 mg)

SFI AM
MW 1127.07 g
made 2425 50μL aliquots
of ~70mM (maybe ~75 since we only had 24 aliquots) or 23.5ish

$\frac{1\text{ mg}}{25\text{ aliquot}} = \frac{0.04\text{ mg}}{1\text{ aliquot}} \rightarrow \frac{0.04\text{ mg}}{50\,\mu\text{L}}$

$\times \frac{1\text{ g}}{1000\text{ mg}} \times \frac{1\text{ mol}}{1127.07\text{ g}} \times \frac{1000000\,\mu\text{L}}{1\text{ L}}$

$\approx 7.098 \times 10^{-4}$ M
or 70 mM

Experimental Notes

diluted 2 aliquots (each 50μL of 75mM) into 10 mL Tyrodes[2] for final [SBFI] = 1.5 mM
bubbled slices for a few hours... plan to remove a few after 2 hours to check label and play w/ pH
removed 3 slices a moved to imaging chamber w/ 155 mM Na^+

Imaging Notes

- we selected slice with 4 obvious MNs (in vertical column on screen) and two less focused ones in upper right
- plan is to vary pH in this prep to observe how it effects SBFI fluorescence
- then run through calibration sol'n if time allows
- intensifier set to 0.929
  video gain at 0.808

in general, each cal sol'n pH ≈ 7.00, and I added 2-3 drops of 1M KOH to bring closer to 7.2.

Misc
There is 22.4 mg wabain left. To divide in 5 cal sol'n, use 4.48 mg each

need more monesin → final [ ] = 10 μM

FW = 692.9 g/mol
to get 10 μM in 100 mL,
I need 10^{-6} mol or 0.6929 mg in each 100 mL solution (or in each 40 μL portion). So I need 0.6929 mg per 40μL → make 1 mL → so 17.3225 mg for 1 mL
34.645 mg for 2 mL ⇐ * a bit chunky (started using this for 60NA onwards)

40 μL → 100 mL ?
10 μM

measured @ bath

al Time	Video Time	pH	temp	[Na]	notes
00	0:02 - 0:19	7.01	29.7°C	155mM	no drugs
24	0:19 - 0:41	7.01	29.7°C	155mM	no drugs
31	messed up initially 0:56 - 1:13	7.01	29.8°C	"	"
45	1:13 - 1:33	6.30	29.9°C	"	add several pipette-fuls of 0.1 M HCl to lower pH at 15:35
50	1:33 - 1:53	6.52	30.0°C	"	added a few drops of 1 M KOH to slowly raise pH between each measurement
55	1:53 -	6.66	29.9°C		
00	- 2:28	6.88	29.7°C		
05	2:28 - 2:46	7.15	29.8°C		
15	2:46 - 3:02	7.27	29.9°C		
25	3:02 - 3:19	7.38	29.8°C		
33	3:19 - 3:34	7.5ish	29.9°C		
45	3:34 - 3:50	7.66	29.8°C		
55	3:50 - 4:07	7.84	29.9°C		
0	4:07 - 4:27	7.98	30.0°C		
30	4:27 - 4:44	7.2	29.9°C		added a lot of HCl to bring back to normal pH (looks like cells are gone)

pH takes FOREVER to stabilize ... still varies sometimes after waiting 10 min.

when I removed the slices, there was a ton of precipitation. I cleaned this a lot before putting in new slices.

After the pH test, I looked at other slices that had been incubating in the SBFI for the past couple hours (11AM until 4:45PM). Between 4:45 and now (5:45) I've been rinsing them in oxygenated Tyrode's.

Under the scope, these slices (7) look awesome. Motor column is obvious in all cases, though not more than ~10 cells labeled in each case. I'm looking at them using same sol'n at end of table on previous page. I set the intensifier to 0.935 and kept video gain the same.

Real Time	Video Time	pH	temp	[Na]	notes
~~17:55~~ 5:13	4:44 - ~~5:05~~	~~7.23~~	30.1°C	155mM	same sol'n as end of previous pg.
18:00	5:13 - 5:36	7.23	30.1	"	"
18:08	5:36 - 5:53	"	"	"	"
18:25	5:53 - 6:12	7.33	29.8	"	added ionophores + 0.5mM ouabain at 18:10; significant movement of prep due to pressure issues... had to translate stage around (but kept same focal plane)
18:45 ~~18:40~~	6:12 - 6:27	7.34	29.8	"	after 35 min
19:10	6:27 - 7:00	7.36	29.8	"	after 1 hr
19:25	7:00 -	7.37	29.8	"	after 75 min
19:38	- 7:38	7.09	30.4	120	switch to 120 Na @ 19:28 (after 10 min)
19:43	7:38 - 7:52	7.09	30.4	120	after 15 min
19:53	7:52 - 8:05	7.11	30.3	90	switch to 90 Na @ 19:48 (after 5 min)
20:08	8:05 - 8:19	7.12	30.3	90	after 10 min
20:23	8:19 -	7.21	30.0	60	switch to 60 Na @ 20:13 (after 10 min

Real Time	Video Time	pH	temp	[Na]	notes
20:40	8:49-9:01	7.23	30.1	30	switch to 30 Na @ ~~20:30~~ (after 10 min)
20:45	9:01-9:17	7.22	29.9	30	after 15 min
20:52	9:17-9:33	7:33	29.7	0	switch to 0 Na @ ~~20:46~~ 20:47 (after 5 min)
20:57	9:33-10:19	7.31	29.8	0	after 10 min
21:02	10:19-10:34	7.34		0	after 15 min
21:08	10:34-10:50	7.43	30.0	155	switch to 155Na @ 21:03 (same as sol'n used at 18:25) (after 5 min)
21:13	10:50-11:06	7.42	30.0	155	after 10 min
21:18	11:06-11:29	7.43	30.0	155	after 15 min

↑

afterwards I just translated around to look at other MNs... some look much better than mine now, though mine ~~as~~ are still O.K...

Ming-fai Fong
30 May 2010

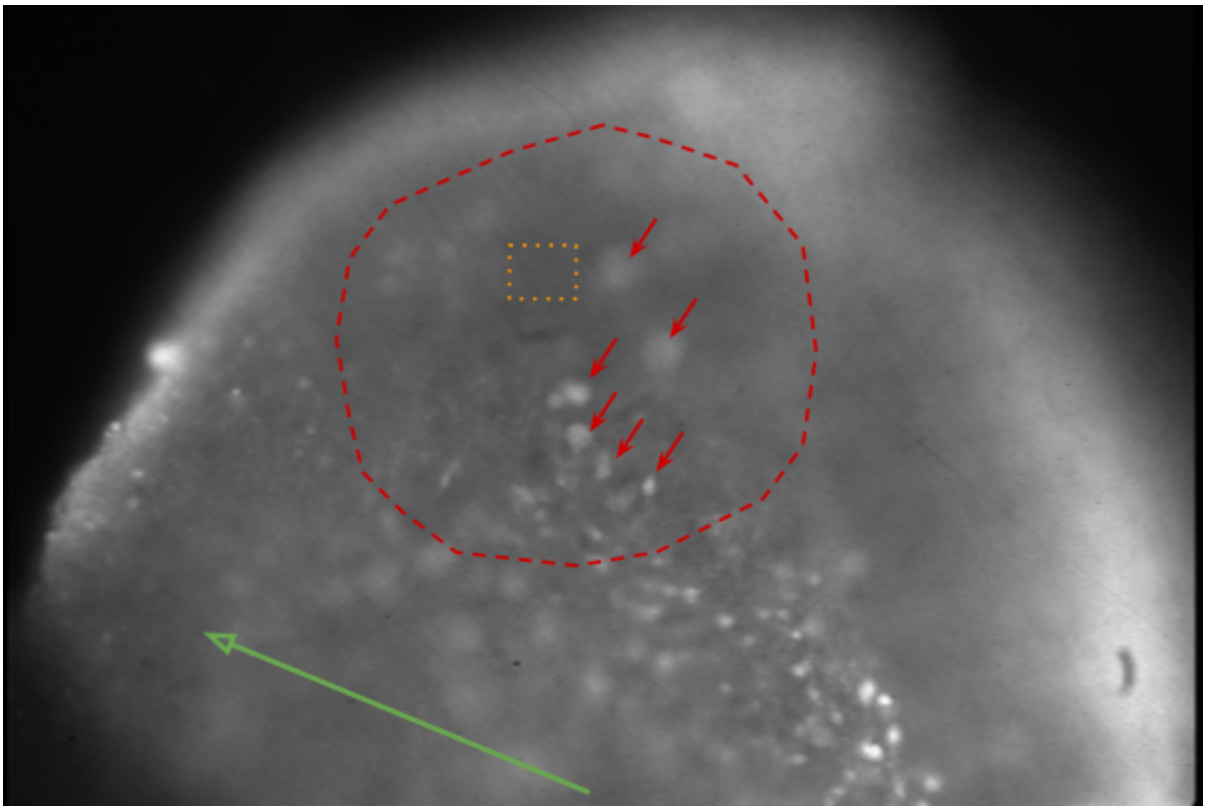
Topic: pH Analysis from SBFI_Con7
Keywords: SBFI, pH

Introduction

I conducted a calibration Na imaging experiment using SBFI AM. Before beginning of the experiment, I conducted a series of pH tests to examine how the dye changes with pH at the standard extracellular [Na] of 155mM. The membrane was not perforated for these tests.

Imaged Cells

I used the six motor neurons shown below across all images. For background subtraction, I used the area indicated by the orange dotted line.

