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GeneClamp 500B

THEORY AND OPERATION

Part Number 2500-118 Rev F, September 2002, Printed in U.S.A.

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E-mail: tech@axon.com

Declaration of Conformity

Manufacturer: Axon Instruments, Inc.
3280 Whipple Road
Union City, CA 94587
USA

Type of Equipment: Scientific Instrument (Picoammeter)

Model Number: GeneClamp 500B

Year of Manufacture: 1997

Application of Council Directives:
EC EMC Directive 89/336/EEC as amended
EC Low Voltage Directive 73/23/EEC as amended

Harmonized Standards to which Conformity is Declared:
EMC: EN 55011: 1990, Class B
EN 50082-1: 1998
IEC 801-2: 1991
IEC 801-3: 1984
IEC 801-4: 1988
Safety: EN 60950-1: 1993

I, the undersigned, hereby declare that the equipment specified above conforms to the above Directives and Standards.

Authorized Signature and Date: (Signature on file)

Explanation of symbols
Explication des symboles
Erklärung der verwendeten symbole
Explicación de símbolos

Symbol Symbole Symbol Símbolo	Description Description Beschreibung Descripción
	Direct current Courant continu Gleichstrom Corriente continua
	Alternating current Courant alternatif Wechselstrom Corriente alterna
	On (Supply) Allumé (alimentation) An (Netz) Encendido (suministro)
	Off (Supply) Éteint (alimentation) Aus (Netz) Apagado (suministro)
	On (Supply) Allumé (alimentation) An (Netz) Encendido (suministro)
	Off (Supply) Éteint (alimentation) Aus (Netz) Apagado (suministro)
	Protective conductor terminal Borne du conducteur de protection Schutzleiterpol Terminal de conductor protector

Disclaimer
This equipment is not intended to be used and should not be used in human experimentation or applied to humans in any way.

Warning
If this equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Power-Supply Voltage Selection and Fuse Changing

Supply Voltage
The GenClamp 500B can be directly connected to all international supply voltages. The input range is from 100 to 240 V~. No range switching is required.

Changing the Fuse
The GenClamp 500B uses a 250 V~, T2A, 5 x 20 mm fuse.

In the event of fuse failure, disconnect the power cord.

Before changing the fuse investigate the reason for its failure.

To change the fuse:
1. Disconnect the power cord.
2. Use a screwdriver or a similar device to rotate the fuse holder counterclockwise.
3. Replace the fuse with another fuse of the same rating.
4. Reconnect the power cord.

Basic Equipment Setup and Safety
1. Supply and Earthing
   Connections: Use the included IEC power cord to connect the instrument to a GROUNDED power receptacle.
2. Mounting: Table or rack.
3. Assembly: The headstage connects to the instrument through the rear panel, 15 pin
   D-sub connector marked "ME1 PROBE" and "ME2 PROBE".
4. Use: Do not operate this equipment with covers or panels removed.
5. Cleaning: Wipe the headstage connector with a damp cloth to clean salt spills. Avoid spilling liquids on the headstage.
   The Teflon input connector should be kept very clean. Effective cleaning can be done by spraying with alcohol or swabbing carefully with deionized water. If possible, avoid the use of Freon since it is thought to be detrimental to the environment.

Safe Environmental Conditions
1. Indoor use.
2. Mains supply fluctuations: not to exceed ±10% of the nominal voltage.
3. Temperature: between 5 ºC and 40 ºC.
4. Altitude: up to 2000 m
5. This instrument is designed to be used under laboratory conditions. Operate in a clean, dry environment only. Do not operate in a wet or damp environment.

Static Precautions
The headstage can normally be safely handled. However, if you are in a laboratory where static is high (*i.e.*, you hear and feel crackles when you touch things), you should touch a grounded metal object immediately before touching the headstage.

Safety Precautions
In TEVC mode the driving electronics on ME2 can supply a current limited high voltage pulse into the headstage electrode connector.

For continued safety, do not touch the electrode tip in TEVC mode.

WARNING!! Shipping the GeneClamp 500B
The GeneClamp 500B is a solidly built instrument designed to survive shipping around the world. However, in order to avoid damage during shipping, the GeneClamp 500B must be properly packaged.

In general, the best way to package the GeneClamp 500B is in the original factory carton. If this is no longer available, we recommend that you carefully wrap the GeneClamp 500B in at least three inches (75 mm) of foam or "bubble-pack" sheeting. The wrapped GeneClamp 500B should then be placed in a sturdy cardboard carton. Mark the outside of the box with the word FRAGILE and an arrow showing which way is up.

We do not recommend using loose foam pellets to protect the GeneClamp 500B. If the carton is dropped by the shipper, there is a good chance that the GeneClamp 500B will shift within the loose pellet packaging and be damaged.

If you need to ship your GeneClamp 500B to another location, or back to the factory, and you do not have a means to adequately package it, Axon Instruments can ship the proper packaging material to you for a small fee. This may seem like an expense you would like to avoid, but it is inexpensive compared to the cost of repairing an instrument that has sustained shipping damage.

It is your responsibility to package the instrument properly before shipping. If it is not, and it is damaged, the shipper will not honor your claim for compensation.

LIMITE DE RESPONSABILITE
CE MATERIEL N'A PAS ETE CONCU POUR DES EXPERIENCES SUR LES ETRES HUMAINS; ET NE DOIT DONC PAS ETRE UTILISE A CETTE FIN.

ATTENTION
L'EMPLOI DE CE MATERIEL D'UNE MANIERE DIFFERENTE A CELLE SPECIFIEE PAR LE FABRICANT AFFECTERA LE NIVEAU DE PROTECTION FOURNIT PAR L'APPAREIL.

Sélection du voltage et changement du fusible

Voltage d'alimentation
Le GeneClamp 500B peut être directement branché sur toutes alimentations comprises entre 100 et 240 V~. Aucun changement n'est nécessaire afin de sélectioner le voltage de l'appareil.

Changement du fusible
Le GeneClamp 500B emploie un fusible de 250 V~, T2A, 5 × 20 mm.

En cas de rupture du fusible, débrancher la prise de courant.

Avant de changer le fusible, chercher la raison de la panne.

Pour changer le fusible:
1. Débrancher la prise de courant.
2. A l'aide d'un tournevis ou autre outil de ce genre, faire tourner le support du fusible dans de sens opposé des aiguilles d'une montre.
3. Remplacer le fusible par un fusible de même valeur.
4. Rebrancher la prise de courant.

Installation du matériel et sécurité
1. Branchement: Employer le fil electrique IEC fourni pour brancher l'appareil a une prise de courant comprenant UNE TERRE.
2. Pose: Table ou rack.
3. Montage: La tête de l'amplificateur ("headstage") est connectée à l'appareil sur le panneau arrière, par l'intermediere d'une prise D-sub à 15 fiches portant l'indication "ME1 PROBE" et "ME2 PROBE".
4. Emploi: Ne pas utiliser ce matériel sans son couvercle et ne pas le couvrir lors de son utilisation.
5. Nettoyage: Essuyer la prise du "headstage" avec un linge humide pour nettoyer les traces de sel. Eviter de renverser des liquides sur le "headstage".La prise d'entrée en Téflon doit être maintenue trés propre. Un nettoyage efficace consiste à vaporiser de l'alcool ou á essuyer soigneusement avec de l'eau désionisée. Si possible, éviter l'emploi de Fréon, ce produit étant considéré comme nuisible pour l'environnement.

Conditions à respecter pour un emploi sans danger
1. Emploi à l'intérieur.
2. Fluctuations du réseaux d'alimentation: ne doivent pas dépasser ±10% de la tension nominale.
3. Température: entre 5 °C et 40 °C.
4. Altitude: jusqu'à 2000 m.
5. Cet appareil a été étudié pour l'emploi en laboratoire et il doit être situé dans un environnement sec et propre. Ne pas l'utiliser dans un environnement mouillé ou humide.

Précautions statiques
Le "headstage" peut être maniée sans danger. Cependant, dans un laboratoire avec un niveau élevé d'electricité statique (c'est-à-dire lorsque vous sentez et voyez des décharges électriques), touchez un objet métallique pour une mise à la terre avant de toucher le "headstage".

Precautions de securite
Dans le mode TEVC le appareil electronique peut vous donner un pulse de haut voltage qui est limitee pour l'alimentation seulement a le connecteur des electrodes de la tete de l'amplificateur.

Pour votre securite ne touchez pas le point de l'electrode en le mode TEVC.

ATTENTION!! Expédition de le GeneClamp 500B
Le GeneClamp 500B est un appareil de construction robuste, étudié en vue d'expéditions dans le monde entier. Cependant, l'appareil doit être correctement emballé pour éviter tout domage pendant son transport.

En général, la meilleure façon d'emballer le GeneClamp 500B est de le mettre dans son carton d'origine. Si celui-ci n'est plus disponible, il est recommandé d'envelopper soigneusement le GeneClamp 500B dans au moins trois inches (75 mm) de mousse ou de feuilles d'emballage à bulles. Le GeneClamp 500B ainsi protégé devra alors être placé dans un carton solide. Indiquer la mention FRAGILE sur l'extérieur de la boîte ainsi qu'une flèche vers le haut montrant la position verticale.

Il n'est pas recommandé d'employer des boulettes de mousse pour protéger le GeneClamp 500B. En cas de chute de la boîte durant son transport, le GeneClamp 500B pourrait se déplacer à l'intérieur et être endommagé.

Si vous devez expédier le GeneClamp 500B à un autre endroit, ou le renvoyer au fabricant, et si les matériaux d'emballage nécessaires corrects ne sont pas disponibles, ces derniers peuvent être obtenus chez Axon Instruments pour un prix minime. Bien que ceci puisse sembler être une dépense que vous pourriez éviter, elle est cepandant insignifiante en comparaison à celle que coûterait la réparation d'un appareil endommagé pendant le transport.

La responsabilité vous incombe de bien emballer l'appareil avant son expédition. Si ceci n'est pas fait, le transporteur ne pourra pas satisfaire vos réclamation de compensation en cas d'avaries.

UNZULÄSSIGE VERWENDUNG
DIESER APPARAT IST NICHT VORGESEHEN, BEI MENSCHLICHEN VERSUCHEN VERWENDET ZU WERDEN UND AUCH NICHT AN MENSCHEN IN IRGENDEINERWEISE ANWENDBAR.

WARNUNG
WEN DIESER APPARAT IN EINER ART UND WEISE ANGEWENDET WIRD, DIE NICHT VOM HERSTELLER SPEZIFISCH ERWÄHNT WIRD, KANN DIE SCHUTZVORRICHTUNG DES APPARATES BEEINTRÄCHTIGT WERDEN.

Spannungswahl für die Stromversorgung und Auswechseln der Sicherung

Netzspannung
Der GeneClamp 500B kann direkt an alle internationalen Netzspannungen angeschlossen werden. Die Eingangsspannung reicht von 100 bis 240 V~. Ein Umschalten des Spannungsbereichs ist nicht erforderlich.

Auswechseln der Sicherung
Der GeneClamp 500B verwendet eine 250V~, T2A, 5 × 20 mm Sicherung.

Im Falle des Ausfalls der Sicherung das Netzkabel ausschalten.

Vor dem Auswechseln der Sicherung den Grund für ihren Ausfall untersuchen.

Schritte zum Auswechseln der Sicherung:
1. Das Netzkabel ausschalten.
2. Die Fassung der Sicherung mit einem Schraubenzieher oder einem ähnlichen Werkzeug entgegen dem Uhrzeiger drehen.
3. Die Sicherung mit einer anderen Sicherung mit gleicher Nennleistung ersetzen.
4. Das Netzkabel wieder anschließen.

Grundlegende Hinweise zu Installation und Sicherheit der Ausrüstung
1. Netz- und Erdungsanschlüsse: Das Instrument mit dem beigefügten IEC Netzkabel an einen Erdungsschalter anschließen.
2. Anbringung: Tisch oder Rahmengestell.
3. Montage: Der Vorverstärker ("headstage") wird über einen mit der Aufschrift "headstage" gekennzeichneten 15 Pin D-Unterstecker an der Rückwand des Instrumentes verbunden ME1 PROBE und ME2 PROBE
4. Gebrauch: Dieser Apparat darf nicht mit abgenommenen Abdeckungen oder Platten in Betrieb gesetzt werden.
5. Reinigung: Zur Reinigung von verschüttetem Salz den Vorverstärkeranschluß mit einem feuchten Tuch abwischen. Das Verschütten von Flüssigkeiten auf den "headstage" ist zu vermeiden.
   Der Teflon-Eingangsstecker sollte in sehr sauberem Zustand gehalten werden. Durch Besprühen mit Alkohol oder vorsichtigem Abtupfen mit entionisiertem Wasser ist eine wirksame Reinigung möglich. Die Benutzung von Freon ist nach Möglichkeit zu vermeiden, da diese Substanz als umweltschädigend angesehen wird.

Umweltsichere Betriebsbedingungen
1. Verwendung in Innenräumen.
2. Netzschwankungen: darf nicht ±10% der Nennspannung überschreiten.
3. Temperatur: zwischen 5 °C und 40 °C.
4. Höhe: bis zu 2000 m.
5. Dieses Instrument ist für den Gebrauch unter Laborbedingungen vorgesehen. Nur in sauberer, trockener Umgebung in Betrieb setzen. Nicht in nasser oder feuchter Umgebung in Betrieb setzen.

