PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2008, 35(4): 465~470
www.pibb.ac.cn

Design of Voltage-clamp-controlled Current-clamp*

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Abstract Previous research revealed that distortion is detected in transient voltage signal recorded with traditional patch clamp amplifier under current clamp mode, which is essentially resulted by electronic design of the headstage of the patch clamp. A new kind of headstage is designed to modify the defect, the circuit of which not only measures the transient potentials as the classical voltage follower does but also is quite suitable for the standard voltage-clamp mode. Furthermore, the technique of voltage-clamp-controlled current-clamp is applied for modifying the conventional patch-clamp amplifier, the variable low-pass filter is added into the circuit to reduce the response speed of voltage-clamp module, thus the transient potentials changes can be measured while membrane potential is kept at a constant value. Bridge balance circuitry is designed to eliminate the voltage drop while the variable current injected into the electrode. And fast capacitance compensation stage of conventional PCA is modified to neutralize the capacitance and accelerate system response speed for current-clamp mode. The experimental results on cell model demonstrate that modified PCA meets the requirement of monitoring transient potential changes in electrophysiology research.

Key words patch-clamp amplifier, current-clamp, voltage-clamp-controlled current-clamp, low-pass filter, bridge balance, capacitance compensation

The patch-clamp technique is a fundamental and powerful method for studying the electrophysiological properties of cell membranes. Conventional patch-clamp amplifiers (PCAs) are applied to record not only the ion channel currents in voltage-clamp (VC) mode but also the membrane potential in current-clamp (CC) mode [1, 2]. Investigations of the membrane potential events in different excitable cell systems have been carried out in the previous researches[3, 4]. However, the experimental results demonstrated that fast transient potential signals, such as action potentials (APs) and excitatory postsynaptics potentials (EPSPs), would be remarkably distorted when recording with the conventional PCAs in CC mode[5].

The generation of the error current and distortion of membrane potentials by PCAs in CC mode is the consequence of the function principles and electronic design of the headstage[5, 6]. In the conventional PCAs, the CC mode (Figure 1) is accomplished by employing a feedback between the current monitor signal and the electrode potential. The feedback acts rapidly to keep the current at zero by varying the electrode potential appropriately. In this way, the high input impedance voltage measuring amplifier is accomplished. However, the input impedance is only improved to a limited extent and an error current is absorbed by the PCA in the CC mode. The error current flow through the PCAs alters the recorded voltage signals in two ways: a voltage drop across the series resistance and by altering the amount of the current charging the membrane capacitance[5]. Moreover, it is impossible to obtain actual voltage signals by performing the off-line corrections of distorted signals, because the related parameters adopted in off-line correction could not actually reflect the practical conditions of the cell membrane during the recording[7].

Therefore, the best way is to modify the circuit of the PCA for CC recording in order to overcome the problems and the limitations. Peters *et al*.[8] designed a low frequency voltage clamp (lfVC) system that modifies the conventional current-clamp mode of PCA for recording the transient potential events while the average membrane potential is kept constant. Later, Sutor *et al*. [9] presented an extended technique which named "voltage-clamp-controlled current-clamp" (VCcCC) and implemented it using a discontinuous

*This work was supported by a grant from The National Natural Science Foundation of China (30327001).
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Received: July 31, 2007 Accepted: October 25, 2007

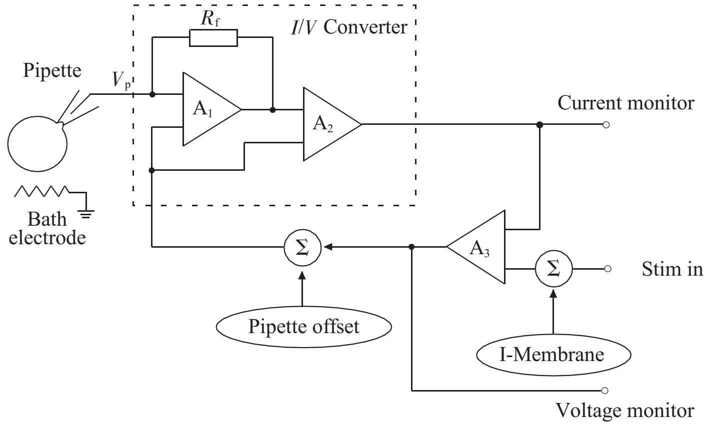

Fig. 1　Schematic of conventional patch-clamp amplifiers in CC mode

single-electrode voltage-clamp amplifier. In this paper, we redesign a new headstage to overcome the above mentioned limitations of the headstage of PCAs in CC mode. Moreover, the VCcCC technique is implemented to modify the electronic circuit of the conventional PCA. The improved PCA can be used to record the transient potential changes accurately at the preset membrane potentials.