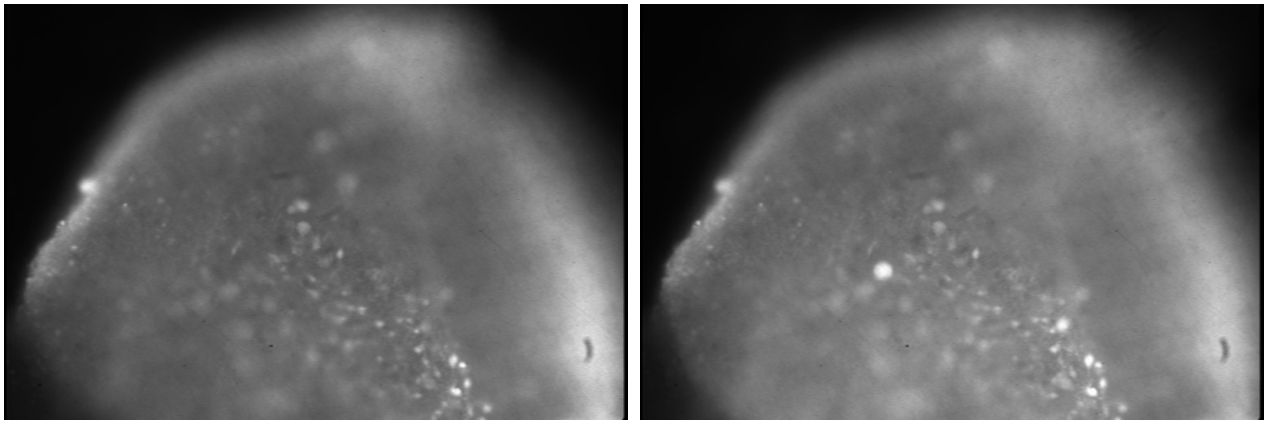


AVG_con7_pH_1500_340 (SBFI_Con7, images from pH test, 15:00, 340nm)

Top of image is lateral, bottom is medial. Motor column is indicated by red dotted line. Motor neurons indicated by arrows (though two in the upper right are in a more shallow focal plane than the other four). Dorso-ventral axis is indicated by solid green arrow. Note that there are some small, brightly-labeled cells in the dorsal aspect of the cord. Some more medial neurons (probably interneurons) are also labeled but out of focus. In this image, pH was at 7.01 and extracellular solution was a low-Cl version of Tyrode's with [Na] = 155mM. Bath temperature was 29.7 ºC. There were no drugs.

(a) **(b)**

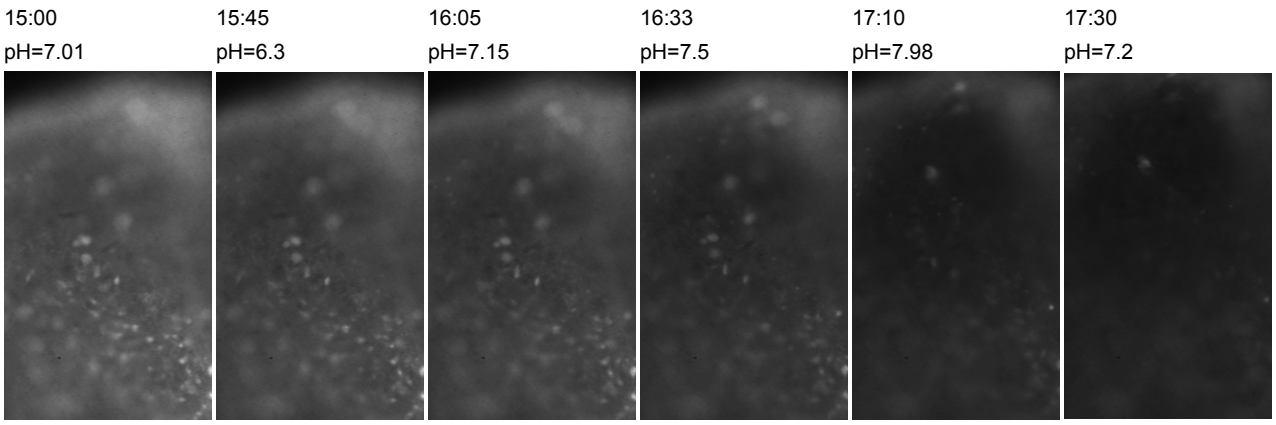
Ming-fai Fong
30 May 2010



AVG_con7_pH_1500 (SBFI_Con7, images from pH test, 15:00)
Slice from lumbrosacral cord of E10 chick embryo loaded with SBFI AM and fluorescently imaged with excitation wavelengths of (a) λ=340nm and (b) λ=380nm.

Analysis

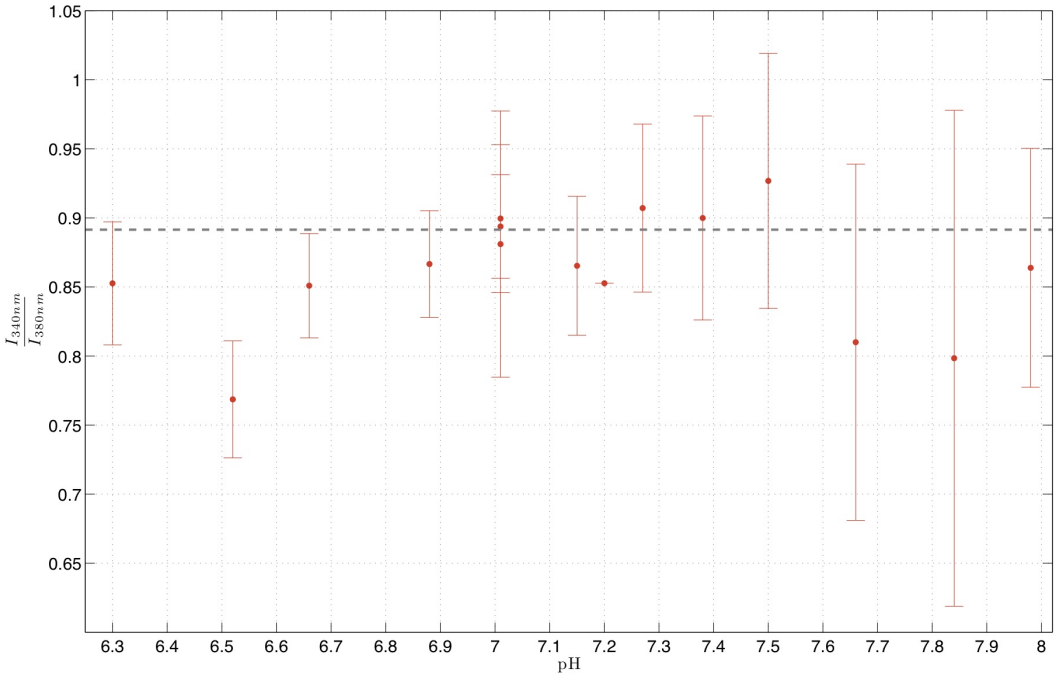
Some representative images are shown below.



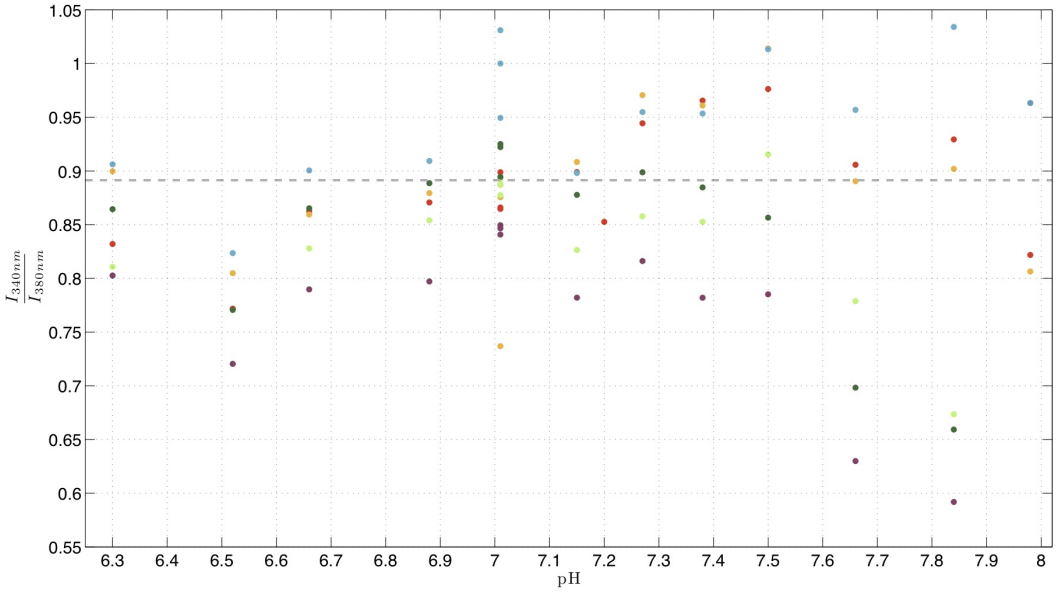
After pH is brought nearly to 8, it becomes nearly impossible to see the cells anymore, and fluorescence doesn't recover even when pH is brought back to a reasonable level. I suspect this is because the pH changes were so radical. To be sure of that the loss of efficacy of the SBFI isn't simply due to time, I need to conduct a similar experiment where I do NOT mess around with pH but instead leave the perfusion on for ~3 hours and check fluorescence every 15-20min.

