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 experiment on 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 L5 2 20x at Room temp W/ND 50 on 380 filter (340 then 380)

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

superficial of few cells

one more time 10:33 (1"04 – 1"34)

then moving more caudal part of L5 2 (1"34 – 2"26)

intensities 888 video 826

Now transverse cut

1st piece not main one but – int 993, video 826 at 11:40 AM (2"26 – 2"49)

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

then more superficial – but ~~use for calibration~~ see dendrites will (3"07 – 3"26)

962 intensity ← Now for real at deep plane (3"26 – 3"46) & will use for calibration (12:00 PM) start here

@ 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

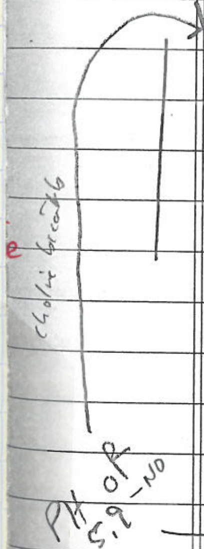
get dendrite of saturated cell isolated from cell body

Date

@ 1:18PM 0 Na + ionophores started
@ 1:22PM 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
@ 2:20PM 30 Na + ionophores (4"07-4"29)
@ 2:25PM 10 min " (4"29-4"45)
@ 2:30PM 15 min " (4"45-4"59)

start perfusing 60 mM Na at 2:34 gets to bath at 2:3?
@ 2:41 5 min 60 mM Na (4"59-5"21) low pH!
↳ 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 at 155 Na+ after clearing fluorescent gunk. 6:02-6:30
@ 3"25 again (after pHing from 7.13-7.21) 6:42-7:11
at 3:32PM start 60 mM Na sol again (w/o gunk) at bath by 3:34 extra look
pH of 5.9 → @ 3:39 5 min 60 mM Na + ionoph (7"14-7"33)
@ 3:50 again 10 min after 60 mM Na } after pHing correctly (? ?)

Page

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

rough white matter — left LSZ — start: 0 end: 21'
eld of view adjacent to nub)
bg @ 340nm: $\bar{I}$ = 28 on cell @ 340 nm: $\bar{I}$ = ~~131~~ 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
first and third. for baseline we'll image from both, but
then do [Na] changes in just the first one.

perfusion just re-started:

* third slice: 340 @ 1:03 - 1:15 — dendrites look great in this one!
(11:44a) 380 @ 1:15 - 1:24

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

* first slice: @ 380, intensifier saturates at 1,030 → tune down to 1,002 to stay in range (for 0Na condition)
~~#1~~ ~~[illegible]~~
(std sol'n)

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

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

26 Jan 2010

*first slice (cont.) std. sol'n

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

(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: All solutions have gramicidin + monesin right in beginning

- -

a
ns
3:35

(13:36) begin perfusing ØNa
reaches bath @ 13:38

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

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

(14:08) 30 minutes after ØNa
4:08 - 4:28

- -

Na
gins
4:11

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

NOTE: perfusion appears slightly quicker than previous...

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

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

- -

Na
egins
14:24

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

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

26 Jan 2010

ectives
ollect optical data from lidocaine cord

rding Log

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

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

perfusion just re-started:

* third slice: 340 @ 1:03 - 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 1,030
~~#1~~ ~~[illegible]~~ tune down to 1,002
(std sol'n) to stay in range
(for 0Na condition)

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

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

26 Jan 2010

*first slice (cont.) std. sol'n

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

(13:05) put 10 μM (350μL into 350 mL) 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 solutions have gramicidin + monesin right in beginning

- - - - - - - - - - - - - - - - - - - -

a
ns
3:35

(13:36) begin perfusing ØNa
reaches bath @ 13:38

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

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

(14:08) 30 minutes after ØNa
4:08 - 4:28

- - - - - - - - - - - - - - - - - - - -

Na
gins
4:11

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

> NOTE: perfusion appears slightly quicker than previous...