1　Methods

1.1　Improvement of headstage

The essence of the voltage follower is a unity-gain buffer amplifier which has an input resistance many orders of magnitude greater than the resistance of electrode and cell, so that the output of it reflects the voltage at the tip of the electrode exactly and the amplifier draws no current from the cell under ideal conditions. Thus, voltage follower has been considered as the first selection for CC recordings, and the PCA working as a voltage follower in CC mode should be regarded as the optimum scheme. That's why the principle and design of the voltage follower is introduced into the modification of the conventional PCAs [5].

Figure 2 shows the schematic diagram of the new headstage for CC mode. The feedback between operation amplifiers (OAs) A1 and A2 makes the headstage for VCcCC mode has a very large input resistance as voltage follower (Figure 2a). The OA A3 and the feedback resistor (R_f in Figure 2a) are used to make a high quality current source for generating and injecting the command current, which is the essence difference between the new headstage and the conventional PCAs headstage. The input of A3 is the sum of the electrode potential V_p and the command voltage V_{cmd}, so the voltage drop across the feedback resistor R_f is equal to the V_{cmd} regardless of V_p, and the corresponding current across the R_f is given exactly by $I = V_{cmd}/R_f$. The closed-loop control system circuit is maintained stable under all conditions and the frequency response characteristic would not be influenced when the feedback factor k is set at 10.

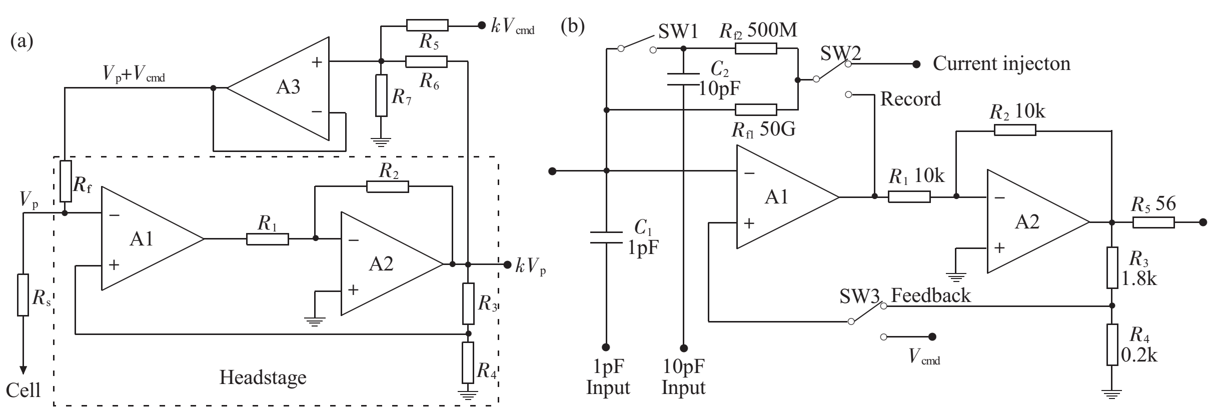

Fig. 2　The new headstage proposed for current-clamp mode

(a) The schematic diagram of the new headstage. The feedback between operational amplifiers (OAs) A1 and A2 is of the voltage-series type. A3 is implemented for current injection. See text for details. (b) Circuit of the new headstage for voltage-clamp mode and voltage-clamp-controlled current-clamp mode. Analog switches SW2 and SW3 are used to set the modes. SW1 is used to set the range of feedback resistors.

The actual circuit of new headstage of PCAs is shown in Figure 2b. The switching between VC mode and VCcCC mode is accomplished by analog switches SW2 and SW3. When the PCA works in VC mode (SW2 is switched to Record while SW3 to V_{com}), the headstage circuit retains the same elements as conventional headstage and the function or performance of the headstage in VC mode wouldn't be influenced. As a consequence, the new headstage is suitable for accurately recording the transient voltage signals in current-clamp mode and is perfectly compatible with the classic VC mode.