Plot of Ratio as a function of pH:

Ming-fai Fong
30 May 2010



This is not really as informative as I would have liked (or expected). A little more information is available when looking at individual cells (indicated by color):



Finally, the most information is available here:

Ming-fai Fong
30 May 2010

time	pH	temperature	chemical recently added	mean ratio	std ratio	diff from 0.8914
15:00	7.01	29.7	--	0.8810	0.0964	0.0104
15:24	7.01	29.7	--	0.8995	0.0535	-0.0081
15:31	7.01	29.8	--	0.8937	0.0374	-0.0023
15:45	6.30	29.9	HCl	0.8526	0.0445	0.0388
15:50	6.52	30.0	KOH	0.7686	0.0424	0.1228
15:55	6.66	29.9	KOH	0.8509	0.0378	0.0405
16:00	6.88	29.7	KOH	0.8665	0.0386	0.0249
16:05	7.15	29.8	KOH	0.8653	0.0503	0.0261
16:15	7.27	29.9	KOH	0.9071	0.0608	-0.0157
16:25	7.38	29.8	KOH	0.8999	0.0738	-0.0085
16:33	7.5	29.9	KOH	0.9268	0.0924	-0.0354
16:45	7.66	29.8	KOH	0.8100	0.1289	0.0814
16:55	7.84	29.9	KOH	0.7983	0.1795	0.0931
17:10*	7.98	30.0	KOH	0.8638	0.0864	0.0276
17:30**	7.2	29.9	HCl	0.8526	n=1	0.0388

*recording at 17:10 only included three cells

**recording 17:30 only included one cell

Ratios do seem to stay fairly stable in the 7.0-7.4 range, which is good. They start looking pretty strange, though, outside of this. Superficially it appears that acidic pH reduces ratios and basic pH increase ratios, but at 7.66 and higher this rule seems to break down.

some additional handwritten notes...

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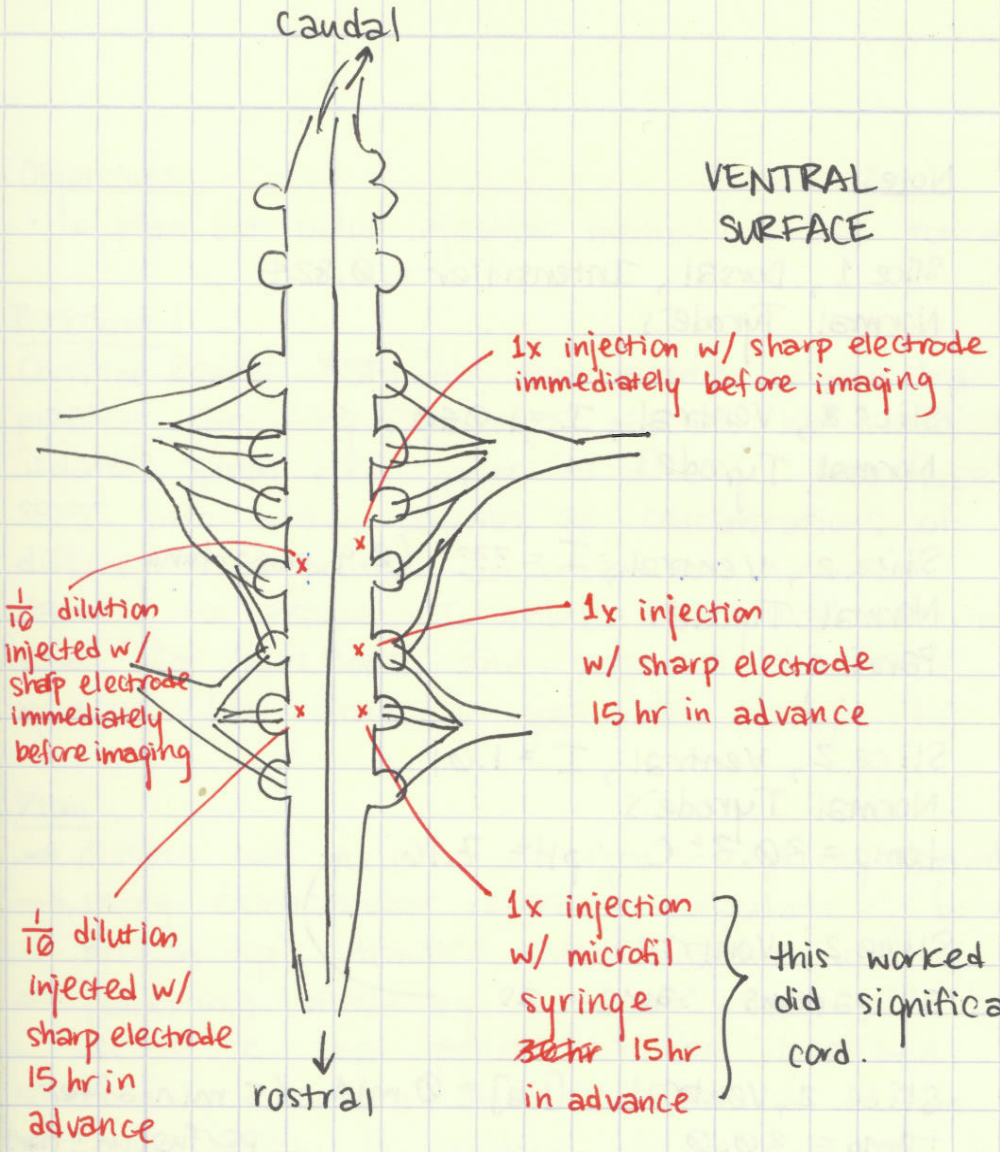
Real Time	Video Time	pH (measured @ bath)	temp	[Na]	notes
15:00	0:02 - 0:19	7.01	29.7°C	155mM	no drugs
15:24	0:19 [redacted] - 0:41	7.01	29.7°C	155mM	no drugs
15:31	0:56 - 1:13 (messed up initially)	7.01	29.8°C	"	"
15:45	[redacted] 1:13 - 1:33	6.30	29.9°C	"	add several pipette-fuls of 0.1 [redacted] M HCl to lower pH a [redacted] 15:35
15:50	1:33 - 1:53	6.52	30.0°C	"	added a few drops of 1 M KOH to slowly raise pH bet each measurement
15:55	1:53 -	6.66	29.9°C		
16:00	- 2:28	6.88	29.7°C		
16:05	2:28 - 2:46	7.15	29.8°C		
16:15	2:46 - 3:02	7.27	29.9°C		pH takes FOREVER to stabilize ... sti varies sometimes after waiting 10 m
16:25	3:02 - 3:19	7.38	29.8°C		
16:33	3:19 - 3:34	7.5ish [redacted] 7.50	29.9°C		
16:45	3:34 - 3:50	7.66	29.8°C		
16:55	3:50 - 4:07	7.84	29.9°C		
17:10	4:07 - 4:27	7.98	30.0°C		
17:30	4:27 - 4:44	7.2	29.9°C		added a lot of HCl to bring back to normal pH (looks like cells are gone)

when I removed the slices, there was a ton of precipitation. I cleaned this a lot before putting in new slices.

04 June 2010



Yesterday I injected SBFI AM into motor column of E10 cord. Today I injected again just caudal. The diagram above shows injection sites.

al Time	Video Time	Notes
:16	0:00-0:14	Slice 1, Dorsal, Intensifier = 0.824 Normal Tyrode's
26	0:14-0:26	Slice 1, Ventral, I = 1.040 Normal Tyrode's
:30	-1.07	Slice 2, Ventral, I = ??? (0.9 something) Normal Tyrode's Panning
:35	1:07-1:26	Slice 2, Ventral, I = 1.01 Normal Tyrode's temp = 30.3° C, pH = 7.16
:40	1:26 - 1:42	Slice 2, Ventral all params same as
50	1:42-2:02	Slice 2, Ventral, [Na] = 0 mM (5 min after perfusion start) temp = 30.0 pH = 7.04
:00	2:02-2:19	Slice 2, Ventral, [Na] = 0 mM (15 min after perfusion start) temp = 30.0 pH = 7.03
:02	2:19-2:34	Slice 1, Ventral, [Na] = 0 mM temp = 30.0, pH = 7.03 I = 1.003
:03	2:34 : 2:49	I = 1.040 ← definitely saturating @ 380 nm
:08	2:49 -3:06	Slice 1, Dorsal, [Na] = 0 mM pH = 7.03, temp = 30.1° C

