

## A simplified electronic circuit for combined single-cell stimulation and recording using loose cell-attached electrodes

Ben W. Strowbridge and R. Todd Pressler, Department of Neurosciences, Case Western Reserve University, Cleveland, Ohio USA

*Correspondence* Dr. Ben W. Strowbridge, Dept. of Neurosciences, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106, (216) 368-6974, Email: [bens@case.edu](mailto:bens@case.edu)

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### 1 **Abstract**

2 While tight-seal patch clamp recordings have found wide use in neuroscience and in other  
3 fields, the requirement to replace the glass pipette after every attempted recording repre-  
4 sents an impediment to high throughput studies such as searching for monosynaptically  
5 connected pairs of neurons. Loose cell-attached recording was introduced in 2000 to circum-  
6 vent this problem since it enabled combined recording and stimulation of visually-identified  
7 neurons without necessitating a tight (gigaohm) seal. Since the stimulus voltages required  
8 to evoke action potentials through low resistance seals are beyond the capacity of most  
9 commercial amplifiers, Barbour and Isope introduced a variation of classic patch clamp  
10 amplifier circuit that is able to deliver stimulus voltages that are effective in triggering ac-  
11 tion potentials under the loose cell-attached patch clamp configuration. The present report  
12 presents the design and operation of a simpler amplifier that contains only two integrated  
13 circuits and is able to effectively stimulate and record action potentials in mitral cells in  
14 rodent olfactory bulb slices. The addition of an accessory analog gating circuit enables man-  
15 ual control of the stimulus voltage with pulse timing controlled by a digital output from a  
16 computer. This system may be useful in studies that require surveying many potential pairs  
17 of neurons for synaptic connections and for sampling and manipulating single-cell activity  
18 in in vivo electrophysiology experiments.

## 19 Introduction

20 Patch clamp methods have had a major impact in cellular neuroscience and have facilitated recordings of activity at both single channel (10) and whole cell (7) recording modes. 21 Because of its high sensitivity and low noise, whole-cell recording has proved especially 22 useful in probing synaptic circuits in acute and cultured brain slices. However there are 23 two primary disadvantages associated with intracellular recordings using the whole-cell 24 recording configuration: dialysis of the intracellular contents of the recorded neuron and 25 the requirement to replace the recording pipette after every recording attempt. In 2000, 26 Barbour and Isope (4) introduced a novel recording configuration for brain slice recording, 27 loose cell-attached recording (LCA), that circumvents both problems and provides a convenient 28 means for both triggering and recording spiking in individual neurons. This method 29 is a variation of traditional cell-attached recording (8, 9, 15, 19) that had been used many 30 groups to monitor neural spiking (e.g., 5, 6, 12). Loose cell-attached recording was developed 31 originally for recording from muscle cells (1, 22). With LCA, the same pipette can be 32 used to record from multiple neurons since the seal resistance required to record spiking is 33 less than needed to detect channel activity. Since there is no whole-cell access with LCA, 34 there is also no intracellular dialysis. The ability to patch multiple neurons using the same 35 pipette is especially useful for locating synaptically-coupled pairs of neurons (5, 16). 36

37 One reason why LCA was not commonly used is that the voltages required to trigger APs 38 (typically 100s of mV) are beyond the capacity of most commonly used patch clamp amplifiers. 39 Blindly stimulating at these voltages without recording the neuronal response is not 40 generally a useful strategy since the effective stimulus intensities can damage the neuron. 41 To be useful, it is necessary to know whether the stimulus applied was effective in triggering 42 an action potential (AP) and then employ the smallest stimulus intensities possible. The 43 primary innovation in the Barbour and Isope (4) study was the introduction of a specialized 44 patch clamp circuit that allowed relatively high voltages to be applied to the membrane 45 patch in the LCA configuration while retaining the ability to detect spiking. With this type 46 of patch clamp amplifier it was often possible to resolve an evoked AP beyond the large 47 stimulus artifact in the recording channel. Even if APs could not be discerned in the on-line 48 recording, evoked spikes could be easily resolved by subtracting the stimulus artifact associated 49 with a failure trial or a just-subthreshold response. The ability to both stimulate and 50 detect APs through LCA recording represented an advance over blindly stimulating through 51 a LCA recording without verification that the stimulus had triggered an AP.

52 While the Barbour and Isope circuit was successful at performing LCA recording, it was 53 moderately complex, containing 8 operational amplifiers (op amps) in separate headstage 54 and remote electronic circuits. The design also included additional features, such as bath 55 ground isolation, that add to circuit complexity. In this report we describe a simpler design

56 that contains only a headstage circuit and requires two op amps. We show this simpler cir-  
57 cuit is effective in both recording and stimulating APs from mitral cells in acute olfactory  
58 bulb slices. The printed circuit board design for the headstage amplifier is freely available  
59 through the GitHub repository (<https://github.com/StrowbridgeLab/LPA>). We also describe an  
60 analog stimulation control device that provides an alternative to specifying stimulus intensi-  
61 ties using software interface controls.