1.2 Principle of VCcCC

The equivalent diagram of the modified PCA system in VCcCC mode is as shown in Figure 3. The membrane potential V_m will be obtained, after the output signal V_p of the headstage is corrected by capacitance compensation unit (compensating the capacitance of the headstage and electrode), R_s compensation unit (correcting the voltage drop across the series resistor) and offset adjustment unit (correcting the offset of the headstage). The membrane potential V_m and the holding voltage V_{hold} are fed into a difference amplifier (A5), and the resulting error signal e is amplified by the proportional controller unit. The output signal y is fed into the current injection unit via the variable low-pass filter unit after being summed with the input command current signal I_{cmd}.

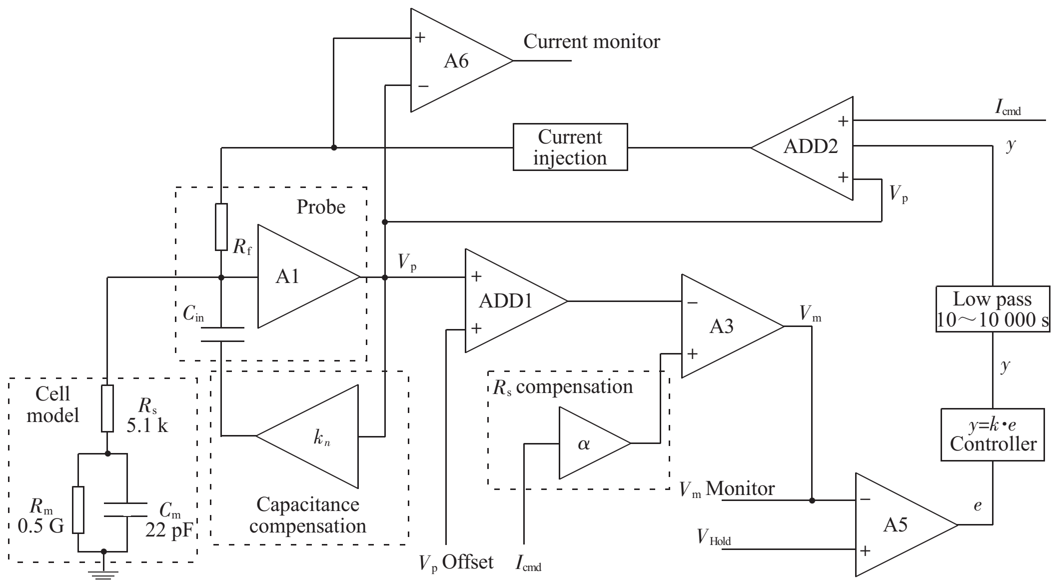

Fig. 3 Schematic diagram of modified patch-clamp amplifier for VCcCC mode recording

1.3 Low pass filter

The low-pass filter is added into the closed loop control system to remove the transient changes of the membrane potentials and to reduce response speed of the VC module. If membrane potential V_m has slow potential changes, the low frequency component of signal y can pass through the low-pass filter and negative feedback to keep V_m at V_{hold}. On the contrary, the high frequency portion of signal y couldn't pass the low pass filter when the V_m has transient potential changes, thus the membrane potential V_m would not be influenced. In this way, the modified amplifier thus performs recording transient voltage changes while holding the membrane potential at a constant value.

In the VCcCC mode, only signals below the preset frequency can be voltage-clamped and the response time of the system must be at least 100 times slower than the frequency response of the signals of interests [9]. To meet the requirement of monitoring membrane potential under different conditions, the time constant of the low-pass filter is selectable. The low-pass filter consists of a 1 000 μF capacitor and a series of selectable resistors of four possible values: 10 kΩ, 100 kΩ, 1 MΩ and 10 MΩ, resulting in the system time constants between 10 s, 100 s, 1 ks and 10 ks. The time constant of the low-pass filter is selected by the combination of two analog switches.