Schutzmaßnahmen gegen statische Aufladung
Der "headstage" kann normalerweise sicher gehandhabt werden. Falls Sie sich jedoch in einem Labor mit höher statischer Aufladung befinden (*D.h.* Sie hören und fühlen beim Berühren von Objekten ein Knacken), sollten Sie unmittelbar vor dem Berühren der "headstage" ein geerdetes Objekt aus Metall anfassen.

Sicherheits Hinweise
Das Gerät kann in der TEVC Anwendung einen stromlimitierten Hochspannungspuls an den Vorverstärkerelektrodenverbindungstecker abgeben. Aus Sicherheitsgrunden sollte die Spitze der Elektrode in der TEVC Anwendung nicht angefaβt werden.

WARNUNG: Versand des GeneClamp 500B
Bei dem GeneClamp 500B handelt es sich um ein solide gebautes Instrument, das beim weltweiten Versand keinen Schaden nehmen sollte. Um jedoch Versandschäden zu verhindern, muß der GeneClamp 500B ordnungsgemäß verpackt werden.

Im allgemeinen läßt sich der GeneClamp 500B am besten im Originalkarton des Werks verpacken. Ist dieser nicht mehr vorhanden, empfehlen wir, den GeneClamp 500B vorsichtig in mindestens 75 mm starkem Schaumstoff oder Bubblepackungen einzuwickeln. Der so eingewickelte GeneClamp 500B sollte dann in einen festen Pappkarton gesetzt werden. Die Außenseite des Kartons ist mit dem Worten ZERBRECHLICH (FRAGILE) und einem Pfeil, der auf die Oberseite des Kartons weist, zu kennzeichnen.

Sollte der Karton vom Spediteur fallengelassen werden, besteht eine gute Möglichkeit, daß der GeneClamp 500B innerhalt der losen Schaumstoffkugelverpackung bewegt wird und dadurch beschädigt werden kann.

Wenn Sie den GeneClamp 500B an einen anderen Ort oder zurück ans Werk senden müssen und Ihnen kein angemessenes Verpackungsmaterial zur Verfügung stehen, kann Axon Instruments Ihnen das geeignete Verpackungsmaterial gegen eine kleine Gebühr zustellen. Sie mögen dies zwar als unnötige Zusatzkosten betrachten, doch ist dieser Aufwand im Vergleich zu den Reparaturkosten fur ein während des Transports beschädigtes Instrument gering.

Sie sind selbst für das richtige Verpacken des Instruments vor dem Versand verantwortlich. Bei einer nicht ordnungsgemäßen Verpackung, die eine Beschädigung zur Folge hat, wird der Spediteur ihren Schadensersatzanspruch nicht anerkennen.

LÍMITE DE RESPONSABILIDADES
ESTE EQUIPO NO ESTÁ DISEÑADO PARA USO EN HUMANOS Y NO DEBE USARSE PARA EXPERIMENTACIÓN O APLICACIÓN EN SERES HUMANOS BAJO NINGUNA CIRCUNSTANCIA.

ADVERTENCIA
SI ESTE EQUIPO SE USA DE MANERA NO ESPECIFICADA POR EL FABRICANTE SE PODRÍA PERDER LA PROTECCIÓN PROVISTA POR EL EQUIPO.

Selección del suministro de corriente y cambio de fusibles

Voltaje de entrada
El GeneClamp 500B puede conectarse directamente a todos los suministros de energía. El límite de voltaje va entre 100 y 240 V~. No es necesario efectuar cambios en el selector.

Cambio de fusible
El GeneClamp 500B utiliza un fusible de 250 V~, T2A, 5 × 20 mm.

En el caso de que un fusible falle, desconecte el cordón eléctrico.

Antes de cambiar el fusible investigue la causa de la falla.

Para cambiar el fusible:
1. Desconecte el cordón eléctrico.
2. Use un destornillador o un dispositivo similar para girar el portafusibles en sentido contrario al de las manecillas del reloj.
3. Reemplace el fusible existente con otro de la misma capacidad.
4. Conecte nuevamente el cordón eléctrico.

Instalación básica y seguridad del equipo
1. Suministro de corriente
   y conexión a tierra: Use el cordón eléctrico IEC incluido para conectar el instrumento a una toma de corriente CON CONEXIÓN A TIERRA.
2. Montaje: Sobre una mesa o en un estante.
3. Ensamblaje: El cabezal ("headstage") se conecta al instrumento en el tablero posterior con el conector de 15 clavijas D-sub, marcado "ME1 PROBE" y "ME2 PROBE" .
4. Uso: No utilice este equipo sin las cubiertas o paneles.
5. Limpieza: Limpie el conector del "headstage" con un paño húmedo a fin de quitar los derrames de sales. Evite derramar líquidos sobre el "headstage". El conector de entrada fabricado de Teflon debe mantenerse muy limpio. Puede hacerse una limpieza efectiva rociando con alcohol o con un algodón humedecido con agua desionizada. En la medida de lo posible evite el uso del gas freón, puesto que es dañino para el medio ambiente.

Condiciones de seguridad ambiental
1. Para uso interior.
2. Fluctuaciones eléctricas en la fuente de suministro: no deben exceder ±10% del voltaje nominal.
3. Temperatura: entre 5 °C y 40 °C.
4. Altitud: hasta 2.000 m
5. Este instrumento está diseñado para ser usado en condiciones de laboratorio. Debe operarse únicamente en un ambiente limpio y seco. No lo use en un ambiente húmedo ni mojado.

Precauciones contra la estática
El "headstage" puede manejarse con seguridad, bajo condiciones normales. Sinembargo, si usted se encuentra en un laboratorio donde la estática es alta (por ejemplo, si escucha y percibe chispas cuando toca los objetos), usted debería tocar inmediatamente un objeto metálico que esté en contacto con tierra, antes de tocar el "headstage".

Precauciones de seguridad
En el modo TEVC el circuito electronico de ME2 puede generar un pulso de alto voltaje limitado solamente por corriente al conector del cabezal del electrodo.

Para su seguridad, no toque el punto del electrodo mientras usando el modo TEVC.

ADVERTENCIA!! Envío del GeneClamp 500B
El GeneClamp 500B es un instrumento de construcción sólida, diseñado para soportar el transporte a cualquier parte del mundo. Sinembargo, para evitar los daños que pudieran ocurrir durante su envío, el GeneClamp 500B debe empacarse adecuadamente.

En general, la mejor manera de empacar el GeneClamp 500B es en la caja original de fábrica. Si ésta ya no se encuentra disponible, le recomendamos que envuelva cuidadosamente el GeneClamp 500B en una funda o sábana de espuma o de "empaque de burbujas" con un espesor mínimo de 3 pulgadas (75 mm). El GeneClamp 500B, envuelto así, deberá colocarse en una caja de cartón resistente. Marque el exterior de la caja con la palabra FRÁGIL y una flecha que indique la posición hacia arriba.

No recomendamos el uso de bolitas de espuma sueltas para proteger el GeneClamp 500B. Si la caja se cae accidentalmente durante el transporte, es muy probable que el GeneClamp 500B se desplace dentro del contenedor con las bolitas de espuma sueltas y se dañe.

Si necesita enviar su GeneClamp 500B a otra localidad, o de regreso a la fábrica, y no posee el empaque adecuado, Axon Instruments puede enviarle el material necesario por un cargo mínimo. Esto podría parecerle un gasto superfluo que preferiría evitar, pero es económico comparado con lo que costaría la reparación de un instrumento que ha sufrido daños durante el envío.

Es su responsabilidad empacar el instrumento adecuadamente antes de enviarlo. Si no lo hace así y resulta dañado, el transportista no será responsable ni aceptará su reclamo de indemnización.

VERIFICATION

THIS INSTRUMENT IS EXTENSIVELY TESTED AND THOROUGHLY CALIBRATED BEFORE LEAVING THE FACTORY. NEVERTHELESS, RESEARCHERS SHOULD INDEPENDENTLY VERIFY THE BASIC ACCURACY OF THE CONTROLS USING RESISTOR/CAPACITOR MODELS OF THEIR ELECTRODES AND CELL MEMBRANES.

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Chapter 1

Introduction

The GeneClamp 500B is a multifunctional high-compliance current- and voltage-clamp system designed for a broad range of recording techniques, including:

- two-electrode voltage clamp of medium- and large-sized cells, such as *Xenopus* oocytes or molluscan neurons
- single-channel patch clamp
- macropatch voltage clamp
- bilayer voltage clamp
- amperometry and voltammetry

The GeneClamp is a sophisticated instrument made easy to operate by means of an on-board microprocessor. Automation has replaced many of the tedious knob-turning tasks with single push-button requests. Built-in automated functions allow the user to zero electrode offsets, measure electrode resistance, subtract leakage current, or zero the output with the touch of a button. For maximum convenience and ease of use, the GeneClamp provides TUNE and TEST pulses appropriate for neutralization of electrode capacitance, optimization of voltage clamp gain, and monitoring the progress of patch-clamp seal formation. Operational errors are sensed automatically, and the user is alerted by error-specific tones. Gain and frequency telegraphs can be read by host computer software via an RS-232 port, and a supplied program CMDGENE allows the user to test many of the functions and capabilities of the instrument. The program can be used in command-line mode. It also can accept commands interactively or from a command file on disk.

The various techniques for which the GeneClamp is designed allow the researcher to address sophisticated questions concerning molecular and cellular physiology. To extract the most from these techniques, meticulous attention to experimental details and their interactions with the theory and operation of the GeneClamp is essential. Therefore, we have written this manual with two goals in mind. The first is to explain the operation of the instrument and many of the principles that underlie its design. The second is to provide the user with a guide to experimental techniques that our customers and scientific consultants have found useful during many years of experience in electrophysiology.

Headstage Safety Issues

We recommend that you never touch a headstage case or attached electrode holder unless the GeneClamp 500B is either in SETUP mode or entirely turned OFF.

The GeneClamp 500B can accept several different types of headstages for ELECTRODE #1 and ELECTRODE #2. The currently available headstages are: HS-2A-x10MGU, HS-2A-x1LU, HS-2A-x1MGU, HS-2A-x0.1LU, HS-2-x0.01MU, HS-2-x0.0001MU and HS-4-x1MGU.

The "G" designator indicates that the headstage case is grounded. The safety issues discussed below do not apply to those headstages that have a "G" designator.

If the headstage does NOT have a "G" designator, the headstage case is connected to the capacitance neutralization circuit. That is, the headstage case is "driven". This is so irrespective of the presence or absence of any other letters in the headstage model designation.

Electrode #2 Background

A consideration for the ELECTRODE #2 headstage is that during VOLTAGE CLAMP operation, internal voltages of up to ±160 V can be connected to the electrode connector of the headstage. With HS-2 and HS-2A series headstages, the maximum current that can be delivered via the electrode connector is limited by a resistor. In an HS-2A-x10MGU headstage, the value of this resistor is 1 MΩ, thus limiting the current to ±160 µA (theoretically, but the actual empirical value depends on the electrode resistance). The other headstages listed above (with one exception) have larger resistor values, thus the current is limited to an even smaller value in these headstages. The exception is the HS-4-x1MGU headstage, which during VOLTAGE CLAMP mode can deliver up to ±10 mA. In SETUP mode, however, the HS-4-x1MGU- current is limited by a 10 MΩ resistor.

During two-electrode voltage clamp the membrane capacitance transients can cause the voltage at the headstage output to transiently reach over 100 V. If one of the electrodes is withdrawn from the cell, a voltage of plus or minus 160 V could be connected to the headstage case, especially if the voltage-clamp GAIN control is well advanced.

Safety Circuits

The GeneClamp 500B implements the following safety circuits:

1) The ELECTRODE #2 headstage case is connected to the headstage output via an .0047 µF capacitor inside the headstage. Thus under conditions where the electrode voltage is steady at plus or minus 160 V, only a single transient current can be discharged from the headstage case.

2) When the voltage clamp command is pulsed, the transient current on the headstage case will follow the repetition frequency of the command pulse. This current is limited by the safety circuits to less than 10 mA.

3) The maximum current that can be delivered via the electrode connector in the headstage depends on the mode and the type of headstage. With an HS-4 series headstage in the ELECTRODE #2 position, the current is limited to ±10 mA. For all other headstages listed above, and for the HS-4 series headstage in the ELECTRODE #1 position, the current is limited to less than ±1 mA.

Chapter 2

Functional Checkout

When you receive your GeneClamp, you should first run a functional checkout to ensure the proper functioning of the instrument. All units are burned-in and thoroughly tested at the factory prior to shipping. If you observe any damage caused by shipping or if you encounter problems with the Functional Checkout described below, please call the factory.

Start-Up Procedure

For the initial checkout, the GeneClamp should be situated on a benchtop away from other equipment. Do not install it in a rack until the checkout is complete. Make sure that the power is OFF. An oscilloscope is the only other piece of equipment required for these tests. A large sheet of aluminum foil is needed to shield the headstages and the MCO-2U model cell. (For a more detailed description of the MCO-2U model cell, see the Reference Guide: General Information section of this manual.)

For all tests described in this manual, the MCO-2U model cell should be switched to the "1 M" position unless otherwise directed.