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

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

- - - - - - - - - - - - - - - - - - - -

Na
egins
14:24

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

(14:29) 5 minutes after 60 Na
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 mins 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
(14:57)	begin perfusing 155 Na reaches bath @ 14:58
(15:03)	5 minutes after 155 Na 7:07 - 7:33 pH = 7.1
(15:10)	12 minutes after 155 Na 7:33 - 7:58
(15:11)	begin perfusing 0 Na (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
(15:32)	19 minutes after 0 Na 9:05 - 9:30

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

add 100 μL of gram + mon to solutions

during this time I made a bit of 122.5 mM Na sol'n ~~(...)~~ (12.5 mL of

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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 122.5 Na
9:30 - 9:47
pH = 6.8 ⟶ added 0.1 M KOH
until pH = 7.15 (at 15:49)

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

(15:55) 18 minutes after 122.5 Na
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 <u>Recording</u>
- 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 } vib. settings
  freq : 8-9 }

  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
  59:29 - 1:20:17 [14:10] 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... agh.
  Pete is re-positioning it.

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

16 Feb 2010

[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 _ 7min

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

[15:10] adding gram + monesin
we're using 100mL 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_15min

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

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

[15:59] 3:14 - 3:35 155_Na_ionophores _ 49min

[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_ 15min ← don't use... 380 to saturated light

[16:28] 3:58 - 4:23
155Na_ouabain_18min

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

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

[16:56] start perfusing 122.5 Na calibration solution w/ 20mg ouabain (0.3448mM)

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

$\frac{580g}{1 mol} \times \frac{0.5 \times 10^{-3} mol}{1L} = \frac{0.290[illegible]}{1L}$ → $\frac{290mg}{L}$ → $\frac{29 [illegible]}{100m[illegible]}$

$200000 \div 58 = 3448$ (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 shoot 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 - 10N'a

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

16 Feb 2010

[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.~~ 00:00 - 00:18 — same stage pos.
  - ⇒ plane 2: ~~0028~~ 00:18 - 00:40; int: ~~0.884~~ 0.900 — same stage pos.
  - ⇒ plane 3: (stage shifted caudal from 1+2 ~~&~~) 00:40 - 01:01 — near nub.
  - ⇒ plane 4: 01:01 - 01:26 (stage shifted rostral from 1+2 ~~&3~~) — same (rostral) pos
  - ⇒ plane 5: 01:26 - 1:46 — same as 4 but with iris turned down and w/ int → 0.956 — 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. sol'n

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

* 155 Na cal sol'n (no ionophores, no ~~oa~~ ouabain) reaches bath @ ~~13:13~~ 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.

10 μM monensin
3 μM gramacidin

* 155 Na + ionophores + ouabain

↑ 0.5 mM (290 mg in 100 mL)

10 μM gramacidin / 3 μM monensin } based on C. Rose Na+ imaging experiments

added ionophores (40 μL monesin, 40 μL gramicidin) + ouabain (290 mg) at 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

↑ 10 μM / 3 μM ↑ 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

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

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

reaches bath at: 15:12

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

~~90 Na - 10 min:~~ pH = 6.20 → added 10 drops 1M KOH

* 90Na + ionophores + ouabain (cont.)

90Na_10min: 5:40 - 5:58 [15:22]

" 15min: [15:27]

* 60Na + ionophores + ouabain

reaches bath at: 15:32

60Na_5min: 5:58 - 6:40 [15:37]

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

60Na_10min: 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_5min: 7:26 - 7:49 [15:58]

30 Na_10min: 7:49 - ~~2~~ 8:16 [16:03]

" ~~15~~ 17min: 8:16 - 8:36 [16: ~~08~~ 10]

* 0 Na + ionophores + ouabain

reaches bath at: 16:17

0Na_5min: 8:36 - 8:55 [16:22]

" _10min: ran out of tape in middle [16:27]
↓ changed to new one.

0Na_15min: 0:00 - 21:05 [16:32]

~~0.5mM~~ 0.5mM

155Na_end + ionophores + ouabain

reaches bath at: 16:42

155Na_end_5min: ~~[illegible]~~ 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 sol'n 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 - ?:??

