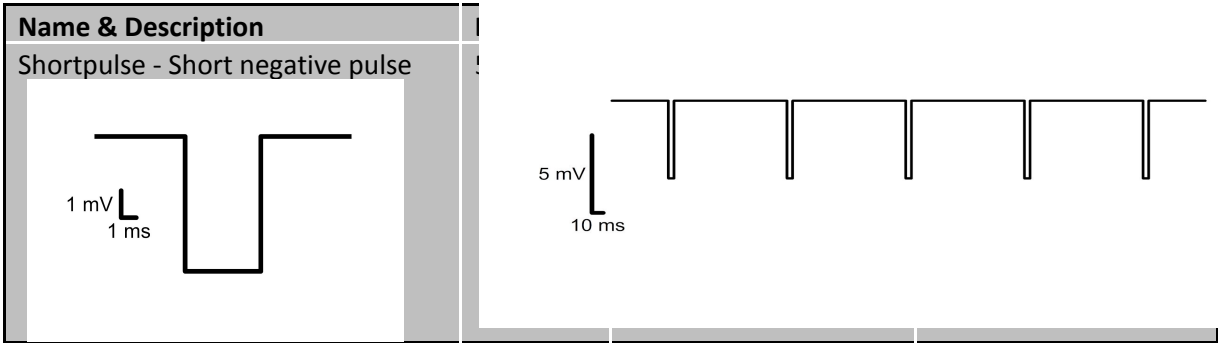# Electrophysiology stimuli for DIGAP

Supplementary Information

Supplementary document for Koos et al., Automatic deep learning driven label-free image guided patch clamp system for human and rodent in vitro slice physiology manuscript.

Stimulation waveform to test electrode resistance:

Name & Description	
Shortpulse - Short negative pulse	

1 mV
1 ms

5 mV
10 ms

Stimulation waveform to analyze the physiological properties of patched neurons:

Name & Description	Length	Amplitude	Repeat interval
IVCC - Incremented long pulse	800 ms	-100 pA	0 s
		increment: 20 pA	

20 pA
100 ms

Batch commands for controlling the HEKA EPC amplifiers:

PROTOCOL "RESET"

This protocol is used for resetting the oscilloscope window in
PatchMaster and switch the amplifier to voltage clamp mode.

```
Command   ( 0.000s): "  O  DispTrace      0; Trace 1"
Command   ( 0.000s): "  O  ResetY"
Command   ( 0.000s): "  O  ResetX"
Command   ( 0.000s): "  O  YScaleInc"
Command   ( 0.000s): "  O  YScaleInc"
Command   ( 0.000s): "  O  YScaleInc"
Command   ( 0.000s): "  O  DispTrace      1; Trace 2"
Command   ( 0.000s): "  O  ResetY"
Command   ( 0.000s): "  O  ResetX"
Command   ( 0.000s): "  O  YScaleInc"
Command   ( 0.000s): "  O  YScaleInc"
Command   ( 0.000s): "  O  YScaleInc"
Command   ( 0.000s): "  O  DispTrace      0; Trace 1"
Command   ( 0.000s): "  E  Ampl3          TRUE"
Wait      ( 0.000s): abs  100.0ms
Command   ( 0.000s): "  E  Ampl1          FALSE"
Wait      ( 0.000s): abs  100.0ms
Command   ( 0.000s): "  E  Mode           3; Whole Cell"
Wait      ( 0.000s): abs  100.0ms
Switch    ( 0.000s): "Oscilloscope"
Switch    ( 0.000s): "Amplifier"
Wait      ( 0.000s): abs  100.0ms
```

PROTOCOL "bHunt"