NOTE: The GeneClamp 500B was originally released with the **MCO-1U** model cell, which is equivalent to the MCO-2U in the "1 M" position. If you own the original GeneClamp 500B, which is not equipped with the STEADY-STATE RESTORE feature, then simply skip the steps of the following Functional Checkout procedure that refer to STEADY-STATE RESTORE or switching the MCO-2U. If you would like to know more about the STEADY-STATE RESTORE enhancement, contact Axon Instruments' Technical Support.

Two-Electrode Voltage Clamp (TEVC) Section (Headstages #1 and #2)

1. If your unit is equipped with a "STEADY-STATE RESTORE" function, turn this switch to the OFF position.
2. Plug in the HS-2A-x1LU headstage into the connector on the rear panel of the GeneClamp labeled Headstage #1 and the HS-2A-x10MGU headstage into the connector labeled Headstage #2.
3. Connect the two headstages to the MCO-2U model cell supplied with the GeneClamp to the indicated parts of the model cell (E #1 and E #2 for Headstages #1 and #2, respectively).

4. Insert one pin of the four-leaded connector supplied with the model cell into the yellow socket at the rear of the HS-2A Headstage; another pin into the CELL socket on the model cell; and the third pin into the case ground (gold-plated socket) of the model cell. Connect the clip lead to the foil shield.
5. Turn on the GeneClamp. The amplifier should be in Setup mode. The upper panel meter should display V1 and the lower should display V2; if not, select the proper display by pushing the V1 or the I2/V2 button under each meter. If the displays do not show a value of 0 mV, zero the offset by pressing the ZERO V1 or ZERO V2 button; the displays should now read 0 mV.
6. Press R1 to measure the resistance of the micropipette E #1, modeled as a 1 MΩ resistor. The upper display should now read approximately 1.6 MΩ. Repeat the same operation for E #2; the display should now read approximately 1.6 MΩ.

NOTE: With the model cell, as with a real cell, the GeneClamp actually measures the resistance of the combination of the micropipette (modeled as a 1 MΩ resistor) and the membrane, (also modeled as a 1 MΩ resistor). During the resistance test the GeneClamp toggles the current on and off so that it can take differential readings of the voltage. This way, the resistance test is not compromised by the electrode offset nor the DC current command. The resistance is calculated using:

$$R = \frac{\Delta V}{\Delta I}$$

The electrodes and membrane of the model cell each have a resistance of 1 MΩ. Consequently, it is correct to assume that the steady-state resistance readings for R1 and R2 will each be 2 MΩ. This represents the sum of the micropipette and the cell membrane resistances. However, when the electrode is intracellular, V2 and V1 do not reach their final values because the model cell does not have time to settle to its final value during each current pulse of the resistance test. Because of this, the resistance reading is lower than expected. This is true for real cells as well.

7. Switch from SETUP to VOLTAGE CLAMP mode. Set the GAIN to 2000, and the STABILITY to 80 µs.
8. Apply a 100 mV command via the HOLDING POTENTIAL knob, and press the "+" button. The meter reading for V1 should be 100 mV, and I2 will read 100 nA.
9. Switch the MCO-2U model cell to the "10 k" position (this mimics a sudden drop in cell membrane resistance). The meter reading for V1 should now read approximately 95 mV, and I2 current will read near 8.5 µA. (See the Two-Electrode Voltage Clamp chapter for discussion of this error.)
10. Switch the STEADY-STATE RESTORE function to ON. Notice that the V1 meter reading returns to 100 mV, and the I2 current to 9.0 µA (since the resistance is actually 11 kΩ here).
11. Switch the amplifier back to SETUP mode.

Test the SCALED OUTPUT Section

12. Connect an oscilloscope to one of the SCALED OUTPUT BNCs on the rear panel. Set the oscilloscope time base to 5 ms/div, the gain to 50 mV/div and trigger to line.
13. Select I1 on the SCALED OUTPUT; set the gain to 10 V/µA and the filter to 2 kHz. You should observe a flat trace.

14. Activate the TUNE function for Electrode #1. This injects a 1 nA current at the line frequency in E #1 which should produce a square wave of 10 mV amplitude. The DC level will move -5 mV as the membrane capacitor in the model cell slowly charges up.
15. Increase the gain on the output to 100 V/μA and note that the square wave gets 10 times larger. Because the DC offset is amplified, it may be necessary to reposition the trace on the oscilloscope.
16. Decrease the filter frequency and note that the square wave begins to round and eventually looks more like a sine wave than a square wave at the lowest frequencies.

Test the Bath Clamp

1. Turn the GeneClamp off, and plug the VG-2A-x100 headstage into the rear-panel labeled BATH CLAMP.
2. With the black wires provided, connect the BATH and SENSE sockets of the VG-2A headstage to the corresponding sockets on the MCO-2U model cell. Insert one pin of the four-leaded connector into the yellow ground socket of the HS-2A-x1LU headstage and one pin into the gold-plated socket (case ground) of the MCO-2U model cell. (The CELL position should be left open.) Connect the clip lead of the four-leaded connector to the foil shield.
3. With BNC cables, connect I_{BATH} OUTPUT and one of the SCALED OUTPUTS to the oscilloscope. Set both channels of the oscilloscope to 0.5 V/div. Use a time base of 2 ms/div and trigger at the line frequency.
4. Turn on the GeneClamp and change to VOLTAGE CLAMP mode. Turn on the VOLTAGE CLAMP TEST FAST oscillator, with the STABILITY adjusted to 80 μs, and increase the GAIN to 10,000. Select I2 and set the SCALED OUTPUT GAIN to 0.1 V/μA, and filter frequency to 50 kHz. Both I_{BATH} OUTPUT and I2 values should be the same.

Patch Section (Headstage #3)

1. Turn the GeneClamp off, and plug the optional CV-5 headstage into the indicated space on the rear panel. If you have either a CV-5-100GU or CV-5B-100GU headstage, you can plug the boost box into the indicated space on the rear panel (unless your unit has been modified with the STEADY-STATE RESTORE switch). This is a physical (not an electrical) connection and can be used as a convenient means of storing the boost box.
2. Connect a cable from one of the SCALED OUTPUT BNCs to one channel of the oscilloscope.
3. Turn the power ON, and set the front panel controls of the GeneClamp as follows:

ELECTRODE CAPACITANCE COMP (all):	Minimum (fully counterclockwise)
DC COMMAND POTENTIAL:	0 mV, OFF*
SEAL TEST:	OFF*
UPPER DC METER:	Select I3 rms
LOWER DC METER:	Select I3
SCALED OUTPUT:	Select I3
SCALED OUTPUT GAIN:	Minimum (press ↓ twice)
LOW-PASS BESSEL FILTER:	50 kHz

LEAK SUBTRACTION:	OFF*
OUTPUT ZERO:	OFF*

*OFF means that all push buttons in that section are not illuminated.

Test the Noise

4. Shield the headstage with a large sheet of aluminum foil. Wrap the foil loosely and completely around the headstage. Connect the foil shield to the ground input of the headstage (gold-plated 2 mm socket at the rear of the probe) using the supplied clip-lead. The easiest way to do this is to connect the clip-lead directly to the foil and to the 2 mm pin inserted into the headstage ground socket. (Alternative ground connections are available from the yellow 4 mm socket on the rear-panel of the main unit, or from the BNC shields.)

5. Determine the noise of the CV-5-100GU or CV-5-100GU using I3 rms display. The expected values under optimal conditions are:

Headstage	pA rms @ 5 kHz
CV-5-100GU	0.15 pA
CV-5B-100GU	0.5 pA

   I3 rms is shown on the panel meter always in a 5 kHz bandwidth using a Butterworth filter that is independent of the front-panel low-pass Bessel filter. The front-panel gain does not affect this reading.

6. Use an oscilloscope to determine the peak-to-peak noise of the CV-5-1GU and the CV-5-100MU headstages. The default gain setting for the CV-5-1GU headstage is x100 V/nA and x10 V/nA for the CV-5-100MU headstage. Set the Bessel filter of the GeneClamp to 10 kHz. Read the noise in volts. The expected values under optimal conditions are:

Headstage	V_{p-p} @ 10 kHz
CV-5-1GU	0.64 V
CV-5-100MU	0.24 V

   If the observed values are more than twice the expected values, then check to see that the foil shield is correctly grounded and that all controls are in the appropriate positions. Also check on the screen of the oscilloscope for line interference at 50 or 60 Hz, or for other noise pickup.

Test the PIPETTE CAPACITANCE COMPENSATION and SCALED OUTPUT

7. Set the instrument panel controls as in #3 above.

8. Set the oscilloscope gain to 0.5 V/div, trigger to line, and the sweep speed to 2 ms/div.

9. Turn on the SEAL TEST 10 mV switch. You should see oppositely going capacitance transients at about 8 ms intervals (about 10 ms for 50 Hz line frequency).

10. Turn the ELECTRODE CAP. COMP. FAST MAG and FAST TAU controls and verify that the capacitance transients change their size and amplitude. Do the same with the SLOW MAG and

SLOW TAU controls but be aware that the amplitude range of the SLOW MAG is much less than the range of the FAST MAG control.

11. Select I3 in the SCALED OUTPUT section. Switch between the GAIN and filter FREQ positions in the SCALED OUTPUT section and verify that the capacitance transient signals seen on the oscilloscope screen are affected. The range of GAIN settings is different for each type of headstage:

Headstage	GAIN
CV-5-100GU	x0.1, x1, x10
CV-5B-100GU	x0.1, x1, x10
CV-5-1GU	x1, x10, x100
CV-5-100MU	x0.1, x1, x10

Do not be alarmed if the OVLD light illuminates. An overload is likely because the CAPACITANCE COMPENSATION is not set optimally.

12. Turn off SEAL TEST. Turn off GeneClamp.

13. Remove the foil shield temporarily. Insert one pin of the four-leaded connector supplied with the model cell into the gold-plated socket at the rear of CV-5 Headstage; another pin into the CELL socket on the model cell; and the third pin into the case ground (gold-plated socket) of the model cell. Connect the clip lead to the foil shield. Now turn on the GeneClamp.

14. Connect the headstage input to the model cell at E #3. Insert the Teflon collet of the model cell into the collar of the headstage. Make sure that the collet is inserted all the way so that the connector pin is inserted fully into the headstage input jack.

15. Shield the headstage and model cell with the foil. Reconnect the foil shield to ground. You should notice an increase in I3 rms.

16. Select V3 on the upper DC meter. Zero the lower panel meter (I3) using the ZERO I3 button. If the meter cannot be zeroed easily within two displayed counts (*e.g.*, ±0.2 pA for CV5-100G) or if it constantly drifts, the foil shield is probably incorrectly connected to ground.

17. Set the DC COMMAND potential to positive (+). The DC COMMAND will depend on the type of headstage tested. The voltage selected will be clamped across the 10 GΩ resistor in the model cell.

Headstage	DC COMMAND POTENTIAL		Meter Reading
CV-5-100GU	100 mV	10.0 pA	±0.5 pA
CV-5B-100GU	100 mV	10.0 pA	±0.5 pA
CV-5-1GU	100 mV	10 pA	±1 pA
CV-5-100MU	1000 mV	100 pA	±5 pA

You have now verified that all the circuits are in working order.

Chapter 3

Tutorial

Grounding the Bath

All voltage measurements are made relative to the bath potential. Consequently, prior to beginning an experiment the user must set the bath potential to ground by means of a bath electrode. In any mode all headstages share the same signal ground. Therefore it is necessary to connect the bath electrode to only one headstage. Typically the bath electrode is a Ag/AgCl pellet that is either directly immersed in the bath or placed in a chamber containing 3 M KCl, which is then connected to the bath by means of an agar bridge containing 3 M KCl. An agar bridge is often used when the chloride concentration of the bath solution is reduced.

Two-Electrode Voltage Clamp

Operation of the TEVC section of the GeneClamp will be described here in the context of a real experiment with an oocyte. If you experience any difficulties, we highly recommend that you work through these procedures using the MCO-2U model cell supplied with the unit and illustrated in the Model Cell section in the Reference Guide. It is assumed that the user has already completed the **Functional Checkout** described earlier in this manual to verify that the unit is functioning properly.

The bath may be grounded by one of two methods. The easiest way is to connect the bath electrode to the yellow ground connection on the back of Headstage #1. This method works well if the currents flowing across the membrane into the bath are not too large. If the user expects large currents, then the preferred method of grounding the bath is to use an active bath clamp, such as the VG-2A-x100 headstage supplied with the GeneClamp, which will maintain the bath potential at zero regardless of the current flow. (See *Bath Error Potential* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION**).

The GeneClamp 500B is supplied with the HL-U holder for the HS-2A headstages so that a range of glass diameters can be accommodated. Use the HL-U for glass with an outside diameter (OD) of 1.0-1.7 mm. To minimize noise, it is important to keep the holder clean and dry. Backfill the micropipette with a small amount of solution. Loosen the end of the holder (from which the wire protrudes) by about 1 or 2 turns to relieve the pressure on the cone washer inside. Slip the wire (or pellet) into the micropipette and slide the micropipette up into the holder until it seats. Tighten the holder gently so that the micropipette is held firmly in place. Only a few millimeters of the silver wire (or pellet) should be immersed.