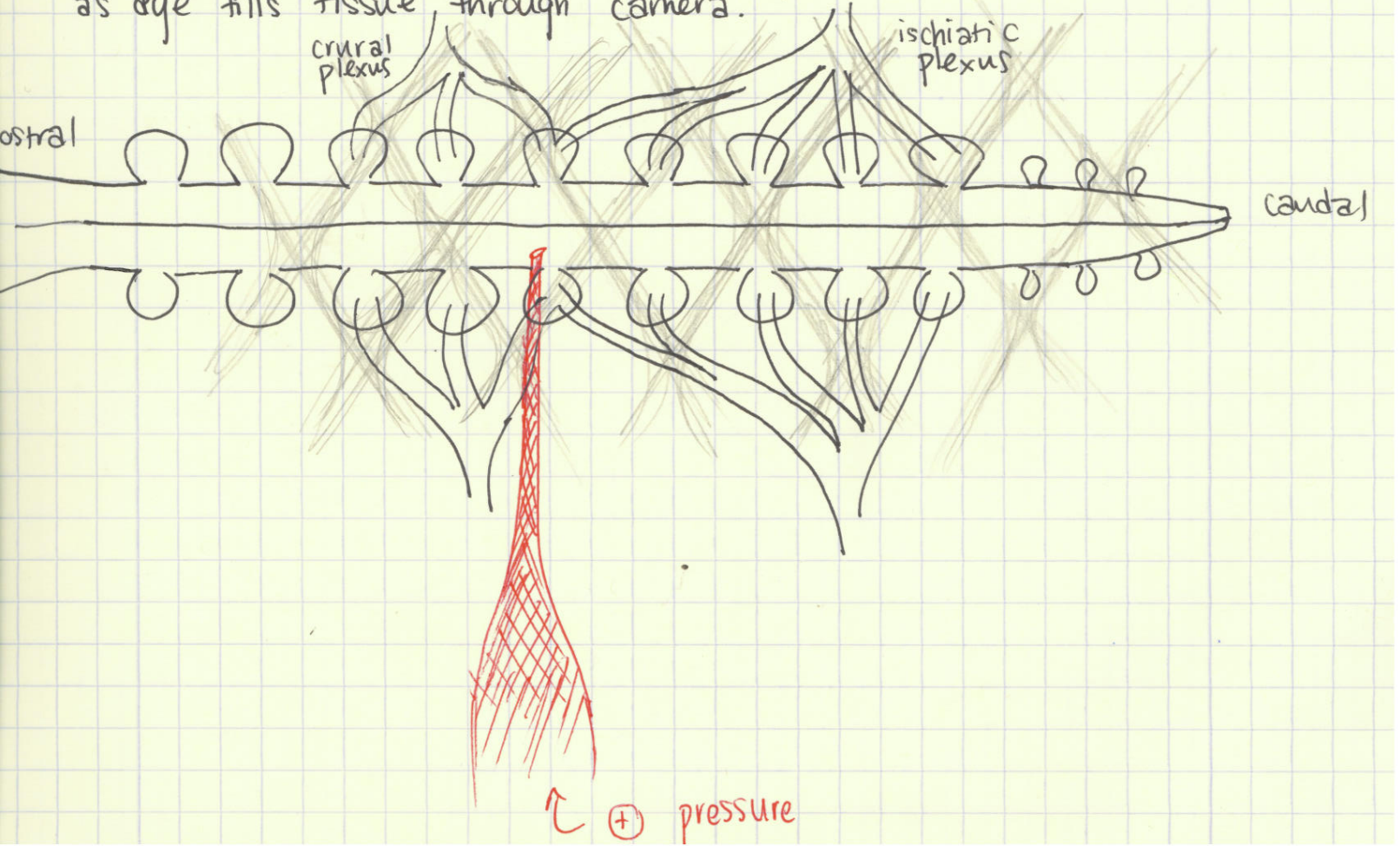
min later still pH 7.04 and temp 30°.

07 June 2010

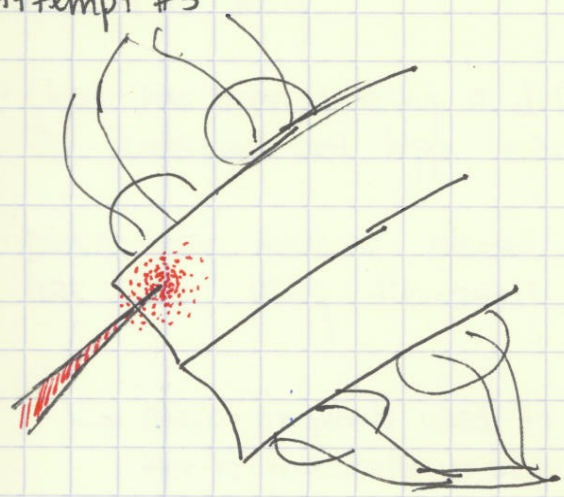
Objectives
- to plan out bolus injection method to use tomorrow

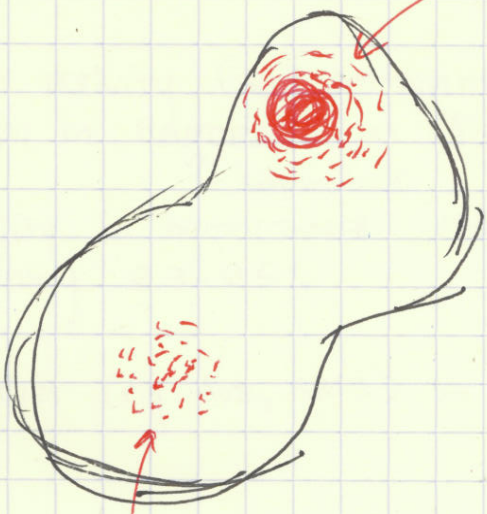
Background
Christine Rose's SBFI AM experiments utilize bolus injection (using patch pipette into living hippocampal tissue. We would like to inject SBFI into isolated spinal cord prior to slicing. Last week I tried to inject SBFI into motor column at concentrations of 75mM (left side) and 7.5 mM (right side) at different time points. In order to minimize damage to tissue, I think a manual micromanipulator should be used for injections and pressure should be applied in more controlled manner (last time I just blew through the capillary line).

Plan
→ dissect out cord and place in optical chamber w/ grid
→ using extracellular recording manipulators, bring a sharp electrode (w/ broken tip) containing dye toward motor column until it just touches surface
⇒ penetrate tissue by ~150 μm. turn off light and position inverted scope so that pipette can be visualized.
⇒ turn on camera and visualize at 340nm or 380nm.
⇒ apply slight ⊕ pressure through syringe and hold for ~1 min. Watch as dye fills tissue through camera.



Attempt #3





I think this is from ~~Attempt #2~~.

07 June 2010

Objectives
- to plan out bolus injection method to use tomorrow

Background
Christine Rose's SBFI AM experiments utilize bolus injection (using patch pipette into living hippocampal tissue. We would like to inject SBFI into isolated spinal cord prior to slicing. Last week I tried to inject SBFI into motor column at concentrations of 75mM (left side) and 7.5 mM (right side) at different time points. In order to minimize damage to tissue, I think a manual micromanipulator should be used for injections and pressure should be applied in more controlled manner (last time I just blew through the capillary line).

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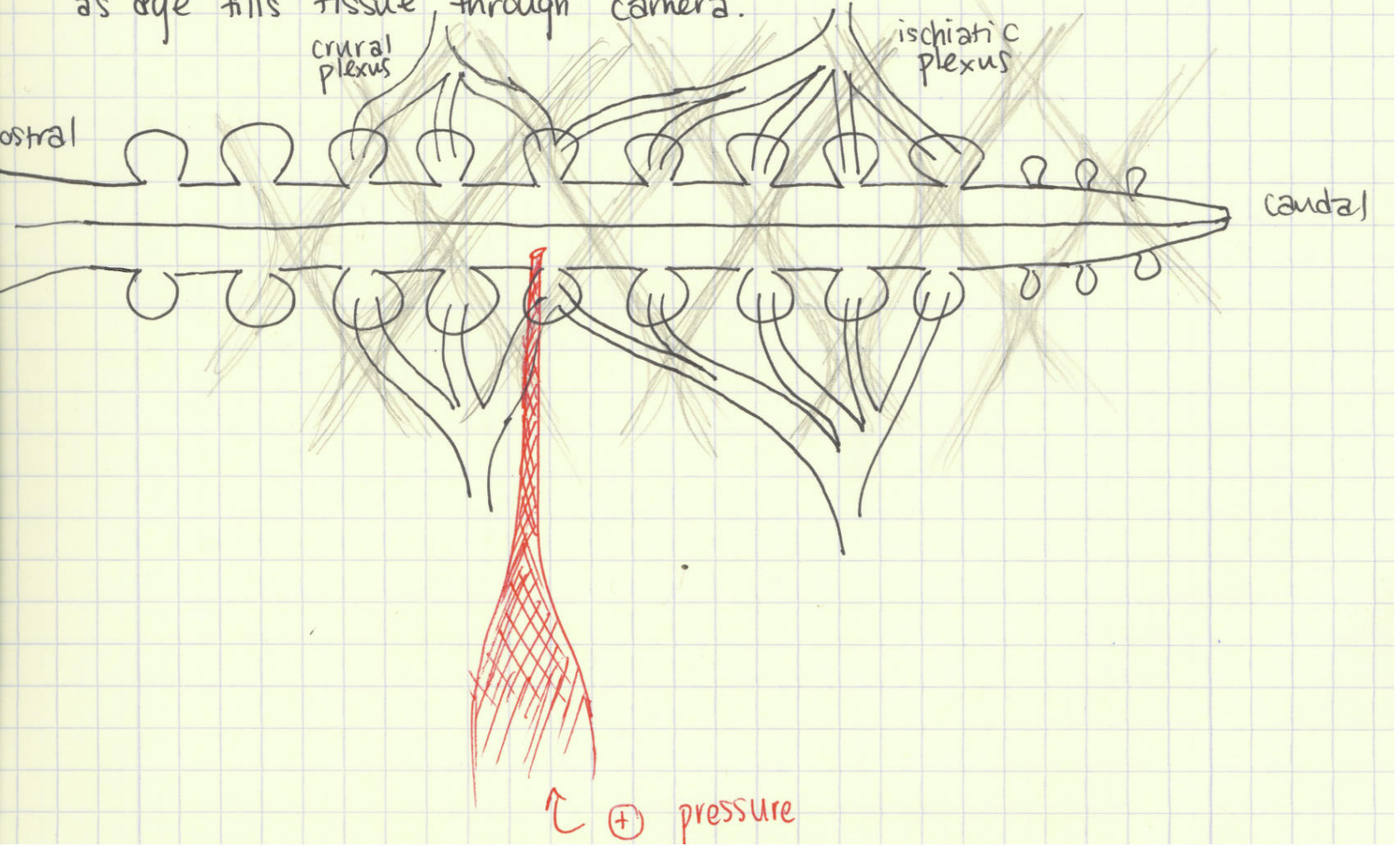
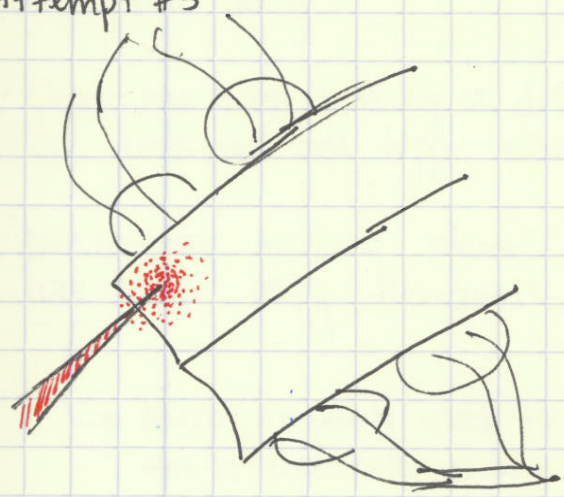
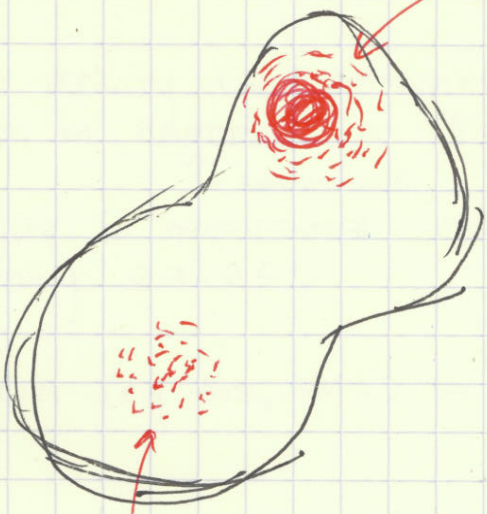
⇒ turn on camera and visualize at 340nm or 380nm.
⇒ apply slight ⊕ pressure through syringe and hold for ~1 min. Watch as dye fills tissue through camera.