## 62 **Methods**

### 63 **Slice Preparation**

64 Horizontal olfactory bulb slices 300  $\mu\text{m}$  thick were made from ketamine-anesthetized P14-  
65 25 Sprague-Dawley rats of both sexes as previously described (2, 3, 11). Slices were incu-  
66 bated for 30 min at 30°C and then at room temperature until use. All experiments were car-  
67 ried out in accordance with the guidelines approved by the Case Western Reserve University  
68 Animal Care and Use Committee. Slices were placed in a recording chamber and superfused  
69 with oxygenated artificial cerebrospinal fluid (ACSF) at a rate of 1.5 ml/min. Recordings  
70 were made between 29-32°C. ACSF consisted of (in mM): 124 NaCl, 3 KCl, 1.23  $\text{NaH}_2\text{PO}_4$ ,  
71 1.2  $\text{MgSO}_4$ , 26  $\text{NaHCO}_3$ , 10 dextrose, 2.5  $\text{CaCl}_2$ , equilibrated with 95%  $\text{O}_2$ / 5%  $\text{CO}_2$ . All  
72 drugs were added to the submerged recording chamber by changing the external solution  
73 source.

### 74 **Whole-Cell Recordings**

75 All whole-cell patch-clamp recordings were made with Axopatch 1C or 1D amplifiers (Axon  
76 Instruments) using borosilicate glass pipettes (TW150F-4, WPI) of impedances ranging from  
77 2-5 mOhm $\times$  pulled on a P-97 pipette puller (Sutter Instruments). Under whole-cell (WC)  
78 current-clamp conditions, recording electrodes contained (in mM): 140 K-methylsulfate (MP  
79 Biochemicals), 4 NaCl, 10 HEPES, 0.2 EGTA, 4 MgATP, 0.3  $\text{Na}_3\text{GTP}$ , 10 phosphocreatine  
80 (pH 7.3 and  $\sim$ 290 mOsm). Whole-cell recordings were low-pass filtered at 5 kHz (FLA-01,  
81 Cygus Technology) and digitized at 10-40 kHz using an ITC-18 simultaneously-sampling  
82 data acquisition interface using custom software written in Visual Basic (Microsoft). While  
83 the most rapid acquisition rates were not required to sample the low-pass filtered intracel-  
84 lular AP response, faster acquisition speeds were useful for generating the rapid TTL output  
85 pulses (25 - 50  $\mu\text{s}$  duration) for controlling the analog switch in the LCA stimulation circuit  
86 (see Results below for details).

## 87 **DIC Imaging**

88 Slices were imaged using infrared differential interference contrast (IR-DIC) optics on Zeiss  
89 Axioskop FS1 or Olympus BX51WI upright microscopes. Transmitted light was restricted  
90 to 710-790 nm using a band-pass interference filter placed above the microscope field stop.  
91 DIC images were captured using a frame-transfer CCD camera (Cohu) and displayed on a  
92 high-resolution monochrome analog monitor (Sony). Individual neurons were visualized us-  
93 ing IR-DIC video microscopy before attempting either WC or LCA recording. Neuronal cell  
94 type was determined based on IR-DIC morphology and soma laminar location.

## 95 **Suppliers**

96 Printed circuit board: Bay Area Circuits, Fremont CA (<https://bayareacircuits.com/>).  
97 Printed circuit design software: DipTrace (<http://www.diptrace.com/>). Electronic parts  
98 and Pomana cases: Digikey, Thief River Falls, MN (<https://www.digikey.com/>). Electrode  
99 holder: A-M Systems, Carlsborg, WA (<https://www.a-msystems.com/>). Electrode glass:  
100 World Precision Instruments, Sarasota, FL (<https://www.wpiinc.com/>). Electronic filter:  
101 Cygnus Technology, Delaware Water Gap, PA (<http://www.cygnustech.com/>).

## 102 **Results**

### 103 **Headstage amplifier circuit**

104 The core circuit we employed is shown in Fig. 1A and is designed to record APs through  
105 the loose patch clamp recording configuration. The headstage circuit (Fig. 1A) was adapted  
106 from reference 4. Both the first-stage FET input op amp (U1, OPA604, Burr Brown/TI) and  
107 the differential output amplifier (U2, INA106, Burr Brown/TI) were mounted on a custom  
108 printed circuit board manufactured by Bay Area Circuits and enclosed within a small alu-  
109 minum box connected to the circuit ground. The circuit ground was connected to the same  
110 silver chloride-coated silver bath electrode as the whole cell amplifier. A 20 M $\Omega$  feedback  
111 resistor (Rf; Slim-Mox; Ohmite) set the transimpedance gain. While many high gain tran-  
112 simpedance amplifiers include a small capacitor across the feedback resistor to minimize  
113 gain peaking, we found that eliminating this extra 1-2 pF capacitance improved our abil-  
114 ity to recognize evoked APs even without artifact subtraction. (Holes for a small capacitor  
115 across Rf are included in the printed circuit board layout but were not populated.) The  
116  $\pm 15$  V power to each op amp was bypassed to ground using 0.1  $\mu$ F ceramic capacitors  
117 (not included in the schematic diagram in Fig. 1 but included in the printed circuit board).  
118 Through the combination of the input stage (OPA604 with Rf) and the 10x differential  
119 amplifier (INA106), the headstage output voltage reflects a scaling of 5 nA/V.