Micropipette Checkout and Cell Impalement

Make sure that the GeneClamp 500B is in SETUP mode. Turn the CAPACITANCE NEUTRALIZATION control to its minimum (counterclockwise) position. Mount the holders with micropipettes on Headstages #1 and #2. Place an oocyte in the bath and advance the micropipettes until the tips are immersed in the bath. Observe V1 on the upper DC meter and V2 on the lower meter. If either one is non-zero, zero it with the appropriate ZERO V button. Measure the resistance of each micropipette with the R1 and R2 controls on the upper panel meter. Acceptable values for oocyte voltage clamping are in the range of 0.5-5 MΩ. It should be noted that in contrast to most standard intracellular and patch-clamp recording techniques, the low-resistance micropipettes used for voltage clamping oocytes can be used on several oocytes in succession. Just check the resistance before re-using a micropipette on another cell to make sure that it is still at an acceptable value.

Once you have determined that the micropipettes are acceptable, position the micropipettes over the oocyte. If necessary, re-zero the voltages, V1 and V2, and impale the oocyte by slowly advancing the micropipette until it "pops" in, first with the micropipette connected to Headstage #1, and then with the micropipette connected to Headstage #2. You should observe a change in V1 and V2 on the panel meters as you impale the cell. Resting potential values of a healthy oocyte are usually -30 mV or more negative. Although not necessary, at this point it is advisable to place a grounded metal shield between the two micropipettes as close as possible to the bath. However, in order to achieve maximal speed and fidelity of the clamp, make sure that it does not contact the bath.

Voltage Clamp the Cell

Once both micropipettes are in the cell and the resting potential has stabilized to an acceptable value, you are ready to clamp the cell. Make sure that the voltage-clamp GAIN and the STABILITY knobs are at their minimum values (fully counterclockwise). Although you may want to carry out your experiment at a particular holding potential (-60 mV for example), the optimal holding potential for adjusting the voltage clamp GAIN and STABILITY controls is the potential at which no net current should flow, that is, the cell's resting potential. The RMP BALANCE LEDs above the HOLDING POTENTIAL knob in the voltage clamp section allows the user to pre-set the holding potential at the cell's resting potential. If the (-) button next to the HOLDING POTENTIAL knob is not illuminated, push the button. One of the two LEDs should be illuminated. Adjust the holding potential until the two LEDs are both equally dim. At this point the holding potential is now set at the resting potential.

Put the GeneClamp 500B in VOLTAGE CLAMP mode and activate the FAST TEST pulse. Observe I2 on the SCALED OUTPUT. With the voltage-clamp gain at its minimum value, you should observe a slightly rounded square wave. Slowly increase the gain and note that the output begins to look like a capacitive transient. Increase the gain until it reaches its maximum value or until the I2 trace begins to show signs of oscillations. If oscillations appear, slowly turn the STABILITY knob clockwise until the oscillations disappear. Resume increasing the gain as before, increasing the stability control when necessary. Continue this either until the gain is at its maximum value or until increasing the STABILITY control reduces the speed of the clamp too much to be useful.

The cell is now voltage clamped at its resting potential. Turn off the TEST pulse and use the HOLDING POTENTIAL to set the cell's holding potential to whatever value the experiment requires. You can now begin your experiment.

Patch Clamp

Operation of the patch section of the GeneClamp will be described here in the context of a real experiment. If you experience any difficulties, we recommend that you work through these procedures using the MCO-2U model cell supplied. It is assumed that the user has already gone through the **Functional Checkout** described earlier in this manual to verify that the unit is functioning properly.

Use the CV-5-100GU for single-channel recording, the CV-5-1GU for macropatch recording or the CV-5B-100GU for bilayer recording. If the bath electrode is not already connected to ground, connect it to the gold-plated ground socket on the back of the headstage.

The HL-U holder is available for the CV-5 headstages so that a range of glass pipette diameters can be accommodated. Use the HL-U for glass with an outside diameter (OD) of 1.0-1.7 mm. To minimize noise, it is important to keep the holder clean and dry. Backfill the patch pipette with a small amount of solution. Loosen the end of the holder (from which the wire protrudes) by about 1 or 2 turns to relieve the pressure on the cone washer inside. Slip the wire (or pellet) into the patch pipette and slide the patch pipette up into the holder until it seats properly. Tighten the holder gently so that the patch pipette is held firmly in place. Attach some tubing to the stainless-steel tube so that you can apply suction. Only a few millimeters of the silver wire (or pellet) should be immersed.

Making a Seal

Make sure the SCALED OUTPUT is on I3. Attach a patch pipette to the holder and touch it to the bath solution. (Some prefer to apply a slight amount of positive pressure during this operation.) I3 on the SCALED OUTPUT will probably be saturated at plus or minus 13 V. Use ZERO I3 to zero the trace. Measure the patch pipette resistance by pressing R3 on the upper panel meter control and note its value. Typical acceptable values are on the order of 5-20 MΩ for single-channel recording and 0.5-5 MΩ for macropatches. Activate the 100 µV SEAL TEST. (The GeneClamp will automatically de-activate R3 measurement when SEAL TEST is selected.) A rectangular waveform should appear on your oscilloscope. The amplitude will depend inversely on your patch pipette resistance.

Slowly move the patch pipette to the surface of the cell. As you press the patch pipette against the membrane and apply suction, you will get two indications of the formation of a seal:

1) The amplitude of the test pulses will diminish and disappear into the background.

2) The peak-to-peak noise will become much smaller.

Once the seal has been created, or sometimes during seal formation, you may want to determine the seal resistance. This is easily done on the GeneClamp by simply switching off the SEAL TEST and activating the R3 function on the upper panel meter. (The GeneClamp will automatically de-activate SEAL TEST when R3 is selected.) The display will directly report the seal resistance in the appropriate units.

The DC COMMAND POTENTIAL control is used to impose DC shifts on the membrane. As you change the potential you will see corresponding changes in the current.

Select V3 on the upper meter. This will show you the membrane potential. Select the appropriate polarity with the (+) or (-) control on the DC COMMAND POTENTIAL and dial the desired potential. Another way to control the membrane potential is by using the E #3 PATCH COMMAND (20 mV/V) input on the back of the

instrument. Any voltage applied to this input will be proportionately applied to the membrane. For example, if you applied a sine-wave command potential you would see a sine-wave membrane potential.

Patch Pipette Capacitance Compensation

Switch off all external command sources. Activate the SEAL TEST 10 mV function. You will notice large positive and negative spikes on the current waveform. These represent the current required to charge the patch pipette capacitance to each potential. These spikes can be almost fully compensated by the ELECTRODE CAPACITANCE COMPENSATION controls (FAST and SLOW). Eliminate the major portion of the spikes by iteratively adjusting the FAST TAU and FAST MAGNITUDE controls. The spikes should almost disappear into the noise. If slow residual spikes are present, they can normally be eliminated by iteratively adjusting the SLOW TAU and SLOW MAGNITUDE controls. You are now ready to begin recording!

Chapter 4

Interfacing A Computer to the GeneClamp 500B

Although the GeneClamp 500B can be controlled manually, the GeneClamp's true potential as a recording instrument is best utilized by interfacing it with a laboratory computer. This section describes the GeneClamp's many features designed for a computerized setup. When combined with an analog-to-digital (A/D) converter, and Axon Instruments software for the PC or Macintosh computers, the system is functionally superior to conventional systems based on stimulators, digital oscilloscopes, laboratory tape recorders and chart recorders.

Most experiments require complex voltage-step protocols. These are best provided by D/A converters interfaced to a computer. Interfaces from Axon Instruments are ideal, but almost any D/A interface can be used. The output voltage from these D/A converters can be delivered through the rear panel external inputs (CURRENT COMMANDS, VOLTAGE CLAMP COMMAND, PATCH CLAMP COMMAND and SPEED TEST). External commands will be added to internal commands set by the front panel controls.

The scaled and filtered current is available from two identical rear panel BNCs (SCALED OUTPUT). One can be connected to an analog device (*e.g.*, oscilloscope) and the other to the computer's A/D converter, allowing the simultaneous sampling of outputs as the computer is delivering commands through its D/A converters. Several voltage and current output BNCs are also available. (For details see *Output BNCs* in the **REFERENCE GUIDE: INSTRUMENT OPERATION**.)

While you are busy conducting experiments, it is easy to forget to record the settings of switches that specify the input signal selected and the scaled output, making it impossible to analyze the data. Therefore, the GeneClamp telegraphs this information via an RS-232 connection. Almost any computer type is appropriate because nearly all computers include RS-232 serial communications. It is possible to have your software interrogate the GeneClamp before recording data in order to automatically determine the logic-controlled switch settings and types of headstages connected at the time the data is recorded. This information can be included with the data record so that analysis programs can read it and, thus, always have the correct information for analyzing the recording. Future versions of Axon Instruments software, such as pCLAMP 6.0, will provide support for the GeneClamp's RS-232 telegraphs.

The GeneClamp is also designed to be readily integrated into a system containing compatible instruments such as the CyberAmps. Since most computers do not have spare serial ports, the serial port on the GeneClamp enables other compatible instruments to be "daisy chained," *i.e.,* connected to a single RS-232 serial port, without modification of the host computer hardware or software.

Note - Power Off Loading

When the computer is off, the analog inputs of the interface present a low impedance load. The output of instruments connected to the interface when it is off will be pulled towards ground. The GeneClamp will not be hurt by this load. However, the peak-to-peak swing of signals from the SCALED OUTPUT BNC may be restricted if the other SCALED OUTPUT BNC is connected to an interface that is switched off. The same considerations hold true for most tape recorders.

Chapter 5

Low-Noise Patch-Clamp Recording Techniques

To realize optimal noise performance, the user must pay close attention to noise sources. This is because the total rms noise is the square root of the sum of the individual squared rms noise sources. This means that any large noise source will dominate the total noise and make other noise sources insignificant. Therefore, all potentially contributing noise sources must be minimized. Specifically, the headstage, the pipette glass, the holder and the seal contribute significant noise even under circumstances where extraneous noise pickup from the environment is negligible. It is absolutely crucial that the entire preparation be properly shielded, and that hum from power supply, mains and other sources be negligible, *i.e.*, $< 0.1\ pA_{p\text{-}p}$. (Actually, $<0.01\ pA_{p\text{-}p}$ is possible with some effort.) In this section, we suggest some approaches to low-noise recording of single channels.

Glass Type and Coating

The noise from pipette glass itself arises from the lossy characteristics of its walls[1]. Therefore, it is expected that glasses with the lowest inherent dielectric loss will have the lowest noise. Generally, the thicker the wall, the lower its noise. These expectations have been largely born out by actual experiments. Table 1 presents the specifications from a large number of commercially available glasses that have been used for patch voltage clamping. Each of these glasses has been shown to be sealable to cell membranes in several different cells. Aluminosilicate glasses like Corning #1723 and high-lead glasses like Corning #8161 are particularly noteworthy for their low inherent noise but have not found much acceptance for use in patch-clamp studies. Aluminosilicate glasses are hard to pull because of their high softening temperature and some high-lead glasses have been reported to modify channel currents. Since any glass may potentially modify channel currents, one must be aware of this fact and control for it regardless of the glass one uses. We recommend two glasses: Corning #7052 and quartz. Both have been successfully sealed to many different cell types. Quartz, with its significantly lower-loss factor, has produced the lowest noise recordings known to us. However, because of its extremely high-softening temperature, quartz requires a special puller like the P-2000 from Sutter Instrument Company.

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1 When a sine voltage is applied across a perfect dielectric, the alternating current should be 90° out of phase with the voltage. The deviation from 90° is the "loss factor". The loss factor is related to the power dissipated in the dielectric. Since energy is lost in the dielectric, dielectrics (*e.g.*, glasses) are commonly referred to as "lossy".

Pipettes can be obtained from specialty glass houses like:

Clark Electromedical Instruments, P.O. Box 8; Pangbourne, Reading; RG8 7HU, England; (734) 843888

Garner Glass; 177 S. Indian Hill Road; Claremont, California 91711 USA; (909) 624-5071

Jencons Scientific, Ltd.; Cherycourt Way Industrial Estate; Stanbridge Road; Leighton Buzzard, Bedfordshire; LU7 8UA, England; (0525) 372010

Sutter Instrument Company; 40 Leveroni Court; Novato, California 94949 USA; (415) 883-0128

Even if one uses electrically superior glasses, low noise will not be obtained unless the outer surface of the glass is coated with a hydrophobic substance, such as Dow Corning Sylgard #184, dental wax or Sigmacote (a silanizing agent from Sigma Chemical Co.). These substances prevent the bathing solution from creeping up the outer wall of the pipette glass. This is important since a thin film of solution on the outer surface of the glass produces a distributed resistance that interacts with the glass capacitance to produce a noise source that rises with frequency. Since it becomes the dominant noise source, it must be eliminated. While many hydrophobic substances have been used, none, to the best of our knowledge, produces low-noise characteristics equal to Sylgard #184. Sylgard also decreases the capacitance of the pipette wall and so reduces the lossiness of the wall as well. It has been shown experimentally that Sylgard will improve the noise of any glass but it will not turn a poor electrical glass into a good one. Low-loss glasses coated with Sylgard give significantly less noise than poor glasses coated with Sylgard. Obviously, the closer to the tip that the Sylgard can be painted the lower the noise.