Attempt #3



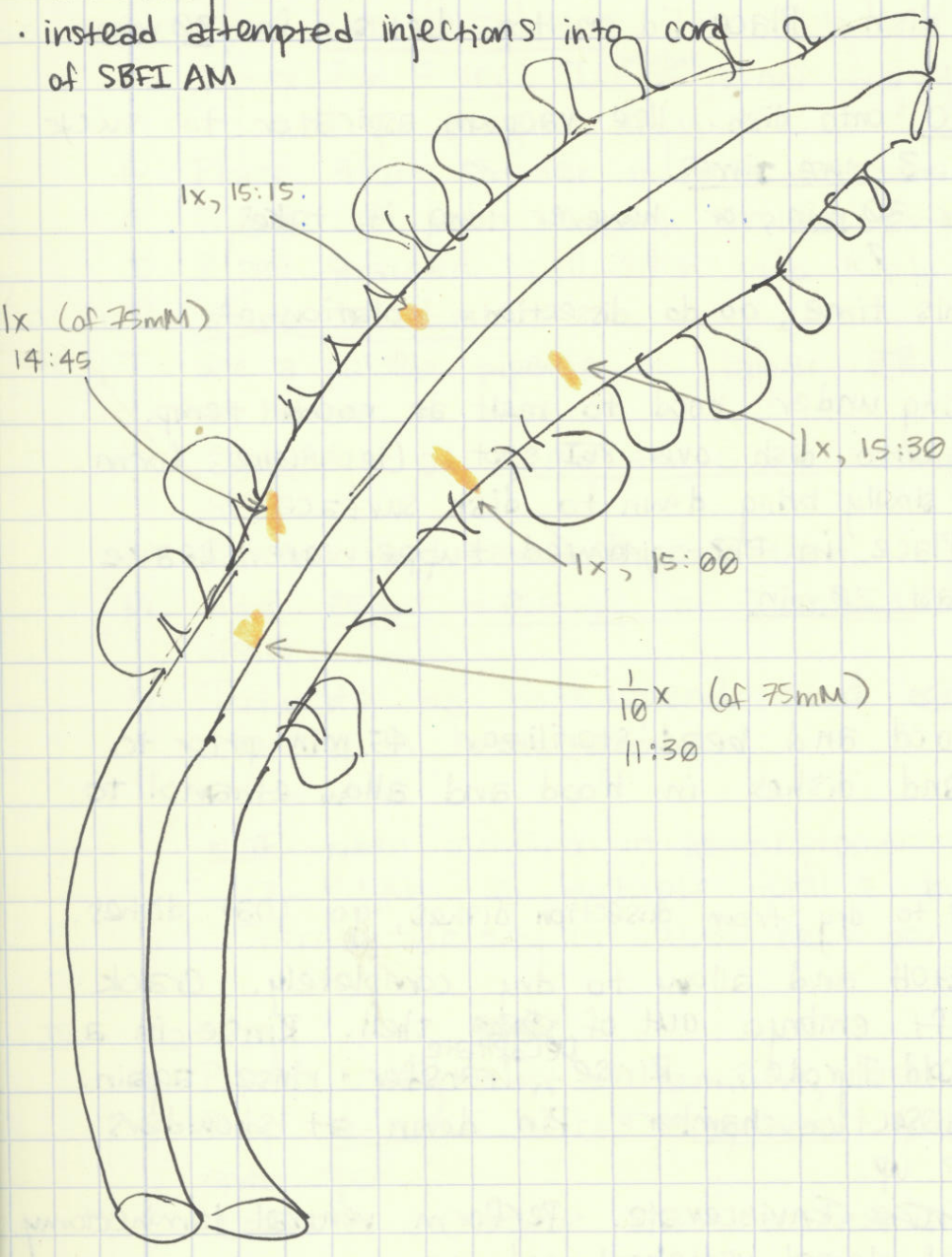


I think this is from ~~Attempt #2~~.

09 June 2010

Objectives
- SBFI experiment

Notes
- nub had fallen out of pipette ~~when~~ overnight some time
- no retrograde label
- instead attempted injections into cord of SBFI AM

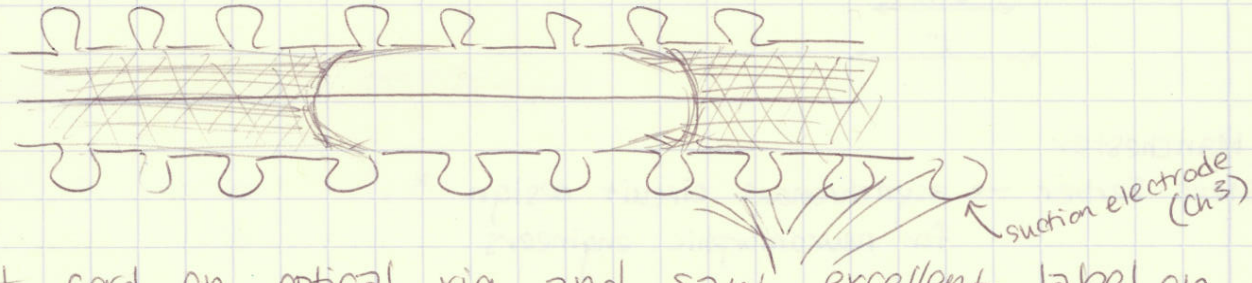


22 June 2010

Objectives
- SBFI lid 5

Background
- Yesterday Pete did a dissection and ~~spinal root~~ ~~back~~ retrograde label with SBFI on a lidocaine prep. He could not suck up the ventral root so he used LS1. spinal nerve. Also tried other side.
- We have ~~the~~ two other retrograde label lidocaine preps that have yielded opposite results. Hopefully this will inform our previous experiments and interpretation

Notes
- removed a segment of ventral meninges



looked at cord on optical rig and saw excellent label on right LS1 side. nothing on other side besides root.

recorded ~~from~~ extracellularly from spinal nerves for hours and saw episode freq ≈
(2mM KCl, 30°C)

optical stuff:
⇒ gain: 0.979 white matter view #1, #2
1.002 " " view #3

⇒ ionophores:
- gramacidin - 3 μM
- monesin - 10 μM (see pg. 93 → 17.3225mg ~~864~~ per 1mL for stock soln) } use 40μL stock of ionophores in 100mL of perfusion solution

⇒ Ouabain 0.5 mM

⇒ gain: 1.070 slices

Real Time	Video Time	Condition	Notes
14:10	0:00 - 0:11	regular Tyrode's through white matter	not warmed → @ RT.
14:23	0:11 - 0:34	"	pH = 7.41, temp = 30.0°C
14:39ish	0:34 - 0:46	same plane as above	pH = 7.41 t = 30.1°C
	0:46 - 0:59	view #2	
	0:59 - 1:14	view #3	
16:56	1:14 - 1:36	slice req. Tyrode's	pH = 731 t = 30.2°C
17:14	1:35 - 1:48	"	pH = 7.36 t = 30.0°C

* CHANGED TO 155Na Calibration sol'n @ 17:14; reached bath @ 17:17

17:25	1:48 - 2:01	155Na, no drugs	pH = 7.22; t = 29.7
17:30	2:01 - 2:19	"	= 7.2; 29.8°C
17:35	2:19 - 2:36	"	