120 The patch clamp electrode was connected through a removable polycarbonate holder  
121 with a 2 mm pin (A-M Systems #672443). A standard 2 mm female test pin jack (Digikey  
122 #J117-ND) was mounted on the Pomona case. The tight fit between the test pin and jack  
123 provided sufficient mechanical stability for repeated loose patch recordings provided the  
124 electrode holder was seated firmly on the jack. The voltage command input to the head-  
125 stage circuit was connected via a RG174 cable. The output of the circuit was connected to  
126 another RG174 coaxial cable after passing through a 50  $\Omega$  impedance-matching resistor.  
127 Both input and output RG174 cables were soldered directly to the headstage printed cir-  
128 cuit board with male BNC jacks mounted on the other end of the cables. Design files for the  
129 headstage printed circuit board are available at the StrowbridgeLab repository on GitHub  
130 (<https://github.com/StrowbridgeLab/LPA>).

131 While the printed circuit board is small enough to fit inside a miniature Pomona box  
132 (Pomona #2400 or #2428), in practice it was more convenient to mount the headstage cir-  
133 cuit board inside a longer box (Pomona #5255) so that the electrode input test pin jack  
134 did not have to be placed directly above the board. The longer enclosure also enabled the  
135 electrode holders to be located at approximately the same position on the end faces of both  
136 the AxoPatch 1D and LCA amplifier headstage boxes, facilitating experiments in which neu-  
137 rons initially recorded using the LCA headstage are subsequently re-patched to establish  
138 a whole-cell recording configuration. The headstage box was mounted on a motorized 3-  
139 dimensional manipulator (461XYZ, Newport) controlled by custom servo power amplifiers.  
140 Custom software enabled a precision computer gaming joystick (Extreme 3D Pro, Logitech)  
141 to move the electrode to form the loose patch recording configuration under IR-DIC visu-  
142 alization (typically through a Zeiss 63x water objective and imaged with a 0.5 inch CCD  
143 camera onto an analog monochrome monitor). The headstage amplifier was powered by  $\pm$   
144 15 volt supplies using a regulated linear triple output power supply module (HBAA-40W-A,  
145 Power-One). The positive 5 volt output line was not used in the headstage circuit but is re-  
146 quired for the accessory analog gating module described below.

## 147 **Circuit operation**

148 The headstage circuit can be operated in three different voltage-clamp modes, two of which  
149 require only an additional low-pass electronic filter and data acquisition interface to form  
150 a complete system. For recording-only applications (*Mode A* in Fig. 1B), the voltage com-  
151 mand input to the headstage circuit is grounded and the output signal is digitized after pass-  
152 ing through a Bessel low-pass filter (FLA-01, Cygnus Technologies). The circuit ground is  
153 connected to the bath ground wire. An example of this recording mode is shown in Fig. 2  
154 from an experiment in which one olfactory bulb mitral cell was simultaneously recorded un-  
155 der the whole-cell (with an AxoPatch 1D amplifier) and loose patch configuration. The neu-

156 ron was stimulated by injecting a depolarizing current step through the whole cell electrode  
157 and the resulting train of APs were easily identified in both whole-cell and LCA amplifier  
158 outputs. Since the LCA amplifier was not used to stimulate the neuron in this experiment,  
159 no artifact subtraction was required in the software routines used to display the LCA output.  
160 The LCA recording pipette contained 124 mM NaCl, 3 mM KCl and 10 mM HEPES (pH 7.4).  
161 Continuous suction (0.25 - 0.4 PSI; 1700 - 2800 Pa) was applied to the pipette throughout  
162 the experiment. Stronger suction was applied when first acquiring the LCA recording con-  
163 figuration. The electrode geometry was similar to those used for conventional whole-cell  
164 recording from small interneurons.