Glass	Loss Factor	Log_{10} Volume Resistivity	Dielectric Constant	Softening Temp. °C	Description
7940	.0038	11.8	3.8	1580	Quartz(fused silica)
1724	.0066	13.8	6.6	926	Aluminosilicate
7070	.25	11.2	4.1	----	Low loss borosilicate
8161	.50	12.0	8.3	604	High lead
Sylgard	.58	13.0	2.9	----	#184 Coating cmpd.
7059	.584	13.1	5.8	844	Barium-borosilicate
7760	.79	9.4	4.5	780	Borosilicate
EG-6	.80	9.6	7.0	625	High lead
0120	.80	10.1	6.7	630	High lead
EG-16	.90	11.3	9.6	580	High lead
7040	1.00	9.6	4.8	700	Kovar seal borosilicate
KG-12	1.00	9.9	6.7	632	High lead
1723	1.00	13.5	6.3	910	Aluminosilicate
0010	1.07	8.9	6.7	625	High lead
7052	1.30	9.2	4.9	710	Kovar seal borosilicate
EN-1	1.30	9.0	5.1	716	Kovar seal borosilicate
7720	1.30	8.8	4.7	755	Tungsten seal borosilicate
7056	1.50	10.2	5.7	720	Kovar seal borosilicate
3320	1.50	8.6	4.9	780	Tungsten seal borosilicate
7050	1.60	8.8	4.9	705	Series seal borosilicate
KG-33	2.20	7.9	4.6	827	Kimax borosilicate
7740	2.60	8.1	5.1	820	Pyrex borosilicate
1720	2.70	11.4	7.2	915	Aluminosilicate
N-51A	3.70	7.2	5.9	785	Borosilicate
R-6	5.10	6.6	7.3	700	Soda lime
0080	6.50	6.4	7.2	695	Soda lime

Table 1. Electrical and thermal properties of different glasses

Holders

The holders supplied with the GeneClamp are made of polycarbonate, which experimentally has been found to produce the lowest noise of about ten substances tested. It was only slightly better than polyethylene, polypropylene or Teflon, but was much better than nylon, Plexiglass or Delrin. Axon Instruments holders avoid metal and shielding, which are noise sources. Holders, however, do become a significant noise source if fluid gets into them. Therefore, great care must be taken when filling patch pipettes with solution. They should be filled only far enough from the tip so that the end of the internal reference patch pipette is immersed. Any solution that gets near the back of the patch pipette should be dried with dry air or nitrogen to keep it from getting into the holder. Holders that become contaminated with solution should be disassembled and sonicated in ethanol or pure deionized water, and then allowed to dry thoroughly before being used again. It is also a good idea to periodically clean the holders by sonication even if no fluids have been observed in them.

The cleanliness of the holder can be checked before each attempt to make a seal. When the holder with a filled patch pipette has been inserted into the headstage connector, and the patch pipette tip is positioned just above the bathing solution, the rms current noise seen on the meter of the GeneClamp (meter switch in the I3 rms position) should not significantly exceed 1 pA.

Seal

The seal will usually be the dominant noise source if it is only a few gigohms. Seal resistances in excess of 20 GΩ must be obtained if exceptionally low-noise single-channel recordings are to be routinely achieved. Seal quality can be monitored by periodically observing the GeneClamp rms noise meter (I3 rms). The noise depends also on the depth of the patch pipette tip below the surface of the bathing solution since the effective patch pipette capacitance increases as the depth of immersion increases. The voltage noise of the headstage interacts with the patch pipette capacitance to produce a noise source that rises with frequency.

Signal Generator

One last potential noise source to consider is the noise in the signal generator that provides the command. In the GeneClamp we have succeeded in minimizing this noise by heavily attenuating the external command. However, it is possible for this noise source to be significant, particularly if the command signal comes from a computer-based D/A converter.

Chapter 6

Reference Guide

Instrument Operation

The major topics in this section are organized alphabetically.

Capacitance Neutralization

Capacitance neutralization is used to compensate for the decreased frequency response of Headstage #1 arising from the combination of the resistance and input capacitance of the micropipette (see **REFERENCE GUIDE: PRINCIPLES OF OPERATION**). In general, the relatively low resistances of micropipettes used with *Xenopus* oocytes (0.5-5 MΩ) mean that this filtering effect is negligible. However, if it does become a problem (*e.g.*, if the micropipette #1 resistance exceeds about 10 MΩ), the GeneClamp can carry out capacitance neutralization for Headstage #1. Pushing the TUNE button causes a -10 nA square wave at the line frequency to be applied at Headstage #1. In SETUP mode, if there is appreciable filtering due to the input capacitance, V1 monitored on an oscilloscope will appear to be rounded (Figure 1-*A*) rather than a square wave.

Turn the CAPACITANCE NEUTRALIZATION knob slowly clockwise until the V1 waveform sharpens up, but does not overshoot (Figure 1-*B*). The appearance of an overshoot indicates that the positive feedback circuit used in the neutralizing circuit is on the verge of instability, and the user should decrease the amount of neutralization used.

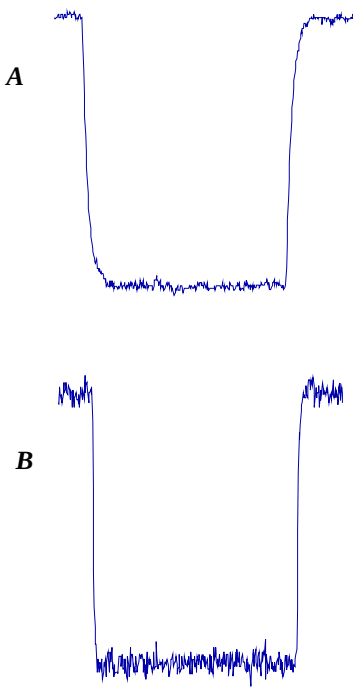


Figure 1. Adjusting capacitance neutralization
V1 waveform prior to ***A*** and after ***B*** applying capacitance neutralization. A high resistance electrode modeled with a 50 MΩ resistor was connected to an HS-2A-x0.1LU headstage.

Command Currents

Command currents can be applied from two internal sources: TUNE and DC CURRENT COMMAND; and two external sources: E #1 CURRENT COMMAND and E #2 CURRENT COMMAND. All sources can be active in SETUP mode, while in CLAMP mode they are inactive.

Tune

The TUNE command generator injects a -10 nA square wave at line frequency through the micropipette connected to Headstage #1 for the purpose of adjusting the negative capacitance compensation, as described previously.

DC Current Command

This control allows the user to inject a steady current down Electrode #1 for current clamping. The value is set by a ten-turn potentiometer which adjusts the DC current clamp value up to one hundred times the headstage current gain (*e.g.*, 100 nA for the HS-2A-x1LU headstage) whose polarity is set by the (+) and (-) buttons. The appropriate span of the potentiometer in nA/turn for a given headstage is read by the microprocessor and indicated by illuminating the 1, 10 or 100 indicators above the command knob. The DC CURRENT COMMAND sums with the external E #1 CURRENT COMMAND and the TUNE oscillator to produce the final command current.

External Current Commands

Two BNC inputs on the back of the GeneClamp (E #1 CURRENT COMMAND and E #2 CURRENT COMMAND) allow the user to inject current down the electrodes connected to Headstage #1 and Headstage #2, respectively. The current injected is given by 10x GAIN nA/V, where GAIN is the headstage current gain (*e.g.*, 10 nA/V for the HS-2A-x1LU headstage).

Command Potentials

Command potentials can be obtained from six internal sources: HOLDING POTENTIAL, TEST (FAST), TEST (SLOW), DC COMMAND POTENTIAL, SEAL TEST (10 mV), and SEAL TEST (100 µV). The first three internal sources are used in TEVC mode, while the other three are used in the patch clamp configuration. In addition, command potentials can be obtained from external sources via three rear panel BNCs: VOLTAGE CLAMP COMMAND (20 mV/V), E #3 PATCH COMMAND (20 mV/V) and E #3 PATCH COMMAND (200 mV/V). The first two external commands are used for TEVC and patch clamping, while the third is useful for voltammetry where there is a need for a larger command voltage (up to ± 1 V).

TEVC Section

Holding Potential

This control allows the user to apply a holding potential in the range of ±1,000 mV whose polarity is set by the (+) and (-) buttons. The value is set via a ten-turn potentiometer whose output is passed through a low-pass filter and buffer to reduce noise and preserve potentiometer linearity. Turn the control fully counterclockwise for a zero command. The output from the filtered and buffered HOLDING POTENTIAL potentiometer is summed with the external VOLTAGE CLAMP COMMAND (20 mV/V) and TEST to form the command potential for the TEVC mode.

TEST Oscillators

This control allows the investigator to apply a -10 mV square wave at either line (FAST) or 1/10 line frequency (SLOW) for the purpose of adjusting the voltage-clamp GAIN and STABILITY controls for an optimal voltage clamp.

Patch Section

DC Command Potential

This control allows the user to apply a membrane holding potential in the range of ±1,000 mV whose polarity is specified by the (+) and (-) buttons. The value is set via a ten-turn potentiometer whose output is passed through a 10 ms low-pass filter and buffer to reduce noise and preserve potentiometer linearity. Turn the control fully counterclockwise for a zero command. The DC command potential is automatically switched off if the ZERO I3 button is pressed. This signal is mixed with the two external commands [E #3 PATCH COMMAND (20 mV/V) and E #3 PATCH COMMAND (200 mV/V)] and SEAL TEST to form the command voltage for the patch headstage.

Seal Test

The SEAL TEST command generators provide a convenient source of test pulses to be used during seal formation. Two values of a test pulse at the line frequency are available: 10 mV and 100 µV. The 100 µV signal is useful for initial monitoring of the seal formation, while the 10 mV signal is useful for adjusting the patch pipette capacitance compensation. Note that the SEAL TEST signal is added to any other command potentials active, such as the DC COMMAND POTENTIAL.

DC Meters

There are two displays on which selected data can be displayed under microprocessor control. The signals available for display are V1, I3 rms, V3, R1, R2, and R3 on the top meter and I1, I2/V2, and I3 on the bottom display. In the case of the I2/V2 display, V2 is displayed in SETUP mode, while I2 is displayed in CLAMP mode. In all cases the illuminated unit display corresponds to the function selected and the headstages used. Overload on any meter is indicated by "EEE" if it is a positive overload or "-EEE" if it is a negative overload.

I3 rms Measurement

To obtain the I3 rms reading, the I3 current signal is AC coupled at 20 Hz and then low-pass filtered at 5 kHz using a four-pole Butterworth filter. The output signal is then rms converted before being displayed on the panel meter. The R1, R2 and R3 signals are derived from the response of the selected probe to a generated test signal, as described below. The middle button on the lower meter is bimodal so that in SETUP mode it displays V2 and in CLAMP mode it displays I2.

Microelectrode Resistance Measurement

The GeneClamp contains microprocessor-controlled circuitry for measuring the resistance of both micropipettes and a patch pipette. R1, R2 and R3 buttons in the DC Meter section execute this function when pressed. In addition, the R3 button can be used to measure the seal resistance of a patch. The upper panel meter will display the resistance in the appropriate units (*i.e.*, MΩ or GΩ). Pressing the button again returns the display to whatever was previously displayed. Pressing R1 or R2 induces a step current of 1 nA x GAIN (headstage current gain), measures the response voltage and calculates the resistance using Ohm's law. For R3, a voltage step is applied to the patch pipette, the resulting current is measured and the resistance is calculated using Ohm's law. In this case, where the resistance can span several orders of magnitude from 0.1 MΩ (a macropatch pipette in the bath) to 200 GΩ (a good seal) the microprocessor determines the appropriate test voltage to apply, ranging from 20 µV to 20 mV, in order to accurately measure the resistance.

The command for either resistance test is toggled on and off (at approximately 3 Hz) and the resistance is calculated using:

$$R = \frac{\Delta V}{\Delta I}$$

Calculating the resistance in this manner eliminates errors due to offsets or DC currents. If R1 or R2 is pressed in VOLTAGE CLAMP mode, a beep will sound. Micropipette resistance can only be measured in SETUP mode.

NOTE: When the CV-5-100MU is connected to the GeneClamp 500B, the units display will be blank when the resistance measured is less than 10 kΩ. The units are kilohms even though the display is blank.

Headstages

Three classes of headstages are available for use with the GeneClamp. The HS-2A and VG-2A headstages are standard and are used in the TEVC configuration, while the CV-5 headstages are optional and are used in the patch clamp configuration. There are several versions of the HS-2A and CV-5 headstages, each suited for specific applications. Alternative headstages are simply plugged into the appropriate connector on the back of the GeneClamp, which automatically recognizes the headstage and adjusts its readouts accordingly. The properties of the headstages are described in detail in the *Headstages* section of **REFERENCE GUIDE: PRINCIPLES OF OPERATION**.

NOTE: HS-4 series headstages can be connected to the GeneClamp. A virtual ground headstage, VG-2, must be used to measure the current. This is because the current-measuring resistor is switched out of the circuit in the HS-4 headstage when it is connected to the GeneClamp.

Installation

The usual headstage assignment is:

Headstage #1: HS-2A-x1LU
Headstage #2: HS-2A-x10MGU
Headstage #3: CV-5-100GU
Bath clamp: VG-2A-x100

In some cases, other assignments may be desirable. Consult *Headstages* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION** for further details.