* ADDED 0.5mM ouabain, 3μM gramacidin, 10μM manesin @ ~~17:43~~ 17:43 *

17:53	2:36-	155Na (begin) 10 min	pH = 7.19; t = 29.8
18:33	- 3:15	155Na (begin) 50 min	pH = 7.13; t = 29.8
18:43	3:15 - 3:28	155Na (begin) 60 min	7.11; 29.9
18:53	3:28 - 3:41	155Na / 70 min	7.10 30.0
19:03	3:41 - 3:56	155Na / 80 min	7.05 30.0
18:13	3:56 - 4:08	155Na / 90 min	7.09 30.1

* ADDED 120 Na @ 19:21; reached bath @ 19:23

eal Time	Video Time	Condition	Notes
9:33	4:08 – 4:19	120 Na 10 min	pH = 7.06 t = 29.8
9:38	4:19 – 4:33	120 Na 15 min	7.07 29.8°
		~~120 Na 15 min~~	

WITCH TO 90Na @ 7:45 ; reaches bath @ 7:47 *

9:52	4:33 – 4:46	90Na 5 min	pH = 7.09 t = 29.9°C
		~~90Na~~	
9:57	4:46 – 5:05	90Na 10 min	pH = 7.09 t = 30.0°C
0:02	5:05 –	90Na 15 min	pH = 7.08 t = 30.0°C

SWITCH @ 8:05 ; REACHES BATH @ 8:07 *

0:12	– 5:42	60 Na 5 min	pH = 7.10 t = 29.6	
0:17	5:42 – 5:56	60 Na	pH = 7.10 t = 29.7	photobleached at 340?
0:22	5:56 – 6:13		pH = 7.0 t = 29.7	

WITCH @ 8:27 ; REACHES BATH @ 8:29 *

0:34	6:13 – 6:28	30 Na	pH
	~~6:28 – 6:44~~	~~30 Na~~	
	6:28 – 6:44		pH = 7.12 t = 29.7
	~~6:44~~ NEW TAPE		
0:44	0:00 – 0:19	30 Na	

Real Time	Video Time	Condition	Notes
20:58	0:19-0:33.	0 Na 5 min	pH = 7.22 t = 30.5
21:~~08~~ 03	~~0:33 - 0:04~~ ???		
21:08	- 1:07	0 Na 15 min	pH = 7.23 t = 30.3
21:13	1:07 - 1:19	0 Na 20 min	pH = 7.25 t = 30.3°C

* SWITCHED TO 155Na @ ~~9:20~~ 9:25; reached bath @ 9:28 *

note: pH of 155Na sol'n (which had been bubbling) fell to 6.85. I ~~had~~ added a few drops of 1M KOH and had to wait ~5 min for pH to adequately fall before switching sol'n.

21:32	~~1:19~~ 1:19 - 1:31	155Na (end) 5 min	pH = 7.31 t = 30.4° C
21:38	1:31 - 1:44	155Na (end) 10 min	pH = 7.30 t = 30.5°C
21:43	1:44 - 1:55	155Na (end) 15 min	pH = 7.30 t = 30.6°C
21:~~48~~ 58	1:55 - 2:06	155 Na (end) ~~25 30 min~~ 30 min	pH = 7.33 t = 30.8° C
(refocused)	2:06 - 2:19	"	"

24 June 20[illegible]

Objectives
⇒ SBFI experiment on control embryo

Preparation
⇒ dissected an E10
⇒ sliced rostral half and incubated for 3 hrs with 15 mM SBFI
⇒ removed pia in the LS1–LS7 area and injected with ~~$~~ various SBFI concentrations (this is for practicing the technique, not for experime

Experimental Notes
→ identified some decently-labeled ~~cells~~ cells in v. horn. let dye wash ~~over~~ out in cont. Tyrode's perfusion for 1 hr.
⇒ general plan:
1. record ~~at~~ with reg. Tyrode's twice.
2. perfuse in 155Na w/o drugs. Record twice.
3. add ionophores + ouabain. ~~let~~ Record at 10 min intervals for 1hr.
4. perfuse in [120Na, 90Na, 60Na, 30Na, 0Na] with drugs for 15 min each. Record at 5, 10, and 15min.
5. perfuse back in original 155Na w/ drugs. Record at 10 min intervals for ~~1hr~~ 30min.

Ming-fai Fong
24 June 2010

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

Ming-fai Fong
04 July 2010

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

Introduction

The following analyses pertain to the following experiment:

2010.06.24 SBFI Con9 Experimental Notes

Regions of Interest

I defined ROIs around two cells that remained visible throughout the experiment. Some examples are below.

I also made some new macros in ImageJ to streamline the process for batch operations. I have listed and described their functions below:

Macro Name	Task Performed
mov2tif	Inputs a directory containing *.mov files. Creates an average of the first 60 frames for each movie, adjusts the minimum intensity to cover full dynamic range, and re-saves as a *.tif file in a designated directory.
batchROImeasure	Inputs directories for averaged images and corresponding ROIs. Measures min/max/mean intensity for each ROI/image combination and tags by ROI area. Saves information as *.txt files in a designated directory.

Computing Ratios

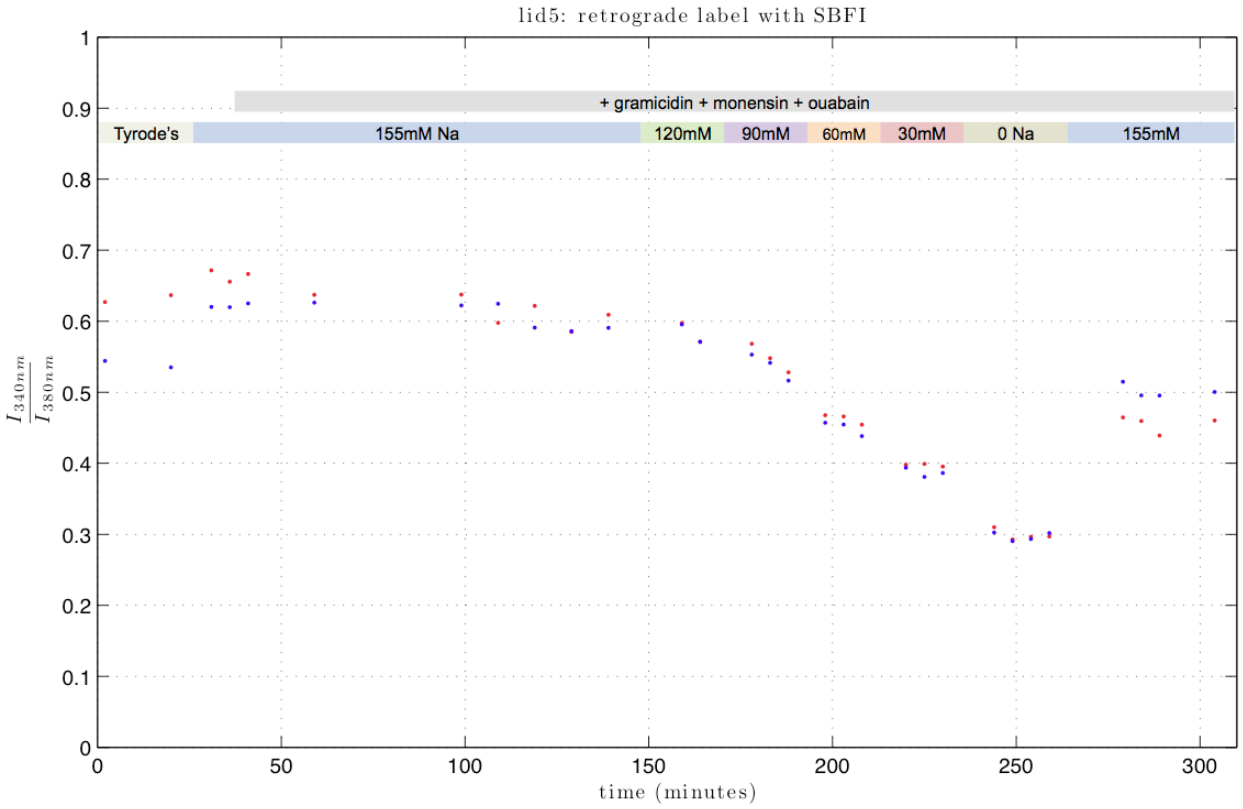
I have written a Matlab script called **ratio_calc** that performs the following tasks:

- reads a folder of ImageJ-generated *.txt files containing measurements of area, mean, min, and max intensities for each ROI (different files in the folder represent different time/concentration conditions)
- performs sorting and background subtraction for mean intensities for each condition, and calculates the appropriate fluorescence ratios
- saves the following information into a data structure called data:
    - headings- original file names with condition information (e.g. 0Na_10min_340)
    - orig340- original mean emitted fluorescence when excited at 340nm
    - orig380- original mean emitted fluorescence when excited at 380nm
    - ratio- contains processed fluorescence ratios
    - times- contains time of fluorescence measurement
- plots fluorescence ratios by time of measurement, labeling different conditions

Ming-fai Fong
15 July 2010

Topic: SBFI Lid5 Analysis
Keywords: SBFI AM, chick embryo, spinal cord, lidocaine

I defined ROIs about two cells for the duration of this recording, computing average intensities in ImageJ, and used Matlab for additional computation and plotting. Results of calibration data are shown below:



Ming-fai Fong
28 July 2010

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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28 July 2010

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

Ming-fai Fong
01 August 2010

Topic: SBFI Con6 (Re-analyzed)
Keywords: sodium imaging, embryonic chick, spinal cord, motoneurons

Introduction

I am re-analyzing a previous experiment to determine how background subtraction effects the calibrated ratios.