165 The other two recording modes allow for simultaneous recording and stimulation of the  
166 recorded neuron through the LCA. While it is possible to directly control the stimulus volt-  
167 age applied to the loose patch using the DAC output of the data acquisition interface (*Mode*  
168 *B* in Fig. 1B), this approach requires frequent interaction with a graphical user interface  
169 to adjust the stimulus intensity throughout each experiment. In practice, using an analog  
170 switch and a 10-turn potentiometer to control the stimulus voltage was preferable (*Mode C*  
171 in Fig. 1B). In this mode, a digital output line from the data acquisition interface controlled  
172 a single pole, double throw analog switch (HI-5043, Intersil). The output of the analog  
173 switch was buffered using a low noise op amp (OP27, Analog Devices) and connected to  
174 the headstage input through a coaxial cable. Since the OP27 amplifier is operated in an in-  
175 verting mode, a negative voltage is applied from the analog switch to generate a positive  
176 stimulus at the loose patch. Depending on stimulus voltage required, a fixed resistor can be  
177 connected in series with the potentiometer resistor to decrease the sensitivity. Adding a 33  
178 k $\Omega$  resistor in series with a 5 k $\Omega$  10-turn potentiometer decreases the sensitivity from 1.5  
179 V/turn to 133 mV/turn with a 15 V supply. This stimulation gain setting for *Mode C* LCA  
180 recordings gave satisfactory results with typical experiments requiring 200 - 500 mV pulses  
181 (50  $\mu$ s duration). While shorter stimulus pulses (25  $\mu$ s duration) provided better separa-  
182 tion between the artifact and evoked AP, the stimulus intensities required to reliably trigger  
183 APs were more variable than with slightly longer pulses; 50  $\mu$ s duration pulses provided  
184 a good compromise in our experiments. The TTL output lines on the the data acquisition  
185 device used in these experiments (ITC-18, Instrutech/Heka/Harvard Bioscience) were opti-  
186 cally isolated. If another, non-isolated data acquisition device is employed optical isolation  
187 should be added to the analog switch circuit. The stimulus control circuit was assembled on  
188 a generic prototyping printed circuit board and powered by the same triple output linear  
189 power supply module as the headstage amplifier.

190 In Figure 2, the same olfactory bulb mitral cell was simultaneously recorded under  
191 whole-cell conditions (with an AxoPatch 1D amplifier) and the custom LCA amplifier operat-  
192 ing in *Mode A*. A series of APs were evoked by injecting a depolarizing current step through  
193 the WC electrode. The resulting spikes are evident in both WC and LCA amplifier outputs.



194 Examples of combined recording and stimulation through the LCA *Mode C* system is shown  
195 in Fig. 2C-D and Fig. 3. Without artifact subtraction, the triggered AP was often detectable  
196 by a slight prolongation of the response. However, in many experiments, evoked APs were  
197 not evident until a failure trial that did not trigger an AP (or slightly subthreshold response)  
198 was subtracted from each response. Figure 2D illustrates peristimulus threshold responses (a  
199 failure and a success evoked by the same stimulus and acquired in the same episode) with  
200 artifact subtraction. Automatic artifact subtraction was implemented using custom Python  
201 software routines in which a specific stimulus response could be identified using a mov-  
202 able cursor and stored in a memory buffer. Dual recording/stimulation LCA experiments  
203 used the same conditions as recording-only experiments (saline-filled pipettes). A potential  
204 complication when performing artifact subtraction is saturation of the LCA output signal,  
205 resulting in distorted artifact responses. While the FLA-01 used to low-pass filter the head-  
206 stage output can provide additional gain, we typically used the filter at either the unity gain  
207 setting or minimal additional gain (e.g., 2X) to prevent saturation at  $\pm 10$  V full-scale ADC  
208 input range using with the ITC-18 acquisition device.

209 Loose patch clamp recording is ideally suited for testing multiple potential presynap-  
210 tic neurons that could be synaptically coupled with a postsynaptic target neuron (5, 16).  
211 In these experiments, the postsynaptic neuron is typically recorded intracellularly in ei-  
212 ther voltage- or current-clamp modes to facilitate detection of small postsynaptic responses.  
213 An example of a combined LCA/WC paired recording is shown in Fig. 3A-B. In this experi-  
214 ment using olfactory bulb slices, a presynaptic mitral cell is recorded and stimulated using  
215 the LCA amplifier in *Mode C* while simultaneously recording from a monosynaptically-  
216 coupled granule cell in the current-clamp configuration using an AxoPatch 1D amplifier. At  
217 perithreshold LCA stimulus intensities, subthreshold trials that failed to trigger an AP also  
218 failed to trigger an excitatory postsynaptic potential (EPSP). Each AP triggered evoked in  
219 the mitral cell triggered an EPSP in the granule cell at approximately the same latency (1.8 -  
220 2 ms) in this example recording.