Case Ground Connector

The metal headstage case and the 2 mm socket at the rear of the headstage are connected to ground.

Shield Drive Connector

A shield drive is available from the 2 mm gold-plated socket on the front of the x1L, x0.1L, x0.01M and 0.0001M headstages. However, using a driven shield is not recommended, since this increases the high-frequency noise. (See *Headstages* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION** for further details.)

Mounting the Headstage

There are two mounting options provided with the HS-2A and CV-5 headstages. One method is to grip the insulated mounting rod in a micromanipulator. This is the simplest method and is often satisfactory. For maximum mechanical rigidity, you can mount the headstage directly to some manipulators using the acrylic mounting plate located on the bottom of the headstage. Put mounting screws through the four outside holes.

If you are not using the mounting rod, you can remove it by twisting it counterclockwise. If you are not using the mounting plate, you can remove it by removing the four screws holding it onto the headstage case.

Cleaning

To clean salt spills from the headstage connectors, wipe with a damp cloth. Avoid spilling liquids on the headstage. The Teflon input connector should be kept very clean. This can be done effectively by spraying it with alcohol or Freon.

Static Precautions

The headstages can normally be handled safely. However, if you are in a laboratory where static is high, you should touch a grounded metal object immediately before touching the headstage. **You should not switch off power to the GeneClamp when changing micropipettes since this will upset the thermal equilibrium.**

Acoustic Pick-up

Rare instances have been reported where the headstage was susceptible to low-amplitude acoustic pick-up. The most troublesome instance was a situation where the audible hum of a nearby isolation transformer was being acoustically coupled to the input of the headstage. Traced to the silver wire of the microelectrode holder, it was solved by trimming off a fraction of the wire, thus changing its resonant frequency.

Optical Pick-up

The Teflon input connector and the glass walls of the hybrid package inside the CV-5 headstages are translucent. High intensity light can get through in sufficient strength to activate the input transistors inside the hybrid. Therefore, you should avoid allowing bright light to fall on the input connector. If you notice line-frequency hum on the current record, it could be due to fluctuating light levels from a bright fluorescent light or equivalent. In general, low light levels are not a problem.

Other Precautions

You should switch off the power to the GeneClamp before plugging in or removing a headstage.

Holder

Features

The HL-U series holder provides for enhanced low-noise mechanically stable microelectrode recordings with or without suction. Because the new holder provides a universal fit for a very wide range of pipette diameters and will fit any of our redesigned headstages, it is named the HL-U.

The barrel of the holder is made out of polycarbonate for lowest noise. There are two different barrel lengths. The shorter barrel length contributes less to the operating noise and, therefore, is ideally suited for single channel patch clamp recordings. Although the longer barrel will contribute more to the operating

noise, the increased length may provide the needed clearance between the headstage and other components in the experimental setup. Maintenance is simple because the holder can be fully disassembled for cleaning and parts replacement.

Mechanical stability of the pipette is assured in several ways. For example, as the pipette cap is closed, the cone washer is compressed on the pipette from the force applied to the front and back of the cone washer. The holder mates with the special threaded Teflon connector on U-type Axon Instruments headstages and is secured in place with a threaded collar.

The holder is designed to emerge along the long axis of the headstage. A right-angle adapter can be purchased if it is necessary for the holder to emerge at 90° from the headstage.

The HL-U holder is designed to be used with Axon Instruments amplifiers, and fit all U-type CV and HS series of headstages. These headstages have a *threaded* white Teflon collet. To minimize the added noise contributed by the holder in single-channel recording, the holder uses a small (1 mm) pin for the electrical connection and a large amount of insulating Teflon. This noise problem is peculiar to single-channel recording.

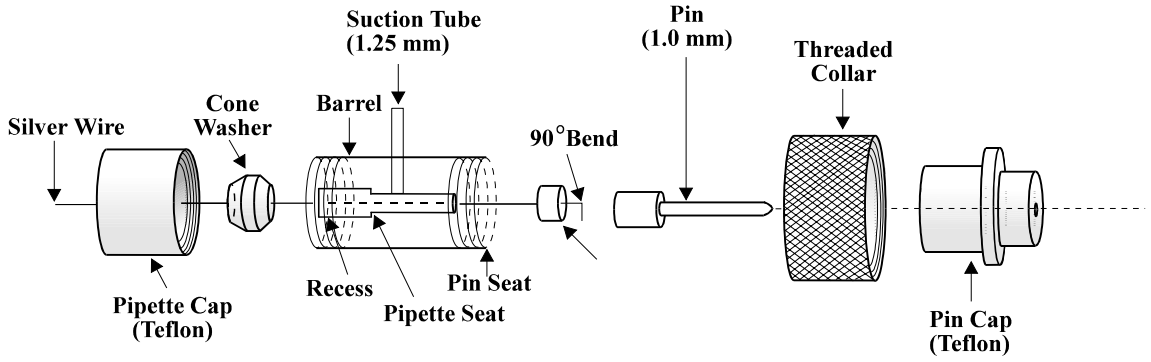


Figure 2. Exploded view of the holder

Parts

The bore size of the HL-U accepts pipettes with an outer diameter (OD) of 1.0-1.7 mm. Pipettes are secured by a cone washer with an inner diameter (ID) that accommodates the pipette OD. Color-coding aids identification of the four sizes of cone washers: 1.0 mm (orange), 1.3 mm (clear), 1.5 mm (orange) and 1.7 mm (clear). Each HL-U is supplied with two barrel lengths, 16 mm and 28 mm.

It has been shown that a Ag/AgCl pellet offers no greater stability than properly chlorided silver wire. Moreover, the diameter of the Ag/AgCl (1 mm) restricts its use to pipettes with a large ID *i.e.*, > 1.1 mm. Therefore, the HL-U is supplied with 0.25 mm silver wire.

Spare components included with each holder are as follows: one 50 mm length of silver wire, 20 cone washers (5 of each size) and one 70 mm length of silicone tubing. Cut into 2 mm lengths, the silicone tubing will yield approximately 30 replacement silicone seals. Additional pipette caps, cone washers, silicone tubing, pins and silver wire can be purchased from Axon Instruments, as well as optional Ag/AgCl pellet assemblies.

Optional Ag/AgCl Pellets

The HL-U holder will accommodate a 1 mm diameter Ag/AgCl pellet that should provide many months of DC-stable recordings. The inner diameter (ID) of the pipette must be > 1 mm. The silver wire is surrounded by a wax-sealed Teflon tube. This ensures that the electrode solution only contacts the Ag/AgCl pellet. Three pellet assemblies are sold as HLA-003.

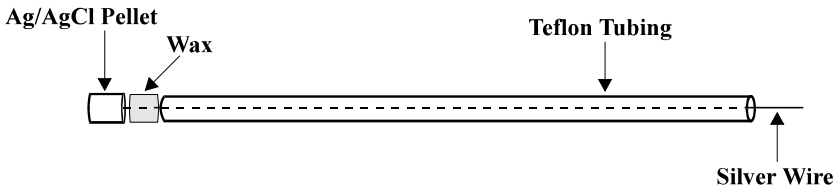


Figure 3. Ag/AgCl pellet assembly

Use

Insertion Of Pipette

Make sure the electrode cap is loosened so that pressure on the cone washer is relieved, but do not remove the pipette cap. Push the back end of the pipette through the pipette cap and cone washer until it presses against the pipette seat. Gently tighten the pipette cap so that the pipette is gripped firmly.

To minimize cutting of the cone washer by the sharp back end of the pipette, you can smooth the pipette edges by rotating the back end of the pipette in a bunsen burner flame before filling the pipette with salt solution.

Cleaning

For lowest noise, keep the holder clean. Frequently rinse the holder with distilled water. If more thorough cleaning is required, briefly wash in ethanol or mild soapy water. Never use methanol or strong solvents.

Filling Pipette

Only the taper and a few millimeters of the shaft of the pipette should be filled with solution. The chlorided tip of the wire should be inserted into this solution. Avoid wetting the holder since this will increase the noise.

Silver Chloriding

It is up to you to chloride the end of this wire as required. Chloriding procedures are contained in many electrophysiology texts[2]. Typically the chlorided wire will need to be replaced or rechlorided every few weeks. A simple, yet effective, chloriding procedure is to clean the silver wire down to the bare metal using fine sand paper and immerse the cleaned wire in CHLOROX bleach for about

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[2]For easy-to-use recipes see Microelectrode Methods for Intracellular Recording and Ionophoresis, by R.D. Purves, London: Academic Press, 1981, p. 51.

The Axon Guide. Foster City, CA: Axon Instruments, Inc., 1993, p. 83.

20 minutes, until the wire is uniformly blackened. This provides a sufficient coat of AgCl to work reliably for several weeks as an internal reference pipette. Drifting or otherwise unstable offsets during experiments is suggestive of the need for rechloriding. The chlorided region should be long enough so that the pipetted solution does not come in contact with the bare silver wire.

Heat smoothing the back end of the pipette extends the life of the chloride coating by minimizing the amount of scratch damage. Another way to protect the AgCl coating is to slip a perforated Teflon tube over the chlorided region.

The chlorided region should be long enough so that the pipette solution does not come in contact with the bare silver wire.

Replacing the Silver Wire

To replace the silver wire, insert the nonchlorided end through the hole of the silicone seal and bend the last 1 mm of wire over to an angle of 90°. Press the wire into the back of the barrel making sure that the silicone seal is flush with the back of the barrel. Slip the threaded collar over the back of the barrel. With the large end of the pin directed toward the bent-over wire screw the pin cap down firmly, but without excessive force. This assures good electrical contact. Screw the pin cap down firmly but without excessive force.

Glass Dimensions

Use the HL-U for pipettes with outside diameter (OD) of 1.0-1.7 mm. The optimal dimensions should match the inner diameter (ID) of the four sizes of cone washers, 1.1, 1.3, 1.5 and 1.7 mm. When the pipette OD falls between two sizes of cone washers, the larger size cone washer should be used. For instance, if the pipette OD is 1.6 mm, then use a cone washer with an ID of 1.7 mm.

Adapters

HLR-U right-angle adapters allow the HL-U series holder to emerge at 90° from the headstage. Use the HLR-U with the HL-U holder.

HLB-U BNC-to-Axon adapter allows conventional BNC-type holders to be used with Axon Instruments U-type headstages. Use the HLB-U with all U-type CV and HS headstages (*e.g.,* CV-4-1/100U and HS-2A-x1MGU). These headstages have a threaded white Teflon collet.

Input Section

Input BNCs

E #1 CURRENT COMMAND

This input has a scale factor of 10 x GAIN nA/V, where GAIN is the headstage current gain. The headstage current gain, GAIN, is coded in the headstages and is indicated on the 1, 10 and 100 DC CURRENT COMMAND indicators on the GeneClamp's front panel. This input is active only in SETUP mode. Current is delivered by the micropipette connected to Headstage #1.

E #2 CURRENT COMMAND

This input has a scale factor of 10 x GAIN nA/V, where GAIN is the headstage current gain. This input is active only in SETUP mode. Current is delivered by the micropipette connected to Headstage #2.

E #3 PATCH COMMAND (20 mV/V)

This is a low-gain command input which sums at the scale factor of 20 mV/V with the front panel commands to form the command voltage. This is the standard input for patch clamping.

E #3 PATCH COMMAND (200 mV/V)

This is a high-gain command input which sums at the scale factor of 200 mV/V with the front panel commands to form the command voltage. This input is useful for cyclic voltammetry.

E #3 SPEED TEST

When switched on by the rear panel switch, the Speed Test injects a current into the headstage input via a 1 pF capacitor in the headstage. Injected current waveform is the derivative of the voltage waveform applied at SPEED TEST input. For example, a 100 Hz 10 V_{p-p} triangle wave will inject a 1 nA_{p-p} square wave into the headstage input. This test is used for verifying the dynamic response of the headstage.

VOLTAGE CLAMP COMMAND

This external voltage-clamp command has a scale factor of 20 mV/V. It sums with the front panel HOLDING POTENTIAL potentiometer signal to form the command signal for the two-electrode voltage clamp.

OVERDRIVING EXTERNAL COMMANDS

The maximum usable limits for safely overdriving the External commands are:

Electrode #1:	±50 V	5 µA	typical
Electrode #2:	±50 V	50 µA	typical
V-clamp:	±50 V	1.0 V	at cell
200 mV/V Patch clamp:	±12 V	2.4 V	at pipette

Microprocessor Control

The GeneClamp is a sophisticated instrument made easy to operate by an onboard microprocessor. By controlling all of the switches, the microprocessor increases the number of features without significantly increasing the complexity of operation. The automation allows you to zero the electrode offset, measure the electrode resistance, measure the seal resistance, subtract leakage current, or zero the output by the touch of a button. Settings for each output signal are retained in memory, restored when the output selection is recalled, or updated when requested.

The microprocessor is always actively polling for an RS-232 command, or a front-panel push-button command. In addition, the microprocessor reads a memory chip (EEPROM) or coding resistor in each headstage, accessing information such as the value of the headstage current-passing resistor. This information, along with the settings of the scaled output, is used to automatically compute and display the correct units for the current outputs and to properly calibrate the resistance measurement.