This protocol is used for creating a new group in the file
structure in PatchMaster, set the test pulse, set the gain of the
amplifier, calculate the offset potential of the electrode, set the
filter, and call the "shortpulse" protocol. This protocol is used
when the pipette is in the bath. The "shortpulse" protocol is
running during the cell hunting phase, and the recorded current
signals measured with the NI board to calculate the pipette tip
resistance.

```
Command   ( 0.000s): "  @  File           "New Group""
Chain     ( 0.000s): "RESET", return
Command   ( 0.000s): "  E  Reset"
Command   ( 0.000s): "  E  Mode           3; Whole Cell"
Command   ( 0.000s): "  E  PulseAmp            -5.0mV"
```

```
Command    ( 0.000s): "  E   PulseDur                  5.0ms"
Command    ( 0.000s): "  E   CSlow                 100.00pF"
Command    ( 0.000s): "  E   RSeries                20.0MOhm"
Command    ( 0.000s): "  E   Gain                7;  0.5 mV/pA -> medium
range"
Command    ( 0.000s): "  E   AutoZero"
Command    ( 0.000s): "  E   Filter2     7.4kHz"
Command    ( 0.000s): "  E   PulseOn              TRUE"
Command    ( 0.000s): "  E   SaveRpip"
Series     ( 0.000s): "shortpulse","",""
Wait       ( 0.000s): abs  100.0ms
Command    ( 0.000s): "  E   Gain               10;  1.0 mV/pA ->
medium range"
Command    ( 0.000s): "  E   Ampl2          TRUE"
Wait       ( 0.000s): abs  100.0ms
Command    ( 0.000s): "  E   Ampl1          TRUE"
Wait       ( 0.000s): abs  100.0ms
Switch     ( 0.000s): "Oscilloscope"
Switch     ( 0.000s): "Amplifier"
Wait       ( 0.000s): abs  100.0ms
Command    ( 0.000s): "  N   Store          TRUE"

PROTOCOL   "bBreakin"

## This protocol is used for compensating the fast capacitive
transient of the pipette. The protocol is called if the pipette tip
resistance higher than 1 GOhm ie. When the gigaseal is formed.

Chain      ( 0.000s): "RESET", return
Command    ( 0.000s): "  E   Mode                3; Whole Cell"
Command    ( 0.000s): "  E   Gain              10; 5 mV/pA"
Command    ( 0.000s): "  E   AutoCFast"
Command    ( 0.000s): "  E   AutoCFast"
Switch     ( 0.000s): "Amplifier"

PROTOCOL   "aBreakin"

##This protocol is used for the cell capacitance compensation and
for Rs compensation. It is called when the whole cell configuration
is achieved.

Chain      ( 0.000s): "RESET", return
Command    ( 0.000s): "  E   Mode                3; Whole Cell"
Command    ( 0.000s): "  E   Gain              11; 5 mV/pA"
Command    ( 0.000s): "  E   CSlow              10.00pF"
Command    ( 0.000s): "  E   RSeries            20.0MOhm"
Command    ( 0.000s): "  E   AutoCSlow"
Command    ( 0.000s): "  E   AutoCSlow"
Command    ( 0.000s): "  E   Gain              11; 10 mV/pA"
Switch     ( 0.000s): "Amplifier"
Chain      ( 0.000s): "IVCC"
```

```
PROTOCOL   "IVCC"

## This protocol is used for switching the amplifier to current
clamp mode and start the recording. From this point, all the
electrophysiological recordings are automatized in PatchMaster.

Command   ( 0.000s): "  N  Store              TRUE"
Command   ( 0.000s): "  E  Ampl1             FALSE"
IF        ( 0.000s): AD-7 > -100.00p
   Command  ( 0.000s): "  E  Mode              4; C-Clamp"
   Command  ( 0.000s): "  E  IHold               0.0pA"
END_IF
Command   ( 0.000s): "E  Mode              4; C-Clamp"
Command   ( 0.000s): "  O  DispTrace       0; Trace 1"
Command   ( 0.000s): "  O  ResetY"
Command   ( 0.000s): "  O  ResetX"
Command   ( 0.000s): "  O  YScaleInc"
Command   ( 0.000s): "  O  YScaleInc"
Command   ( 0.000s): "  O  YScaleInc"
Wait      ( 0.000s): abs  150.0ms
Value     ( 0.000s): Value-4 =  0.0000
Value     ( 0.000s): Value-3 =  0.0000
SetPgf    ( 0.000s): PgfParam-1 =  0.0000
REPEAT    ( 0.000s): sweeps 0.000s
   Sweep    ( 0.000s): "Long square","",""
   IF       ( 0.000s): Value-3 =  0.0000
     IF       ( 0.000s): Value-1 >  1.0000
        Value    ( 0.000s): Value-4 =  Value-2
        Value    ( 0.000s): Value-3 =  1.0000
     END_IF
   END_IF
   IF       ( 0.000s): Value-1 >  16.000
     BREAK    ( 0.000s): repeat
   END_IF
END_REPEAT
Value     ( 0.000s): Value-4 MUL  1.0000G
SetPgf    ( 0.000s): PgfParam-1 = Value-4
Chain     ( 0.000s): "RHEOBASE"
Command   ( 0.000s): "  E  Mode              3; Whole Cell"
Command   ( 0.000s): "  E  VHold             -70.0mV"
Switch    ( 0.000s): "Amplifier"
```

See more: https://www.heka.com/support/tutorials/tutorials_down/pm_tutorial.pdf (Chapter 14).