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 both gram and mon for 100ml solution

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

added at 14:55

a. ~~[struck out]~~ 30 min ~~[struck out]~~ (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: ~~155~~ 120 Na + 0.1mM OUA, 10μM gram, 3μM mon

reaches bath at ~~4~~ 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 mon

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. <u>Transverse: 90 Na (cont.)</u>

c. 15 min (16:36)
5:08 – 5:29

7. <u>Transverse: 60 Na + 0.1 mM OUA, 10 μM gram, 3 μM mon</u>

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. <u>Transverse: 30 Na + 0.1 mM OUA, 10 μM gram, 3 μM mon</u>

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. <u>Transverse: 0 Na + 0.1 mM OUA, 10 μM gram, 3 μM mon</u>

~~a. 5 min~~ reaches bath at 17:20

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

9. <u>Transverse: 0Na (cont.)</u>

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

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

10. <u>Transverse: 155Na + 0.5mM OUA, 10 μM gram, 3 μM mon</u> (again)

~~ac 5w~~ 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

TX
1 mg (GT limit is 100 mg)

SBFI AM
MW 1127.07 g
made ~~24~~25 50 µL aliquots
of ~70 mM (maybe 75 since we only had 24 aliquots) or 23.5ish

$\frac{1\ mg}{25\ aliquot} = \frac{0.04\ mg}{1\ aliquot} \rightarrow \frac{0.04\ mg}{50\ \mu L} \times \frac{1\ g}{1000\ mg} \times \frac{1\ mol}{1127.07\ g} \times \frac{1000000\ \mu L}{1\ L}$

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

Experimental Notes