Example

Below are the four cells used for analysis and the five different background subtraction regions. The same ROIs and BGs were used for all images, but only the 0Na_10min_340nm is shown here as an example.

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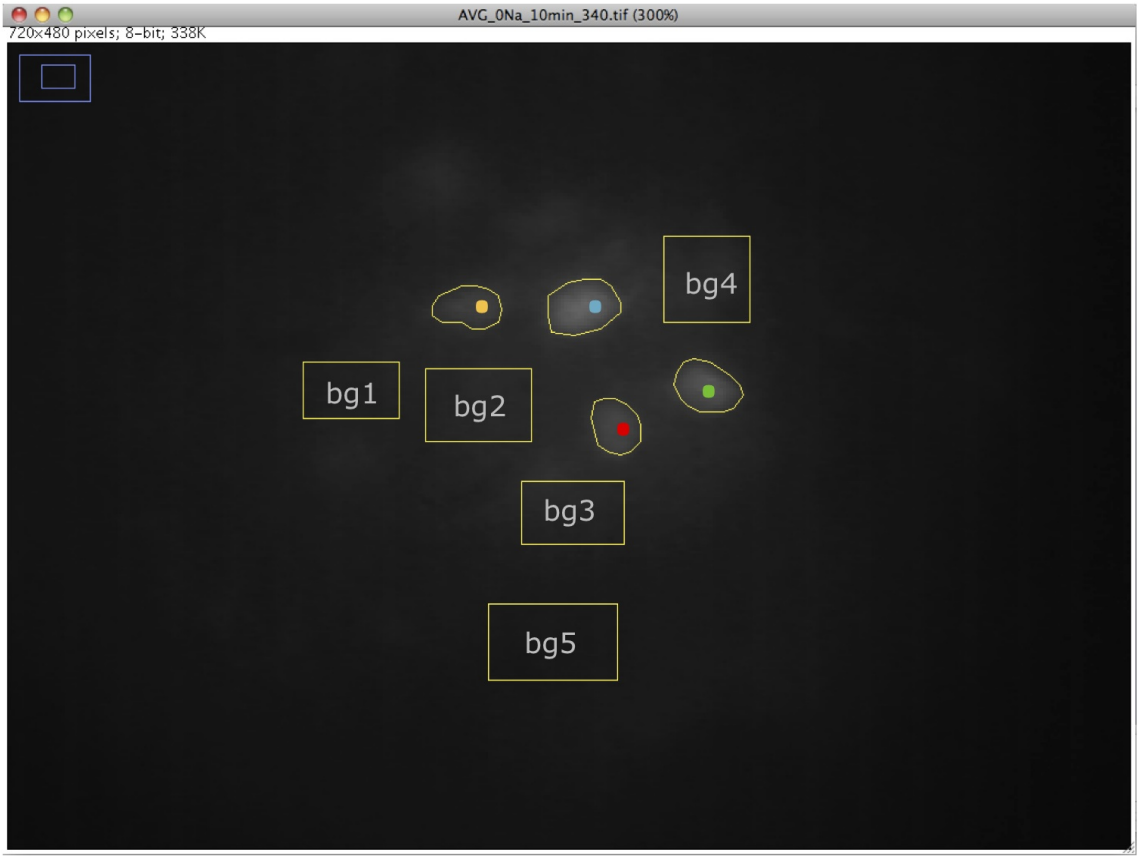
01 August 2010



Calibration Curves

Below are the different calibration curves for different background subtraction regions. (Pre-subtraction fluorescence is the same for all).