1.4 R_s compensation

Experimental evidences had demonstrated that the series resistance (R_s) can distort the membrane potential signals by causing a significant voltage drop [5, 7]. The variable current injected into the headstage causes a corresponding voltage drop across R_s, so the output of the headstage is:

$$V_p = V_m + I_{cmd} \cdot R_s \quad (1)$$

In order to separate the membrane potential V_m from the output potential V_p of the headstage, a subtraction technique named as "Bridge Balance Technique" is used in our circuit. The essence of the technology is to generate a signal that is proportional to the product of the electrode current and series resistance, and then the actual membrane potential is isolated by subtracting the signal from V_p. The circuit in Figure 4 is used to generate the signal and then eliminate it. The multiplying digital-to-analog converters (mDACs) DA1 and DA2 are used to set the proportional factor of R_s compensation as variable resistances[10]. The membrane potential V_m is given by:

$$V_m = V_p + V_{posset} - \frac{R_{DA2}}{R_{DA1}} V_{cmd} \quad (2)$$

Where command voltage $V_{cmd} = I_{cmd} \cdot R_f$.

The commonly used method is to apply repetitive pulses of current to the electrode when it is immersed in the bath. The control codes of DA1 and DA2 in R_s compensation stage are adjusted until the steady-state pulse response is completely eliminated. The value of series resistance can be calculated by the parameters of the compensation circuit. Special attention should be given to that R_s compensation stage can work only under the condition that the series resistance is constant[11].

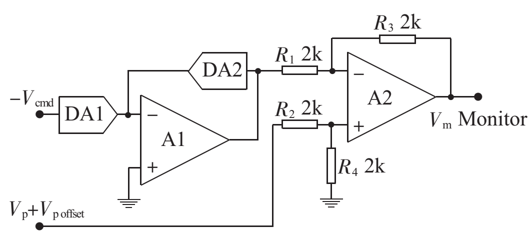

Fig. 4 Detailed Schematic diagram of series resistance compensation

Multiplying digital-to-analog converters DA1 and DA2 are used to set the proportional factor of series resistance compensation stage. Difference amplifier A2 is used to subtract the scaled fraction of the current from V_p.

1.5 Capacitance compensation

The high frequency performance and bandwidth of the amplifier is limited by the presence of the input capacitance of headstage and the stray capacitance of the electrode. The capacitance and the series resistance form a low pass filter, so the high frequency portion of signal is attenuated when the step stimulus signal is injected into the headstage. The special electrical circuit named "capacitance compensation stage" is added to neutralize the capacitance. We modified the C-fast compensation circuit of VC mode, because the principle of the capacitance compensation in VCcCC mode is the same as the C-fast compensation in VC mode. The essence difference of the two modes is that the input signal of compensation circuit is clamped voltage V_c in VC mode while it is the output of the headstage in VCcCC mode. When compensation current injected through the injection capacitor is exactly equal to the current that passes through the input capacitance of headstage and the stray capacitance of the electrode, the influence of the capacitor will be completely eliminated.

The headstage and capacitance compensation stage will form a closed loop control system, when the output of headstage used as the input of capacitance compensation stage and being injected to the electrode. Therefore, the circuit will oscillate if the gain of the capacitance compensation stage exceeds a certain setting. It is recommended to adjust C-fast compensation stage to neutralize all capacitance in the VC mode first, and then adjust the value of the compensation 0.5 pF lower; and finally switch to VCcCC mode (HEKA elektronik GmbH. EPC10 Patch-clamp amplifier user's manual 8.6. 65-65).

2 Results and discussion

A conventional PCA PC-2C (InBio life science instruments, China) [12] was modified to perform intracellular recording in VCcCC mode. In order to estimate the performance of the modified PCA in VCcCC mode, we performed a series of tests on a cell model (CM1, InBio life science instrument, China).

Figure 5 shows the process of the capacitance compensation. A repetitive pulse (2 kHz, $V_{p\text{-}p}$ = 20 mV) was injected into the Cell Model, and then the response was acquired at the V_m monitor. As shown in Figure 5a, the high frequency portion of signal is attenuated when the step signal passes through due to the presence of stray capacitance of electrode and input capacitance of the headstage. After exact compensation the system response is accelerated remarkably (Figure 5b). The current injected into the input of headstage caused the output signal to overshoot when the gain of capacitance compensation stage exceeds the optimum setting (Figure 5c).