diluted 2 aliquots (each 50 µL of 75 mM) into 10 mL tyrode's[2] for final [SBFI] = 1.5 mM
bubbled slices for a few hours... plan to remove a few ~~[crossed out]~~ after 2 hours to check label and play w/ pH
removed ~~one~~ 3 slices & 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 ~~so~~ 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

~~100 mL × 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 40 µL → 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 (pH, temp)

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	0:56 - 1:13 (messed up initially)	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		pH takes FOREVER to stabilize ... still varies sometimes after waiting 10 min.
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)

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 (11 AM until 4:45 PM). 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.

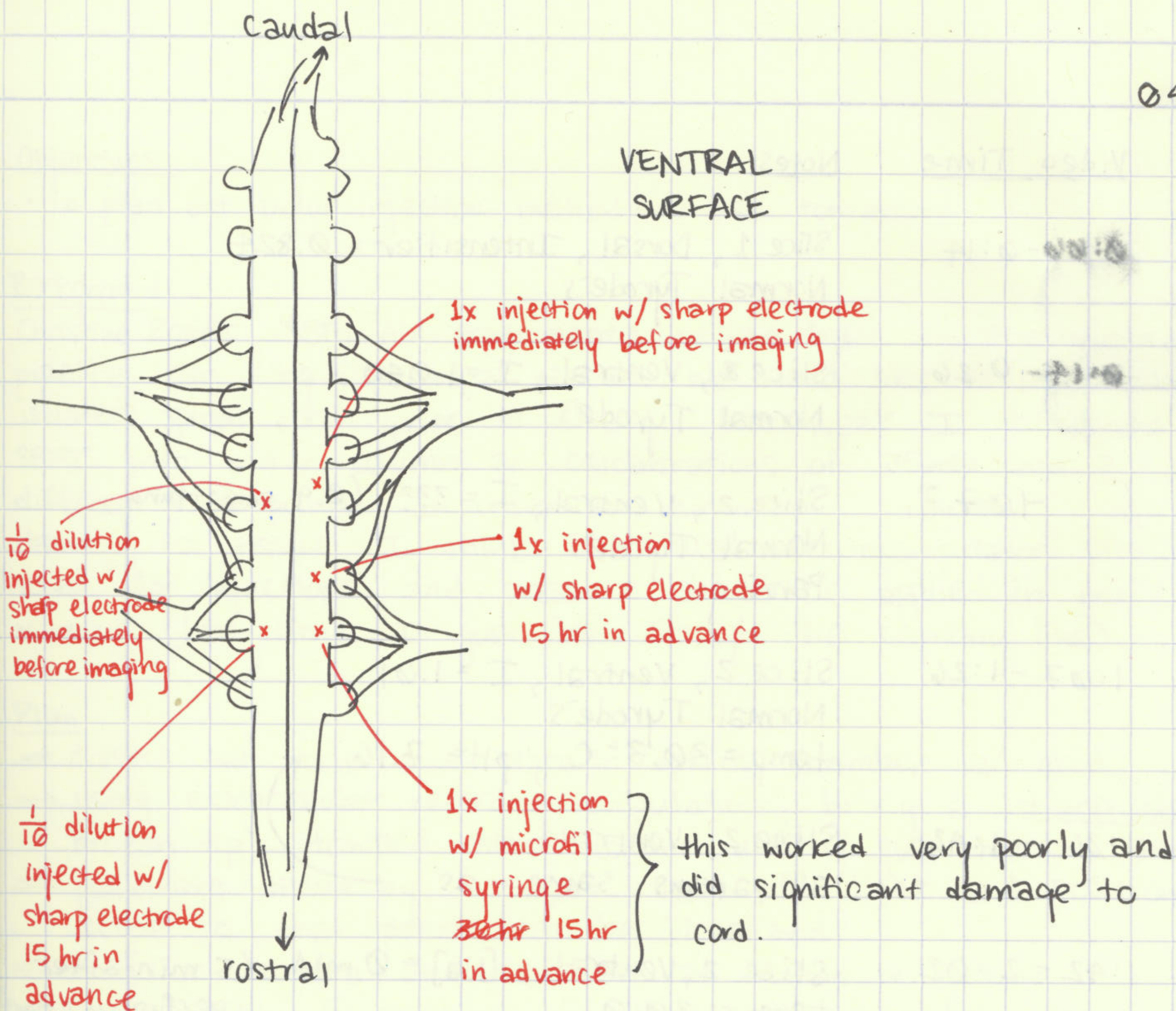
Real Time	Video Time	pH	temp	[Na]	notes
17:55	4:44 - ~~5:05~~ 5:13	7.2~~3~~	30.1 °C	155mM	same sol'n as end of previous pg.
18:00	5:13 - 5:36	7.23	30.1	"	"
18:08	5:36 - 5:53	"	"	"	"
18:25	5:53 - 6:12	7.33	29.8	"	added ionophores + 0.5mM ouabain at 18:10; significant movement of prep due to pressure issues... had to translate stage around (but kept same focal plane)
~~18:40~~ 18:45	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 @ ~~[illegible]~~ 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 @ ~~8 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	11:06-11:24	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...