The microprocessor provides for logical switch selection. Logic switching selects the gain, the Bessel filter frequency and the input signal. The values for each scaled output signal selection are kept separate so that the appropriate value is restored immediately when the input signal selection is changed.

Many operational errors, such as requests that would result in conflicting settings, are automatically circumvented. In addition, two tones of different durations alert the user to the most common operational errors likely to be encountered with any clamp. A long-duration tone is triggered when the function requested is valid but the data are out of range. A medium-duration tone alerts the user that the function is inactive because the headstage is not connected.

When the instrument is switched on, it will always power up in the same state that it was in when it was turned off, except for the mode, which will always be SETUP. Leak subtraction circuitry and output zeroing will always be off during power-up. This prevents their inadvertent use and corruption of experimental results.

The microprocessor and host computer have control over many of the commands and controls, but not the external commands. The only controls that are purely manual and cannot be mimicked by a host computer are: the voltage-clamp GAIN, STABILITY, Electrode #1 CAPACITANCE NEUTRALIZATION and Electrode #3 CAPACITANCE COMPENSATION.

Special Switch Combinations

By pressing special switch combinations it is possible to disable the "click" keypress tones and most of the front panel lights. This may be useful when the clicks or lights are of sufficient intensity to interfere with experiments involving the auditory or visual systems.

To disable the "click" on each keypress, press LEAK SUBTRACT, OUTPUT ZERO and ZERO V1 simultaneously. The disabled mode is maintained even after the instrument is switched off. Pressing the same combination re-enables the "click."

Pressing LEAK SUBTRACT, OUTPUT ZERO and SETUP simultaneously disables all of the front panel lights except for the RMP balance and the OVLD. (Since these two lights are directly driven from the analog circuitry, they cannot be controlled by the microprocessor.) To bring the lights back, press any meter button. The lights will be re-instated at power up.

Mode Selection Section

This section controls the SETUP/CLAMP modes. In the SETUP mode, Headstages #1 and #2 function as independent voltage-measuring circuits and can be used for current clamping. In VOLTAGE CLAMP mode, Headstages #1 is for voltage monitoring and Headstage #2 is for current-passing. To be consistent with the rest of the push buttons, pushing either button toggles that mode. However, the OFF state of both buttons is

defined to be SETUP, so multiple pressing of the SETUP button will stay in SETUP mode, while multiple pressing of the VOLTAGE CLAMP button will alternate between VOLTAGE CLAMP and SETUP. In theory, the SETUP button is superfluous, but one can consider it to be an escape button to unconditionally return to SETUP mode.

The GeneClamp's microprocessor allows only those commands and inputs used with a particular mode to be active in that mode. For example, in VOLTAGE CLAMP mode, all current clamp command inputs are disabled.

Output Section

Scaled Output

All the controls in the Output Section affect only the signal on the Scaled Output. Only one signal can be processed at a time.

A selected signal may be filtered, zeroed and amplified. In addition, I2 and I3 can also have leak subtraction applied to them to eliminate any step leak current due to the command signal. The input to this section is selected using the push-button controls in the SCALED OUTPUT section. Selectable input signals are V1, V2, V3, I1, I2 and I3. Each input selection has a gain, zero, filter frequency and (if applicable) leak subtraction value associated with it retained in memory, and switching the input source will automatically bring in those values associated with the selected signal. The signal path is LEAK SUBTRACT → FILTER → OUTPUT ZERO → GAIN → SCALED OUTPUT BNCs. The scaled output overload LED (OVLD) monitors the output signal at both the pre- and post-filter and will illuminate if the output exceeds 11 volts.

Filter

The filter is a 4-pole low-pass Bessel filter. The attenuation of signals and noise above the -3 dB frequency is 80 dB/decade (24 dB/octave). The Bessel characteristic is suitable for patch and voltage clamping because it introduces < 1% overshoot. The -3 dB frequency can be selected from among 12 different values using push buttons. The selected value is illuminated. The filter cutoff frequencies are: 10 Hz, 20 Hz, 50 Hz, 100 Hz, 200 Hz, 500 Hz, 1 kHz, 2 kHz, 5 kHz, 10 kHz, 20 kHz and 50 kHz.

All low-pass filters slow the rise time of the signal. For filters with < 10% overshoot, the 10-90% rise time is approximately:

$$t_r = \frac{0.35}{f_c}$$

where f_c is the -3 dB frequency in Hertz. For example, the 10-90 % rise time of a 1 kHz filter is approximately 350 µs.

NOTE: If you use an external filter: Some manufacturers specify the -3 dB frequency based on the phase response of the filter instead of its amplitude response, or based on a straight line approximation to the filter characteristics instead of the actual characteristics. You should check your external filter by checking t_r for a step signal applied to its input.

When a signal with 10-90% rise time t_1 is passed through a filter with 10-90% rise time t_2, the rise time of the output signal is approximately:

$$t_r = \sqrt{t_1^2 + t_2^2}$$

Gain

The filtered signal is passed onto an amplifier whose gain is 0.1, 1, 10 and 100. The gain is selected using push buttons, and the selected value is illuminated.

In voltage- and patch-clamp amplifier design, there are conflicting concerns when deciding where to place the Bessel filter and the gain amplifier in the signal pathway. On the one hand, to minimize the noise contribution of the circuitry of the Bessel filter itself, the gain amplifier should come first. On the other hand, if the gain amplifier comes first, the peaks of the amplified signal and noise might get clipped if the gain setting is high. After filtering, it is difficult to distinguish the clipped signals from biological channel currents.

The very effective compromise solution adopted in the GeneClamp 500B is to adjust the pre- and post-filter gain according to the headstage(s) used and total gain requested. This task is automatically performed by the microprocessor.

Leak Subtraction

Leak subtraction involves subtracting a percentage of the command voltage from the response output to remove any DC leakage component of the output. This operation is only valid on the SCALED OUTPUT signal, and only if I2 or I3 is selected. Invalid selection will result in an error beep. Selection of any other signal for the scaled output will automatically disable LEAK SUBTRACT. Re-selection of either I2 or I3 for scaled output will reinstate the previous values for LEAK SUBTRACT. When UPDATE is pressed, a series of twelve x50 ms long, 10 mV hyperpolarizing pulses are applied; the response is measured and the microprocessor automatically determines the scaling factor for the leak subtraction. This entire process takes about 1.2 s. If the LEAK SUBTRACT button is held down for longer than this, the leak resistance is displayed on the top meter until the button is released or 2 seconds have elapsed, whichever is shorter. If the properties of the cell or patch change such that the current leak resistance correction is incorrect, pressing UPDATE will recalculate the scaling factor.

Since the internally generated leak subtraction test pulses are summed with the other command sources, the front panel HOLDING POTENTIAL and the external VOLTAGE CLAMP COMMAND for I2, or the front panel DC COMMAND POTENTIAL and the E #3 PATCH COMMAND for I3, the user must not change the other command sources while the leak subtract calculation is being performed. In addition, the algorithm will not work if I2 or I3 are saturated.

Output Zero

This feature removes any DC component of the output signal. Press OUTPUT ZERO to activate this section at the last update value. Press UPDATE to remove the DC level. If OUTPUT ZERO is off, pressing UPDATE will simultaneously switch it on. Toggling OUTPUT ZERO toggles the last value in and out, but does not update it. If OUTPUT ZERO is active, pressing the OUTPUT ZERO button will deactivate this function and the offset returns. However, the zeroing value is retained in memory and reactivating the function will again zero the output. Each selection has its own stored

zero, so changing the signal selection will automatically bring in the zeroing value associated with the selected input.

Overload Light

The overload (OVLD) light illuminates if either the input to the filter or the output of the final gain amplifier exceeds ±10 V for longer than 100 µs. This signifies that the signal output is being clipped and has become non-linear.

Output BNCs

All output signals are low-pass filtered at 100 kHz by 500 Ω // 3,300 pF passive filter. Thus the output resistance is 500 Ω. GAIN refers to the headstage current gain (*e.g.*, gain is ten for a x10MG headstage).

SCALED OUTPUT

Two identical outputs provide the SCALED OUTPUT signal as processed according to the front panel controls. The two outputs are connected in parallel but they have independent passive filters.

10 x V1 OUT

Provides an amplified version of the Headstage #1 voltage (V1) summed with the contribution from the zeroing circuit. Thus, the output will be forced to zero when the ZERO V1 button is pressed in SETUP mode.

10 x V2 OUT

Provides an amplified version of the Headstage #2 voltage (V2) summed with the contribution from the zeroing circuit. Thus, the output will be forced to zero when the ZERO V2 button is pressed in SETUP mode.

0.1 x V2 OUT

Provides an attenuated version of Headstage #2 voltage (V2) so the high voltage signal during TEVC can be connected to a device whose input range is restricted to ±13 V. This output is summed with the contribution from the zeroing circuit. Thus, the output will be forced to zero when the ZERO V2 button is pressed in SETUP mode.

5 x V3 OUT

Provides an amplified version the patch clamp command voltage.

I_{BATH} OUTPUT

The bath current from the VG-2A bath clamp headstage has a scaling factor of $\frac{10}{GAIN} V / \mu A$. If no bath probe is installed, this output is grounded. For example, the scaling factor is 0.1 V/µA for the VG-2A x100 headstage.

I1 OUT

Headstage #1 current (I1). It has a gain of $\frac{10}{\text{GAIN}} \text{V} / \mu\text{A}$.

0.1 x I2 OUT

Scaled version of the non-saturating Headstage #2 current 0.1 x I2. It has a scaling factor of $\frac{1}{\text{GAIN}} \text{V} / \mu\text{A}$.

I3 OUT

Headstage #3 current summed with the contribution of the zeroing circuit. Thus, the output will be forced to zero when the ZERO I3 button is pressed. It has a scaling factor of 1 x GAIN V/nA.

Push-Button Controls

All functions, command polarities, display options and signal conditioning are controlled via the push-button controls on the front panel. Only certain buttons are usable at a given time depending on the mode selected. The microprocessor "knows" which ones can be used and will produce an error tone if a disallowed button is pushed (see below). Unless otherwise specified, pressing an illuminated button will toggle the selected function and cancel any associated functions. For example, if the ZERO V1 button is pushed, the (+)/(-) DC CURRENT COMMAND and TUNE functions are disconnected and then Electrode #1 is zeroed. The CURRENT COMMAND and TUNE functions remain disconnected after the zeroing has been performed. Detailed descriptions of the functions of the various buttons are described in the appropriate sections of the manual.

A beeper is incorporated in the unit which has 3 functions:

1. Whenever a button is pressed, the beeper will issue a 10 ms long "click" to give an audible confirmation that the button has been pushed.
2. If a button is illegally pressed, a short beep (200 ms) will result. As an example, pressing the ZERO V1 button in CLAMP mode will give this response.
3. If a button is legally pressed, but the function cannot be performed, then a long beep (600 ms) will result. As an example, pressing ZERO I3 when the probe has no leakage load will result in failure to zero the current.

RS-232 Serial Port

The control interface between the host computer and the GeneClamp 500B control transfers instructions to the GeneClamp and status information to the computer. This connection makes use of the extremely convenient RS-232 serial interface. With this interface, it is easy to connect the GeneClamp to any host computer, provided that appropriate data acquisition software is available. The RS-232 output can be used to telegraph information about the SCALED OUTPUT filter, gain, units and input signal selection to the host computer. The RS-232 input, in turn, can be used to set all of the logic-controlled switches.

All communications are initiated by the host. Communications on the serial port must operate at a speed between 300 bits per second (baud) and 19200 baud. To further ease the interface task, the intelligent RS-232 circuitry in the GeneClamp automatically conforms to the baud rate of the host computer. There are

no complicated switches or jumpers to set; the GeneClamp simply works at the existing speed and parity of the RS-232 port of the host computer.

The GeneClamp is also designed to be integrated into a system containing other compatible instruments (*e.g.*, CyberAmp). Since most computers do not have spare serial ports, the serial port on the GeneClamp enables other compatible instruments to be "daisy chained", *i.e.,* connected to a single RS-232 serial port, without modification of the host computer hardware or software. The expandable RS-232 bus used by the GeneClamp is slightly non-standard. The transmit lines have been protected by diodes so that multiple instruments can be connected without damage to the same serial port. A rate of 19200 baud can be sustained if the total daisy-chained cable length is less than 6 m (20 feet). Longer cable lengths are possible if the baud rate is reduced.

All instruments continually listen for commands from the host. An instrument will only respond if the command specifies the instrument's address. This is not a general-purpose bus. It relies on the principle that an instrument will not speak unless spoken to by the host. Only one host is permitted on the Axon Expandable RS-232 bus.

Up to ten compatible instruments may be "daisy chained" to one computer serial port. The RS-232 OUT port of the first device is connected to the RS-232 IN port of the next device, and so on. The interconnection cable should be a simple 25-pin flat cable, such as the RS232-02 cable from Axon Instruments. Most plotter cables (also known as null modem cables) suffice for the communications link from the host to the first instrument on the bus. The RS232-01 cable from Axon Instruments is specifically designed for this task. Complete wiring information for the cable is given in the **RS-232 PORT COMMUNICATIONS** chapter for those users who wish to make their own cable.