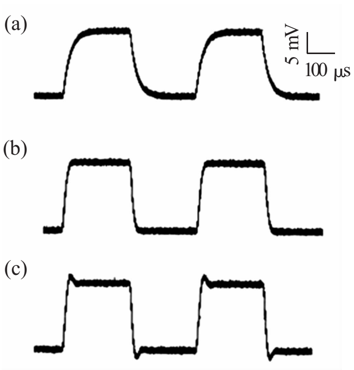

Fig. 5 Results of capacitance compensation
(a) The high frequency component of response is attenuated for the presence of capacitance. (b) System response is accelerated remarkably after exact compensation. (c) The injecting current caused the output signal to overshoot when the gain of compensation stage past the optimum setting.

Figure 6 illustrates the results of the series resistance compensation experiment. The repetitive step signal (200 Hz, $V_{p\text{-}p}$=40 mV) was applied to a 5.1 MΩ series resistance at I_{cmd}, and then the response was acquired at the V_m monitor. Figure 6a reveals that there is a rapid voltage step on the response due to the voltage drop across the series resistance before Rs compensation. When optimum compensation is used, the voltage drop across the series resistance is eliminated in Figure 6b. If the compensation is advanced too far, the voltage drop is overcompensated and a reverse-polarity rapid voltage step appears (Figure 6c).

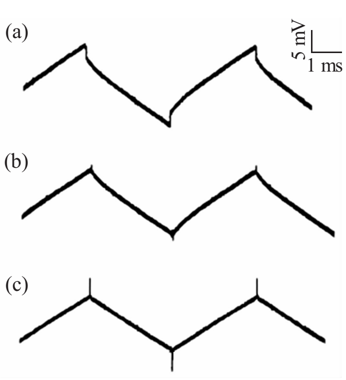

Fig. 6 Results of the series resistance compensation
(a) The response has a rapid voltage step on the response due to the voltage drop across the series resistance before compensation. (b) The voltage drop is eliminated when the series resistance is exactly compensated. (c) The voltage drop is overcompensated and a reverse-polarity voltage step appears.

The most important performance of system is the response speed to the disturbances of membrane potential. Repetitive step signals were injected at the input of the headstage (Cell point in Figure 2a) as the disturbances of the membrane potential, and the response was acquired at the V_m monitor. When the series resistances are 1 MΩ and 10 MΩ, the bandwidth of system before capacitance compensation is 15.8 kHz and 1.5 kHz, respectively. After optimum capacitance compensation, the bandwidth is increased to 42 kHz and 10.5 kHz, respectively[13]. Experimental results demonstrate that the system has a bandwidth of above 10 kHz after capacitance compensation, which is enough for measuring physiological signals.

3 Conclusion

A new headstage for CC mode recording is designed to overcome the electronic design limitations of conventional PCAs headstage. The new headstage is suitable for accurately recording physiological voltage signals as conventional voltage followers. The VCcCC technique was implemented to modify the conventional PCAs. The experimental results of the cell mode demonstrate that the improved circuits can overcome the disadvantage of conventional PCAs in the CC mode and enable monitoring of fast potential changes at preset holding potentials. The new headstage and VCcCC technique will facilitate the measurement of the transient membrane potentials and might increase the accuracy of the system.

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电压钳控制电流钳的设计 *

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摘要 研究证明，传统膜片钳放大器在电流钳模式下记录到的快速电压信号会存在失真，且造成失真的根本原因是由于膜片钳的探头电路设计．为了克服这些缺陷重新设计了一种探头，新探头电路不仅能像传统的电压跟随器一样测量瞬态电压，而且适用于传统的电压钳工作模式．此外，一种命名为电压钳控制的电流钳技术被应用来改进传统的膜片钳放大器．用可变的低通滤波器来调整电压钳模块的响应速度，从而在实现膜电位钳位的同时准确记录快速电压信号．桥平衡电路用来消除命令电流流过串联电阻时带来的电压误差．而传统膜片钳中的快电容补偿环节则被改进用来补偿电极分布电容和探头放大器输入电容并提高电流钳模式下系统的响应速度．细胞模型实验结果表明，改进后的膜片钳放大器能够满足电生理研究中快速电位变化测量的需要．

关键词 膜片钳放大器，电流钳，电压钳控制电流钳，低通滤波器，桥平衡，电容补偿

学科分类号 Q337，TN722.3

* 国家自然科学基金资助项目(30327001).

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收稿日期：2007-07-31，接受日期：2007-10-25