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 ~~3~~ 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°.

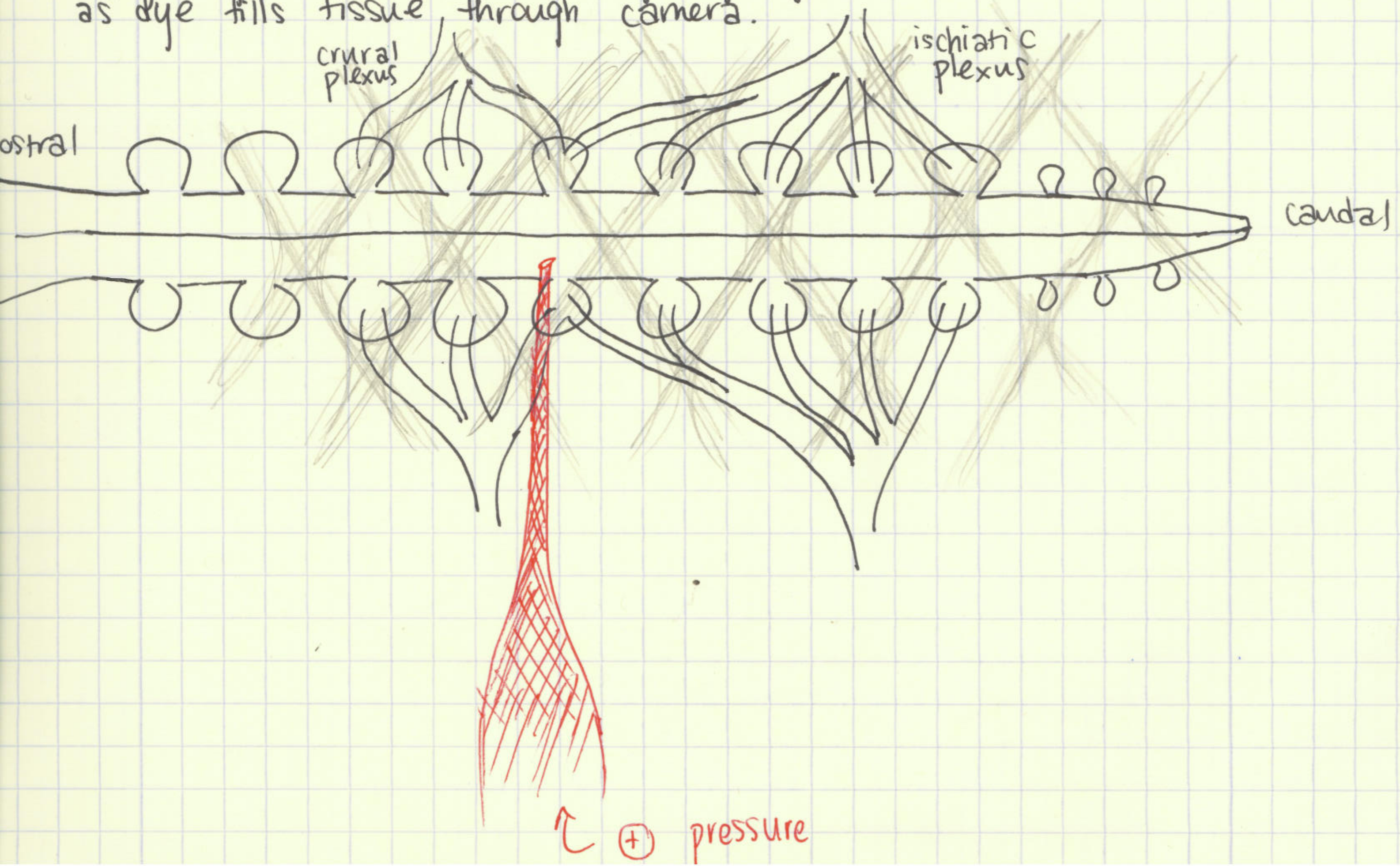
07 June 2010

Objectives
· to plan out bolus injection method to use tomorrow

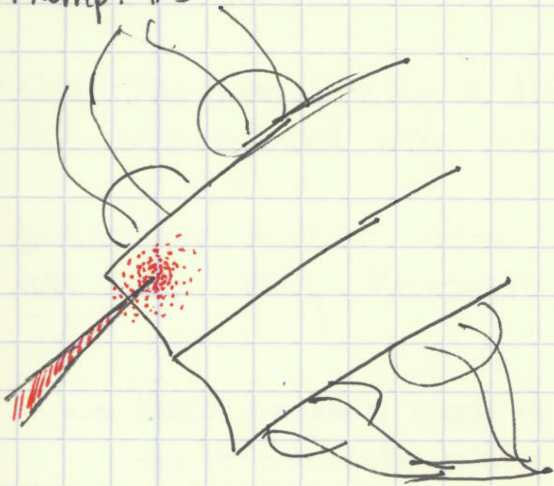
Background
Christine Rose's SBFI AM experiments utilize bolus injection (using patch pipette into living hippocampal tissue. We would like to inject SBFI into isolated spinal cord prior to slicing. Last week I tried to inject SBFI into motor column at concentrations of 75mM and 7.5 mM at different time 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).

(75mM ↑ left side; 7.5 mM ↑ right side)

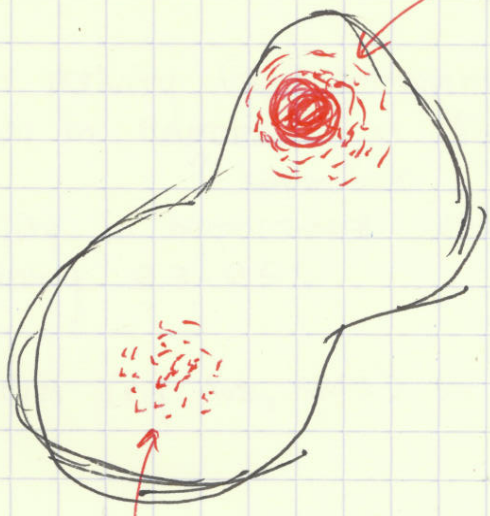
Plan
⇒ dissect out cord and place in optical chamber w/ grid
→ using extracellular recording manipulators, bring a sharp electrode (w/ broken tip) toward motor column until it just touches surface (sharp electrode containing dye)
⇒ penetrate tissue by ~150 μm. turn off light and position inverted scope 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



booyah.