## 221 Discussion

222 In this report, we detail the design and operation of a relatively simple amplifier circuit  
223 that enables combined recording and stimulation under the loose patch clamp recording  
224 configuration. Loose patch stimulation represents a simple method for stimulating one neu-  
225 ron without activating nearby neurons or axons. Loose patch clamp recordings also do not  
226 dialyze the intracellular contents of the recorded neuron, providing another advantage  
227 over conventional whole-cell recordings. While the relatively large (100s of mV) voltages  
228 required to reliably trigger APs is beyond the capacity of most commercial patch clamp am-  
229 plifiers designed for whole-cell or single-channel recordings, the home-built amplifier we

230 employed uses only two integrated circuits and is inexpensive to construct. The electronic  
231 components required for the headstage circuit can be purchased for less than \$25 and all  
232 the design files required to have printed circuit boards manufactured are available on the  
233 GitHub repository. The availability of a simple but workable design for a LCA amplifier that  
234 can be used to stimulate APs in individual neurons may be helpful in studies aimed at iden-  
235 tifying monosynaptically-coupled neuronal pairs in cell cultures or brain slices. This method  
236 could also be applied in vivo to assay the effect circuit and/or behavioral effects of defined  
237 manipulations to individual neurons (e.g., 17).

238 Our circuit design represents an adaptation of a circuit presented in a previous report (4)  
239 and is based on classic patch clamp amplifier designs (10, 21). In the previous LCA report  
240 (4), additional circuitry was employed to transmit both the current signal and command  
241 voltage with low noise from a remote electronics box to the headstage circuit. While we did  
242 not compare the operation of both circuits, we demonstrate that our simpler design had suf-  
243 ficient signal-to-noise ratio to detect APs in olfactory bulb mitral cells and therefore is likely  
244 to be useful in other neurons with large APs such as hippocampal and neocortical pyramidal  
245 cells. The present amplifier also did not include circuit elements that were present in the  
246 previous Barbour and Isope design that prevented input signal saturation and that isolated  
247 the bath ground by passing the reference electrode through a high impedance op amp input.  
248 These three non-essential circuit subsystems (paired transmission lines, anti-saturation con-  
249 trol and bath ground isolation) accounted for the majority of the components (6/8 op amps)  
250 in the previous design. The need for anti-saturation circuitry is reduced in our design be-  
251 cause of the lower feedback resistance used in the transimpedance input amplifier (20 M $\Omega$   
252 instead of 50 M $\Omega$ ).

253 Searching for monosynaptically-coupled pairs of neurons in brain slices is a common  
254 application of patch clamp recording (e.g., 11, 13, 14, 16, 18, 20). With whole-cell record-  
255 ings, glass electrodes typically cannot be re-used after a recording attempt because of the  
256 difficulty in achieving a second gigaohm seal once the positive pressure applied to the elec-  
257 trode is released. The inability to re-use patch clamp electrodes represents a significant  
258 hurdle during large-scale studies that employ paired recordings since at least one electrode  
259 must be removed for every connection tested. The ability to re-use electrodes is one of  
260 the primary advantages of LCA recording. With loose patch clamp recording, multiple po-  
261 tential presynaptic neurons can be tested more rapidly while maintaining a conventional  
262 whole-cell intracellular recording on the postsynaptic neuron. In our experience using  
263 rodent olfactory bulb slices, not having to replace the presynaptic recording electrode af-  
264 ter every attempted paired recording attempt increased throughput by approximately 4  
265 times. The primary trade-off with this approach is the inability to visualize the presynap-  
266 tic neuron without re-patching it to achieve a whole-cell recording configuration. Another  
267 trade-off of LCA recordings is the variable threshold voltage required to trigger APs. By



268 periodically monitoring the stimulus voltage required to trigger spikes, we have main-  
269 tained monosynaptically-coupled paired recordings for up to 50 minutes (using LCA for the  
270 presynaptic neuron and whole-cell recording for the postsynaptic neuron).

271 A limitation with loose patch stimulation using the present circuit is that for some ex-  
272 periments, a failure trial needs to be acquired and subtracted from each record to reveal  
273 stimulus-evoked APs. In many of recordings, differences in the raw current trace are suffi-  
274 cient to enable the operator to determine if an AP was triggered (see Fig. 3B). In some ex-  
275 periments, off-line artifact subtraction was required to unambiguously determine when stim-  
276 uli were effective in triggering APs. In principle, artifact subtraction could be automated by  
277 using a second analog switch (and a second TTL output) that reverses the pulse stimulus po-  
278 larity. In this approach, the acquisition software could apply a stimulus pulse with the same  
279 amplitude but opposite polarity at the end of each episode, then automatically display the  
280 artifact-subtracted current waveform.

281 The threshold voltage required to trigger an AP tended to vary throughout most exper-  
282 iments. Since applying large stimulus voltages through the LCA amplifier can degrade the  
283 health of the patched neuron, it was helpful to continuously track the threshold stimulus  
284 voltage and employ just-suprathreshold intensity stimuli. Even when adjusting the stimu-  
285 lus voltage to be just-suprathreshold, we occasionally triggered spontaneous spiking in the  
286 recorded neuron. In most cases, pausing the stimulation for several minutes abolished the  
287 spontaneous spiking.