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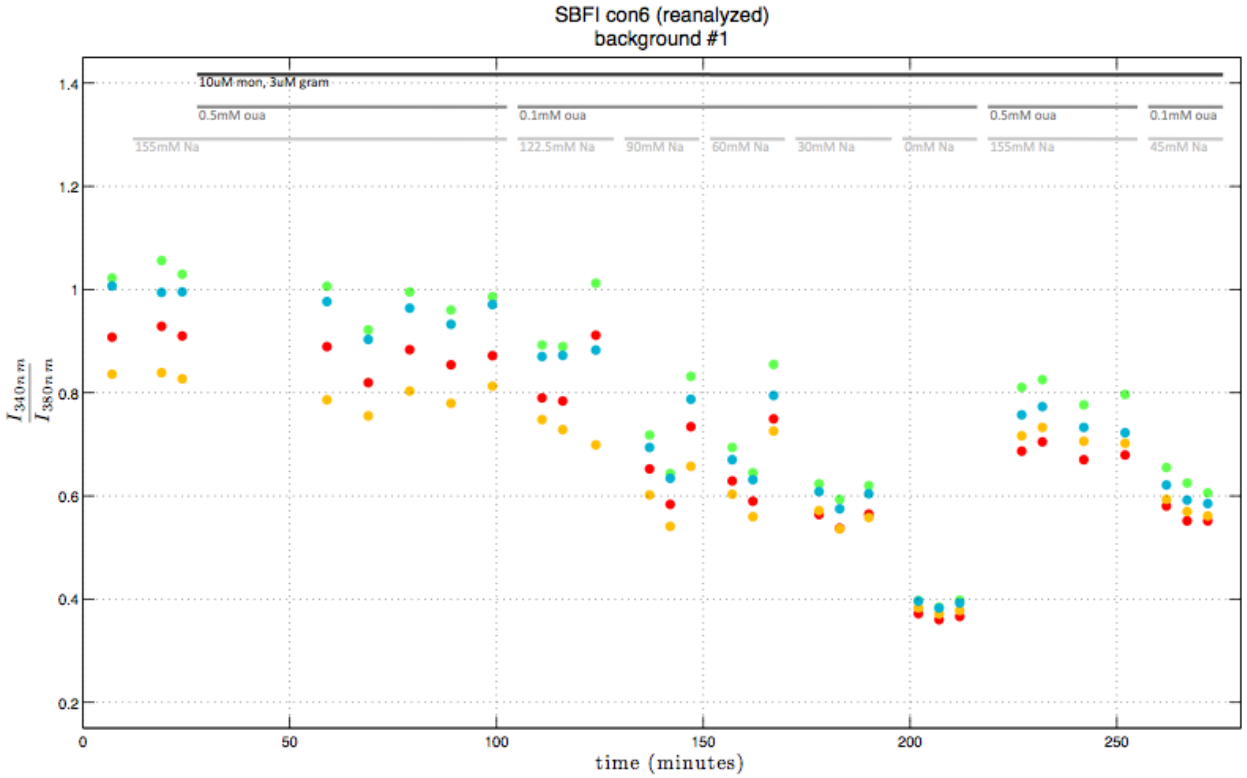
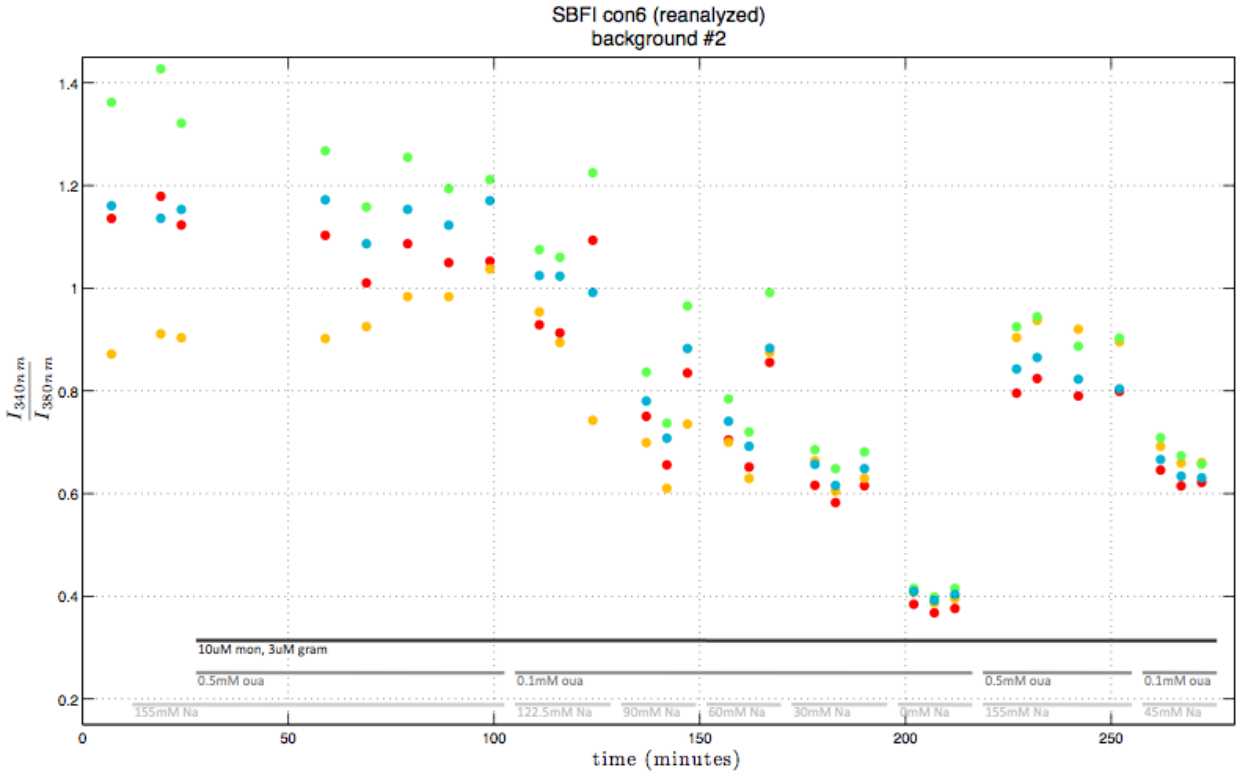
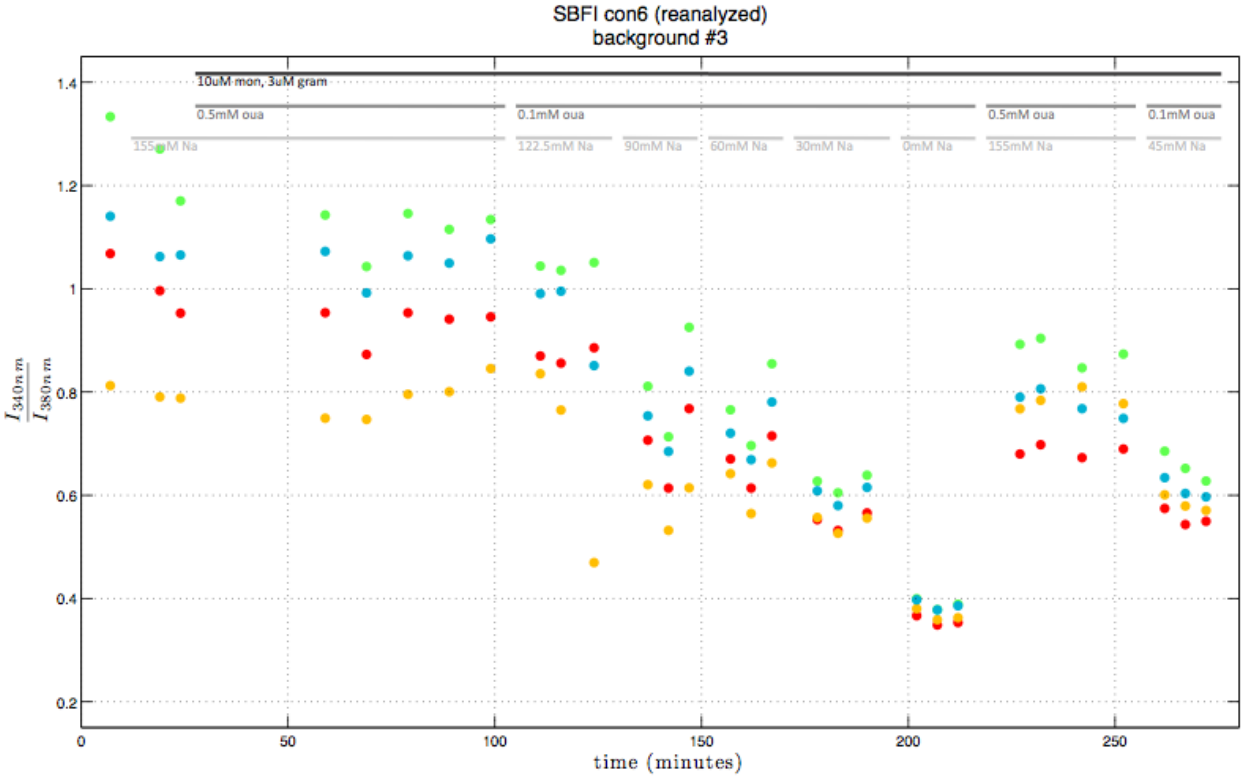
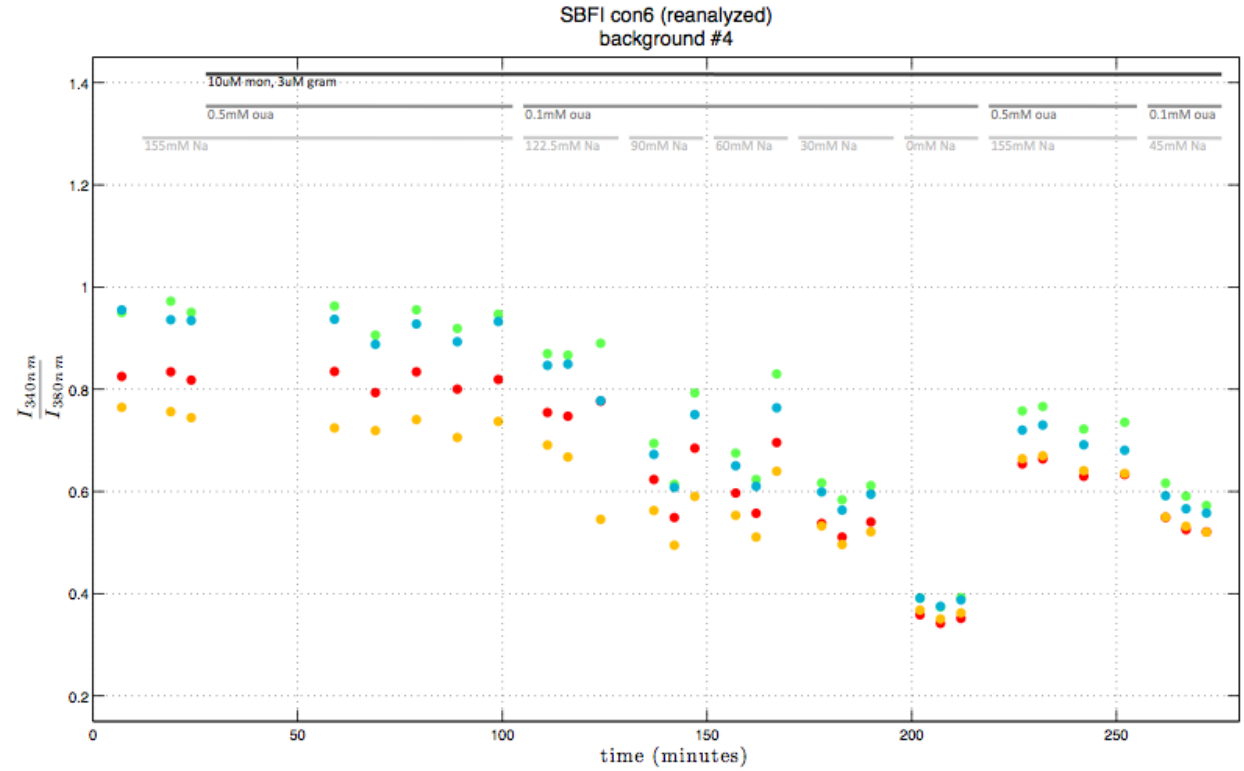
01 August 2010



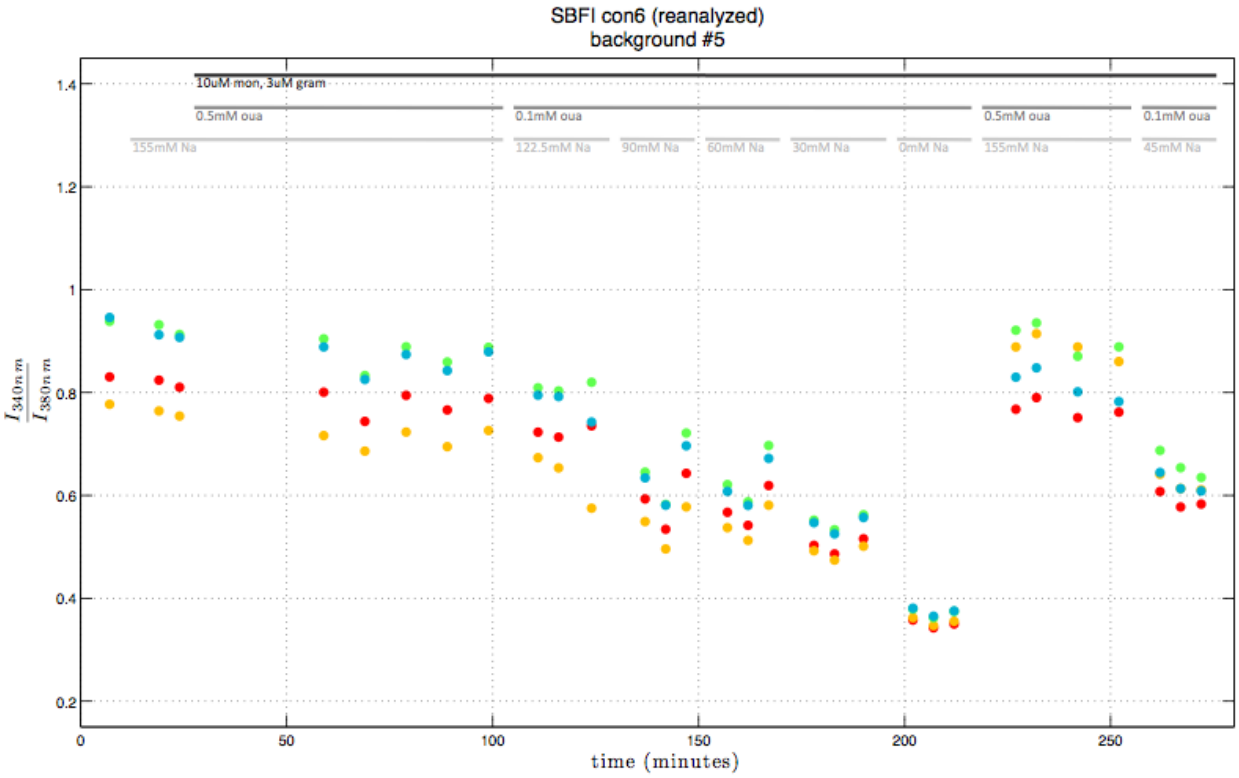


Ming-fai Fong
01 August 2010





Ming-fai Fong
01 August 2010



Ming-fai Fong
13 August 2010

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