I think this is from Attempt #2.

07 June 2010

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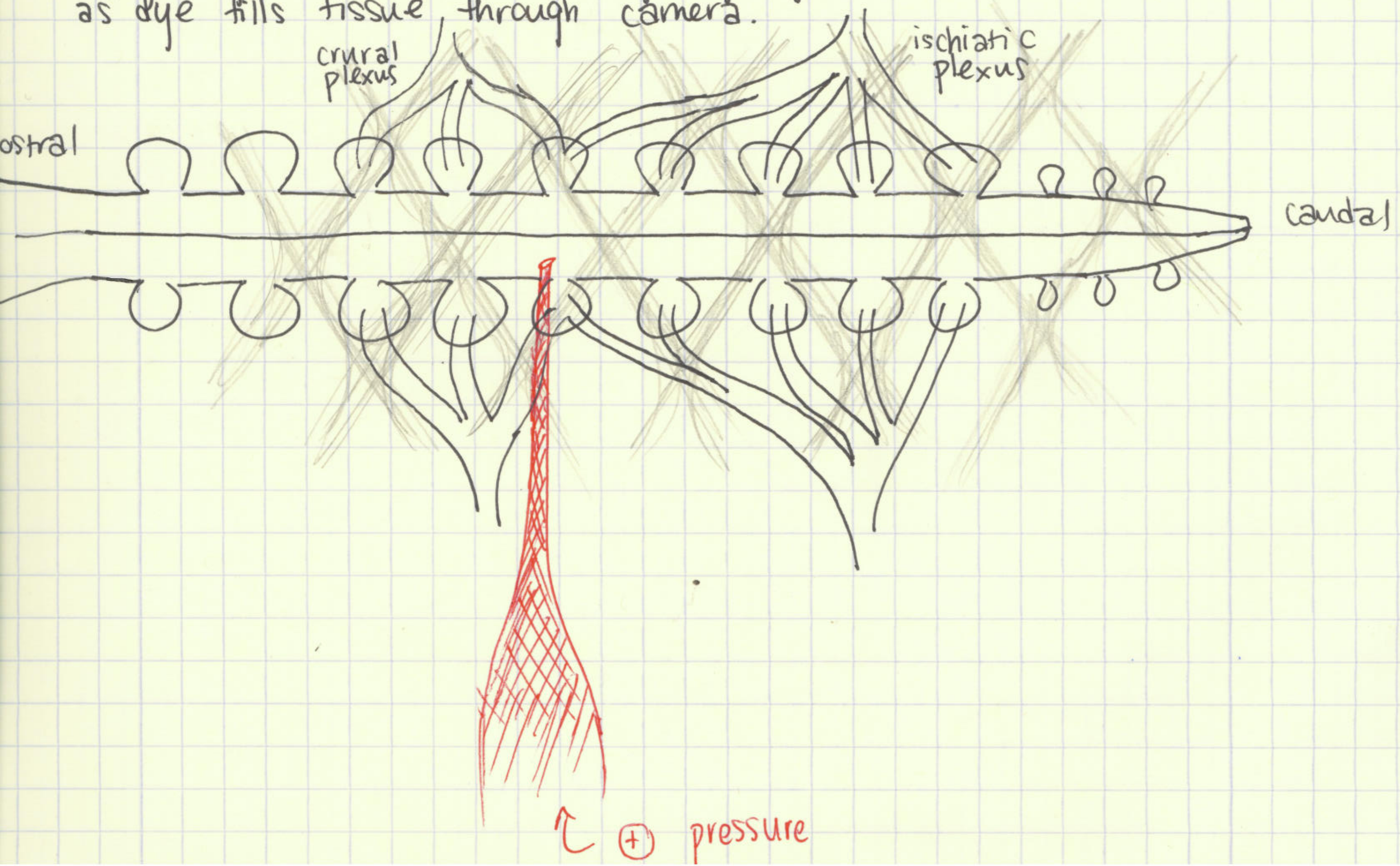
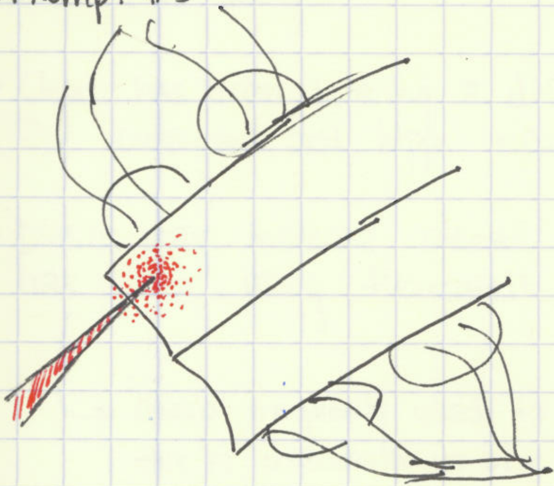
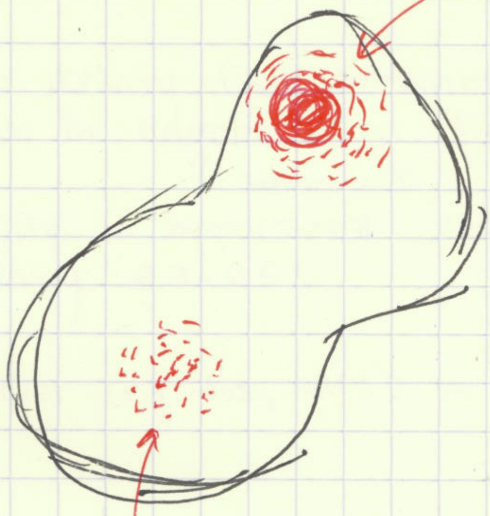