288 One possible method to reduce the work required to manually track the threshold stim-  
289 ulus is to apply a weak second voltage pulse to monitor seal resistance. This could be  
290 implemented using a second analog switch connected to the same virtual ground summing  
291 point on the inverting input to U3. By monitoring seal resistance, one could determine  
292 if the change in threshold stimulus voltage required to trigger APs could be predicted by  
293 changes in seal resistance. If changes in seal resistance explained the variation in the stimu-  
294 lus voltage required to triggered APs, the acquisition software could provide a continuously  
295 updated estimate of the threshold stimulus required. Alternatively, the acquisition software  
296 could directly control the stimulus intensity using a digital-to-analog converter or a digital  
297 potentiometer.

## 298 Figure Legends

299 **Figure 1: Loose cell-attached amplifier circuit diagrams** A, Diagram of the core head-  
300 stage amplifier used for LCA recording and stimulation experiments. The input to the  
301 amplifier is connected to polycarbonate electrode holder (red). The headstage circuitry is  
302 implemented on a custom printed circuit board and mounted inside a small Pomona alu-  
303 minum box attached to a micromanipulator. The input and output of headstage circuit are  
304 connected via thin coaxial cables (RG174) soldered directly to the printed circuit board.  
305 All resistors are standard 1% 0.25 watt values. The circuit also included 0.1  $\mu$ F ceramic  
306 bypass capacitors on the  $\pm 15$  V power supply lines to each op amp (not included in the cir-  
307 cuit diagram in A). B, Diagrams of different recording modes possible using the headstage  
308 circuit shown in A. In *Mode A*, the command input is grounded, allowing a recording-only  
309 configuration. The headstage output is typically amplified by 1-10X and low-pass filtered  
310 at 10-20 kHz using a Cygnus Technology FLA-01 filter. In the experiments presented here,  
311 the ADC + DAC data acquisition system was an Instrutech ITC-18. In *Mode B*, The DAC out-  
312 put of the data acquisition system is used to generate the command signal to the headstage  
313 circuit. In *Mode C*, the data acquisition system only provides a timing signal for the stimu-  
314 lation pulses. Stimulus intensity was controlled by a 10-turn potentiometer connected to an  
315 analog switch (U4) and buffered by a low noise op amp (U3). Buffering the command input  
316 using this circuit enables additional command inputs (not shown) to be combined into the  
317 voltage signal driving the headstage amplifier.

318 **Figure 2: Simultaneous intracellular and loose cell-attached recordings** A, Diagram  
319 of the *Mode A* recording configuration. B, Simultaneous whole-cell (black trace) and LCA  
320 (blue trace) recordings of the response of a mitral cell (MC) to a depolarizing current step  
321 injected through the whole-cell (WC) recording electrode. Inset shows an enlargement of  
322 the first two APs recorded in the LCA system. C, Diagram of the *Mode C* recording confi-  
323 guration. D1-D2, Examples of simultaneous intracellular (black traces) and LCA recordings  
324 (blue traces) of responses in the mitral cell to brief (50  $\mu$ s duration) voltage pulses applied  
325 through the LCA pipette. The LCA command voltage was set to the perithreshold intensity  
326 so that the same stimulus elicited both single APs and failures. (The middle response in D1  
327 and the first response in D2 were failures.) The stimulus artifact (recorded from a single fail-  
328 ure trial) were subtracted from each response.

329 **Figure 3: Paired recording between olfactory bulb mitral and granule cells** A, Diagram  
330 of recording configuration with a *Mode C* recording from the mitral cell (black traces) and  
331 an intracellular (whole-cell current clamp) recording of the granule cell (red trace). Four  
332 voltage pulses were applied through the LCA electrode at perithreshold intensity which trig-  
333 gered three APs in the MC (the response to the third pulse was a failure). Each of the MC  
334 APs triggered an EPSP response in the granule at approximately the same latency (1.8 - 2

335 ms). LCA recording from the MC shown both with artifact subtraction (top black trace) and  
336 without artifact subtraction (bottom black trace).