Attempt #3





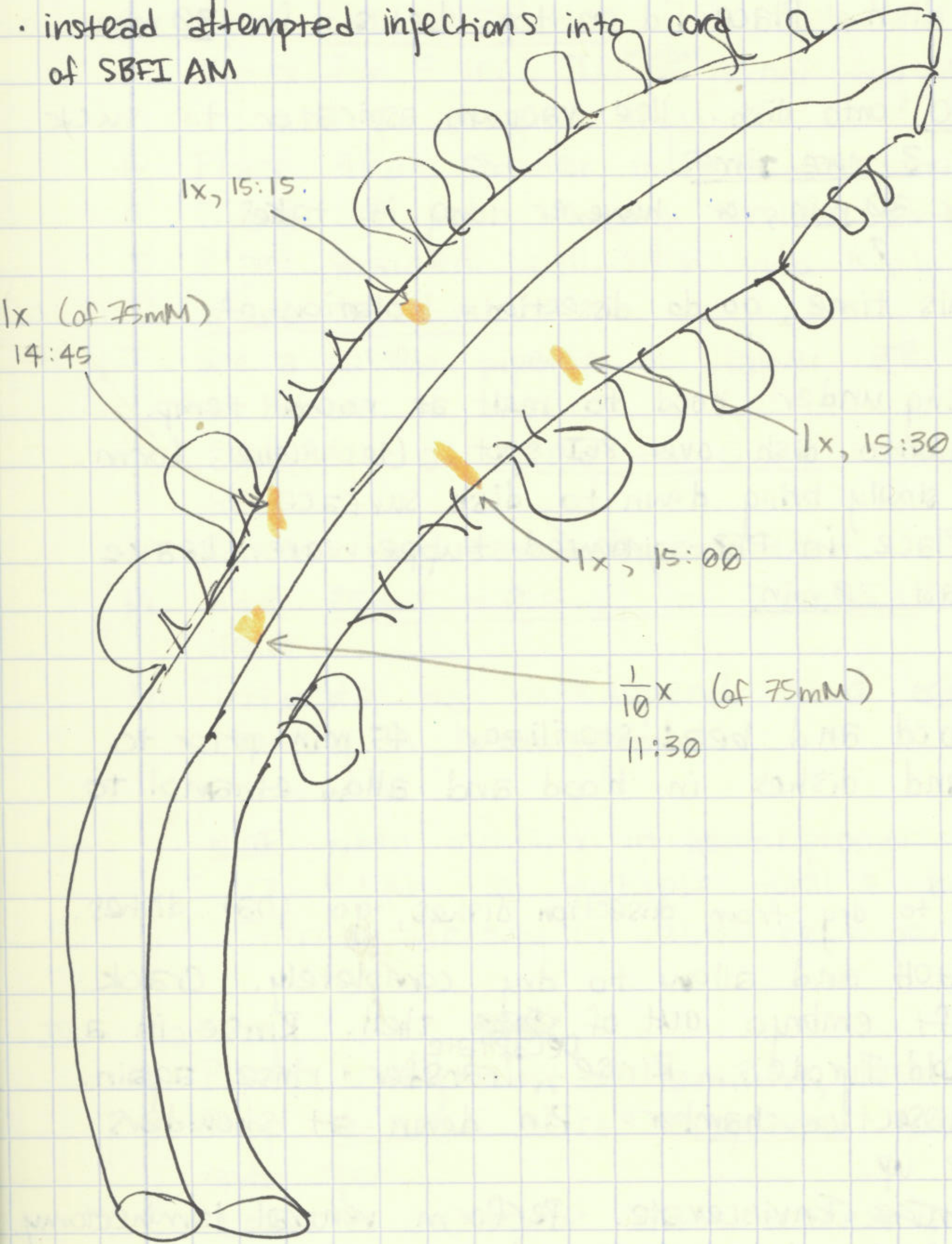
booyah.