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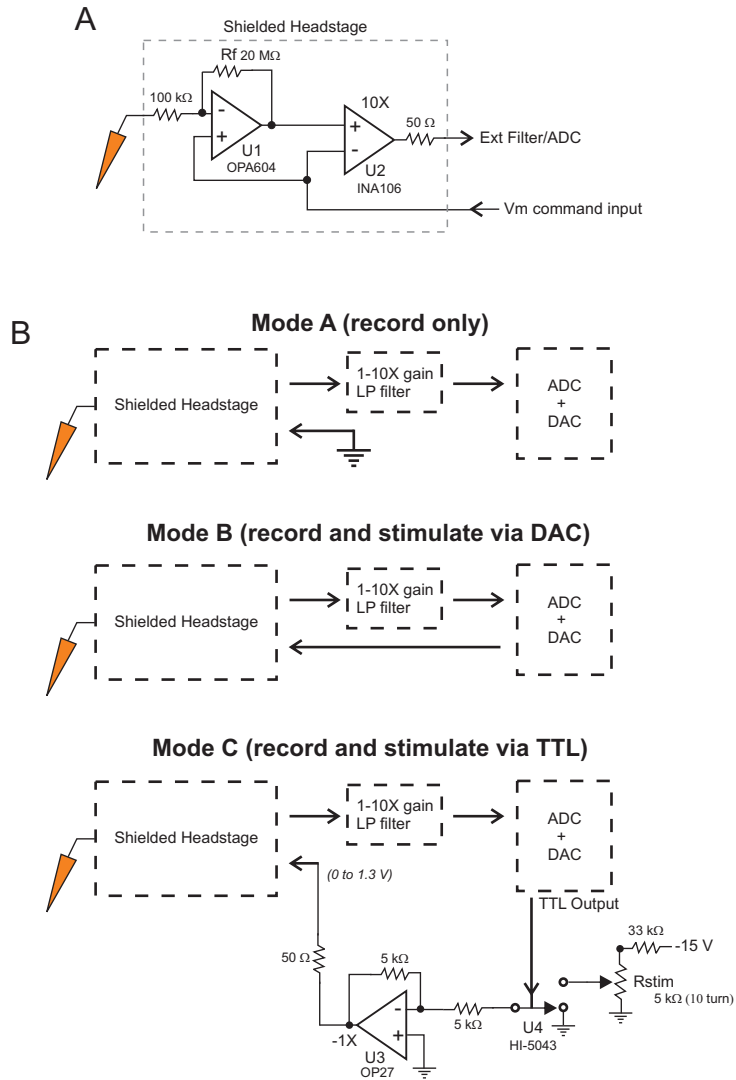