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

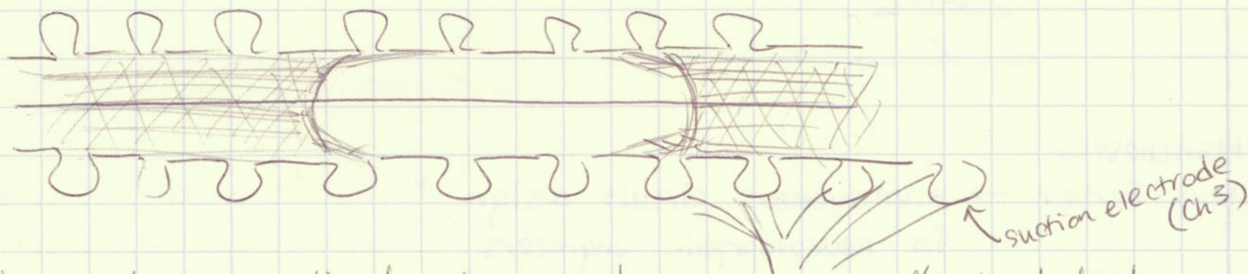
- SBFI lid5

Background

- Yesterday Pete did a dissection and ~~spinale 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 ~~ethe~~ 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 per 1mL for stock sol'n) } use 40μL stock of ionophores in 100mL of perfusion solution

⟹ Ouabain 0.5 mM

⟹ gain: 1.070 slicer

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 reg. Tyrode's	pH = 7.31 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

Real Time	Video Time	Condition	Notes
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, 3 μM gramacidin, 10 μM monensin @ ~~60:43~~ 17:43 *

Real Time	Video Time	Condition	Notes
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
18:03	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

Real 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 ~~120 Na 15 min~~ →	7.07 29.8°	

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

Real Time	Video Time	Condition	Notes	
9:52	4:33 - 4:46	90Na 5 min	pH = 7.09 t = 29.9°C	
9:57	4:46 - 5:05	~~90Na~~ 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 *

Real Time	Video Time	Condition	Notes	
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	

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

Real Time	Video Time	Condition	Notes
0:34	6:13 - 6:28	30 Na	pH
	~~[illegible]~~ 6:28 - 6:44	~~30 Na~~	pH = 7.12 t = 29.7
	~~[illegible]~~ 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	??? ~~0:33-1:07~~		
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 @ ~~9:26~~ 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 1 M KOH and had to wait ~5 min for pH to adequately fall before switching sol'n.

Real Time	Video Time	Condition	Notes
21:32	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 20 min~~ 30 min	pH = 7.33 t = 30.8° C
(refocused)	2:06 - 2:19	"	"

24 June 201

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 ~~the~~ 30min.