Figure 1 17 Sept 2017 2:00 PM



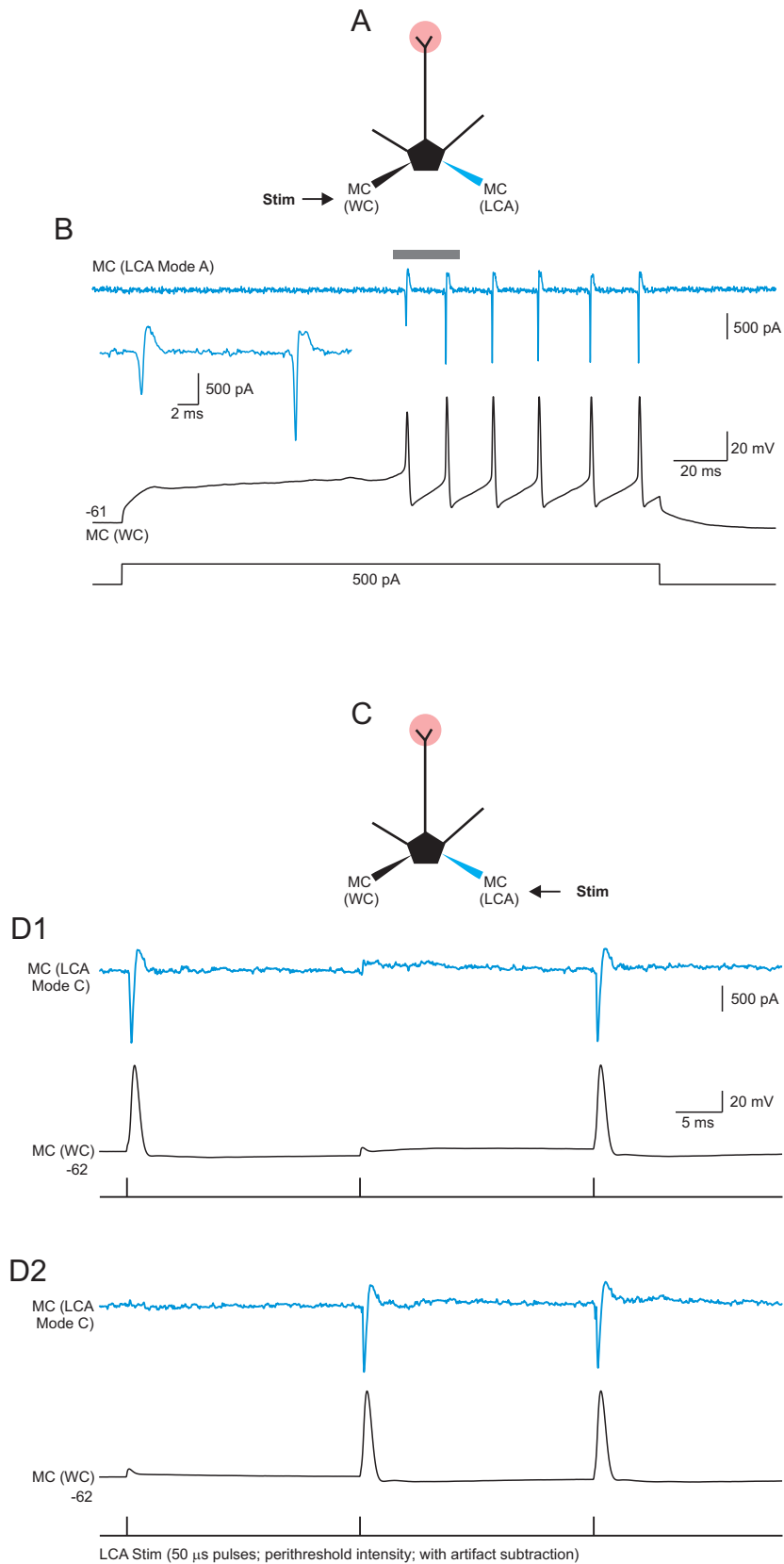


Figure 2

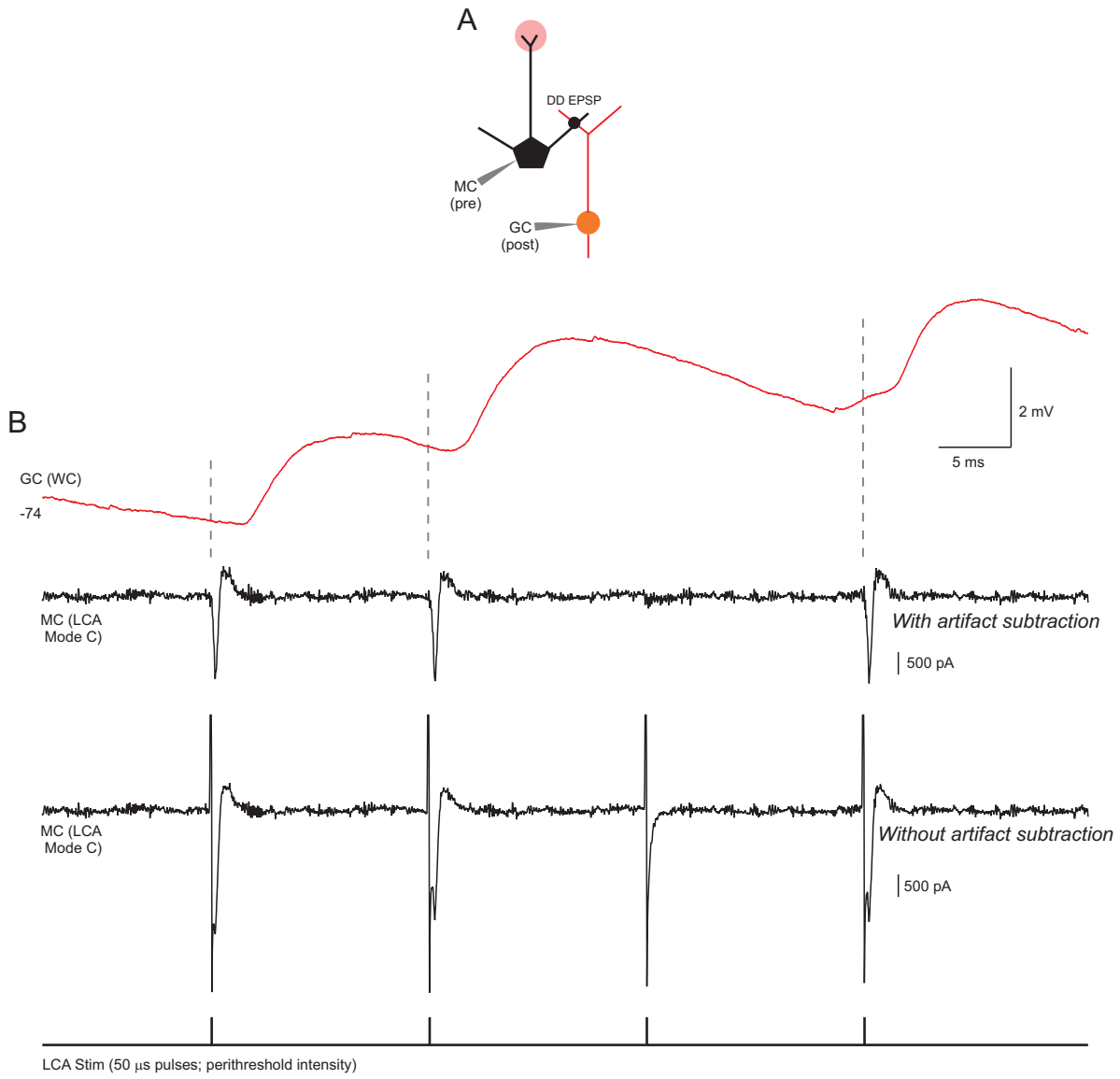


Figure 3