So that the host computer can distinguish between the instruments, each one has a switch on the back that must be set to a unique address between zero and nine. Device addresses can be allocated randomly as long as the addresses are unique.

RS-232 Port Communications

Communications with the GeneClamp are made via the RS-232 serial port. All commands and responses are in plain ASCII. Certain conventions adhered to by the GeneClamp and other Axon products are described in this section and in the **SOFTWARE CONTROL** chapter.

All instruments on the Axon Expandable RS-232 bus must use the "AT" command protocol described on page 90. To enable the GeneClamp to determine the baud rate, each command string from the host computer must commence with the letters "AT". The GeneClamp 500B calculates the baud rate from the first bit in the letter "A". It calculates the parity from the letter "T". Parity can be even, odd, high or low. One or more stop bits are acceptable.

Serial Communications Cables

To connect to a PC compatible with either a 9-pin or a 25-pin D connector, we recommend using Axon Instruments RS232-01 cable or a "null-modem" cable from a good vendor. This is the type of serial cable used to connect a computer to a pen plotter. It is not the type of cable normally used to connect a modem.

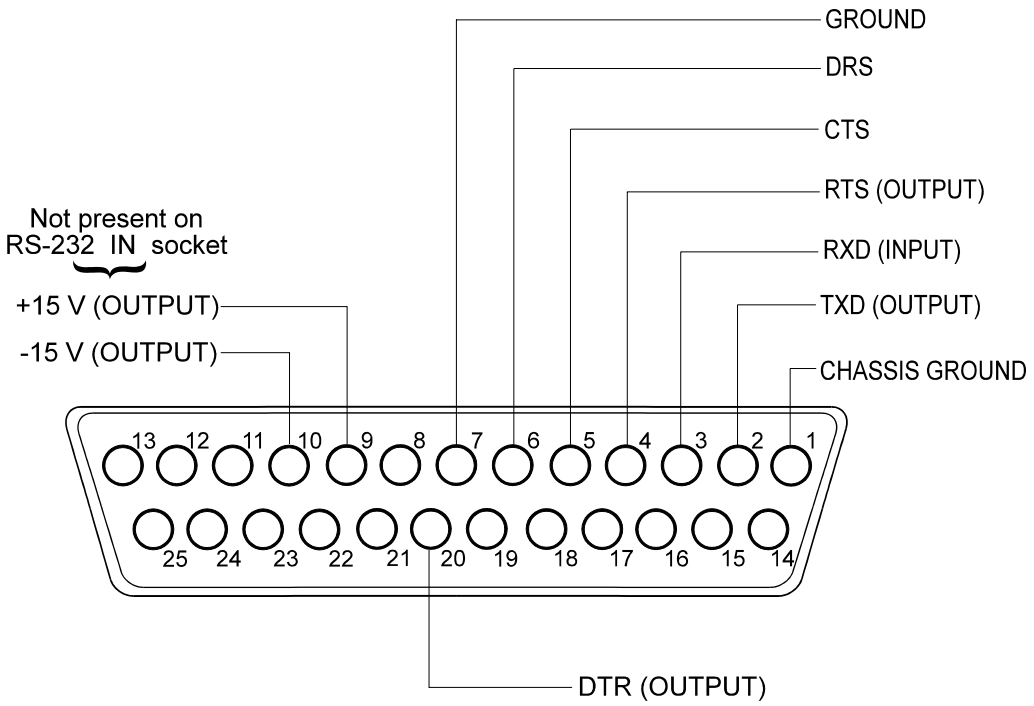


Figure 4. Pin Connections of the DB25 Female RS-232 IN and OUT Connectors

With a Macintosh II, most serial printer cables should work well with the GeneClamp. The RS232-03 cable from Axon Instruments is suitable for connecting the GeneClamp to a Macintosh II.

If problems arise first check the cable connections with a meter to make sure that they correspond to the connections shown in the table below. Most serial communications problems stem from an incorrectly wired cable furnished (at low cost) by some vendors.

To construct a cable to connect a computer to the GeneClamp, the minimum set of connections are as follows.

For an IBM PC-AT with a 9-Pin Connector

IBM 9-pin			**25-pin GeneClamp RS-232 IN**	
Pin number		*connected to*	*Pin number*	
2	(RXD)		2	(TXD)
3	(TXD)		3	(RXD)
5	(GND)		7	(GND)
6 and 8	(DSR, CTS)		20	(DTR)

For an IBM PC or PC/XT with a 25-Pin Connector

IBM 25-pin			**25-pin GeneClamp RS-232 IN**	
Pin number		*connected to*	*Pin number*	
1	(Shield)		1	(shield)
2	(TXD)		3	(RXD)
3	(RXD)		2	(TXD)
7	(GND)		7	(GND)
5, 6	(CTS, DSR)		20	(DTR)

For a Macintosh II with an 8-Pin Connector

Mac II 8-pin			**25-pin GeneClamp RS-232 IN**	
Pin number		*connected to*	*Pin number*	
2	(DCD)		20	(DTR)
3	(TD)		3	(RXD)
4	(GND)		7	(GND)
5	(RD)		2	(TXD)
8	(Link to GND)		1	(Shield)

Two-Electrode Voltage Clamp (TEVC)

In VOLTAGE CLAMP mode the GeneClamp functions as a two-electrode voltage clamp with Electrode #1 for voltage sensing and Electrode #2 for current passing. The voltage-clamp output compliance of ±130 V allows for a fast clamp capable of handling large currents, such as might result from heterologous expression of ion channels in a *Xenopus* oocyte. Two different controls are used to adjust the performance of the clamp circuit to achieve the fastest clamp possible without the clamp becoming unstable. They are the voltage-clamp GAIN and STABILITY controls.

Gain

This knob controls the loop gain of the voltage clamp, and is adjustable from 20-10,000 (or 2000-1,000,000 with STEADY-STATE RESTORE active). High gain leads to a faster clamp and better accuracy. One adjusts the GAIN value in conjunction with the STABILITY control to achieve the highest gain possible and still maintain stability.

Stability

This control allows the loop gain to be stabilized under high gain by introducing a phase lag into the feedback loop to compensate for electrode cross-coupling phase lead and non-idealities in the phase response of the cell membrane. Its value ranges from 3-2,000 µs.

The procedure of adjusting the voltage-clamp GAIN and STABILITY controls to achieve the best clamp possible for a given cell is called tuning the clamp, and is best carried out at the potential where no net current flows across the cell membrane, *i.e.*, the resting potential. This ensures that there will be no large current flows across the membrane that could damage the cell as the gain of the clamp is increased. In SETUP mode, the HOLDING POTENTIAL potentiometer is adjusted so that both RMP (resting membrane potential) LEDs are equally dim. At this point the holding potential is pre-set to the resting potential of the particular cell being used, so selecting VOLTAGE CLAMP mode will not cause a large current transient that could damage the cell.

Make sure the voltage-clamp GAIN knob is at its minimum position (fully counterclockwise), enter the TEVC mode and activate the TEST FAST command, which applies a -10 mV command potential from the resting potential, and monitor the voltage clamp current output (I2). The output should initially look like a rounded square wave. Slowly increase the gain and note that I2 begins to sharpen up and look like a capacitive transient (Figure 5-*A*). As the gain increases, you may notice the appearance of oscillations or ringing (Figure 5-*B*). Increasing the amount of phase lag with the STABILITY control will remove this (Figure 5-*C*) and allow you to increase the gain even more. However, the phase lag will decrease the high frequency gain of the loop and thus slow the clamp down a bit. Use the minimum amount of phase lag that permits the clamp to be operated at full gain. At this point turn off the TEST command. The voltage clamp is now optimized for the cell and the holding potential can now be set to the desired potential.

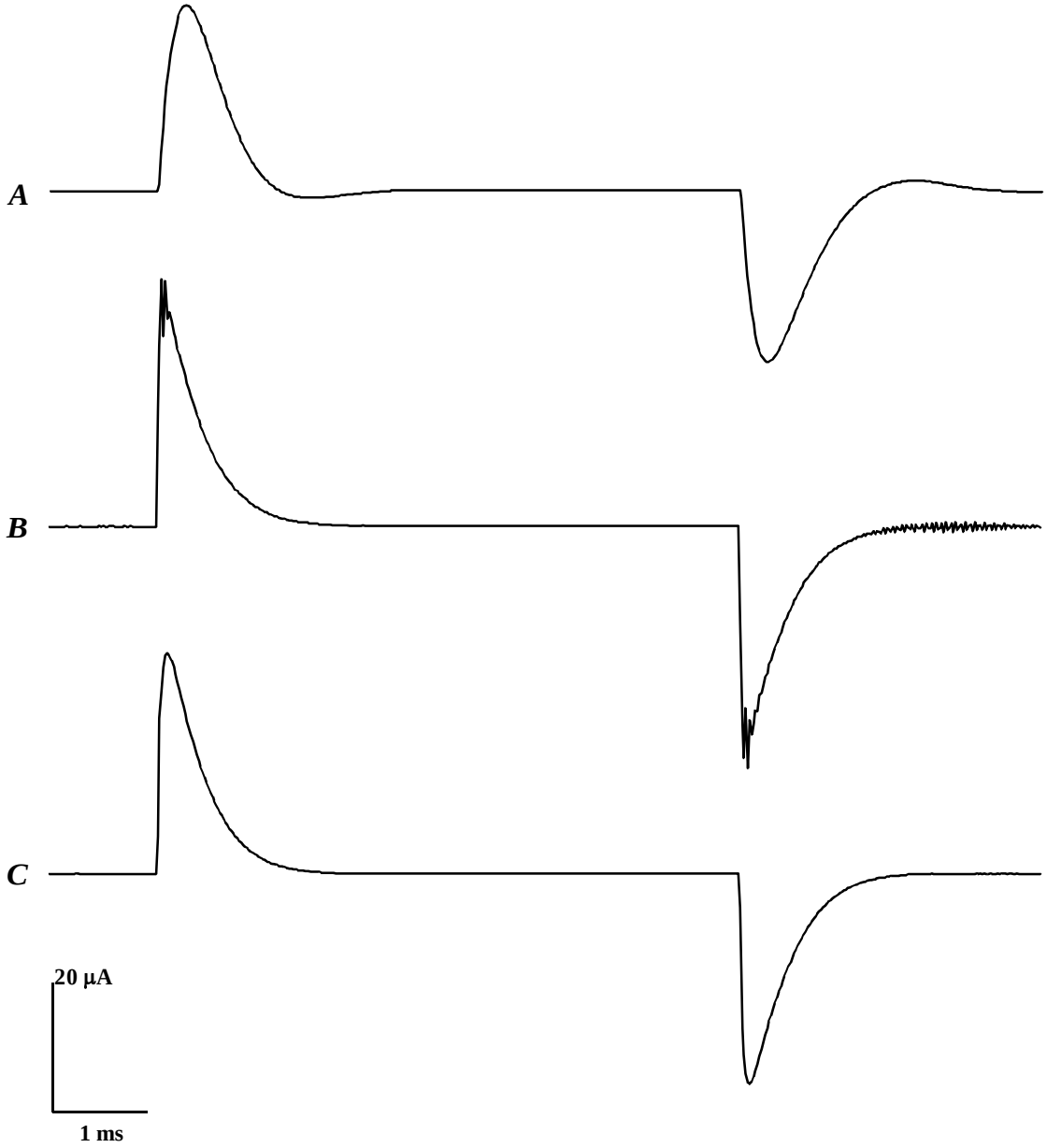


Figure 5. Tuning the two-electrode voltage clamp

Zeroing Buttons

There are four zeroing buttons on the GeneClamp which are used to remove any DC offset from the signal being processed. An "intelligent" zeroing algorithm is used to minimize transients while the zero is found.

If the signal DC level is more than 10 mV away from zero, the correction voltage is incremented in 10 mV steps to automatically adjust the DC level towards zero. Once the DC level is within 10 mV of zero, the algorithm uses a successive approximation technique to find the exact correction value required to remove the DC level. The four signals that can be zeroed are Electrode #1 (V1), Electrode #2 (V2), patch current (I3), and the various SCALED OUTPUTs (V1, V2, V3, I1, I2, I3). The instrument "remembers" the offset applied, and in the case of V1, V2 and I3, holding down a given zeroing button for greater than 500 ms will cause the offset to be displayed on the appropriate panel meter. In the SCALED OUTPUT section each selection has its own stored zero, so changing signal selection will automatically bring in the appropriate zeroing voltage. For example, if V1 was selected and zeroed, and then V2 was selected and zeroed, when V1 is re-selected the scaled output for V1 will still be zero.

Power-Supply Voltage Selection & Fuse Changing

Supply Voltage

The GeneClamp 500B can be directly connected to all international supply voltages. The input range is from 85 to 264 V_{AC}. No range switching is required. Alternatively, the instrument can be powered by a DC voltage of 110 - 340 V_{DC}. In the DC case, the SEAL TEST is inoperative.

Changing The Fuse

The GeneClamp 500B uses a 2.0 A, 250 V slow acting 5 x 20 mm fuse.

Before changing the fuse investigate the reason for its failure.

To change the fuse:

1) Disconnect the power cord.

2) Use a screwdriver or a similar device to rotate the fuse holder counterclockwise.

3) Replace the fuse with another fuse of the same rating.

4) Reconnect the power cord.

General Information

Grounding and Hum

A perennial bane of electrophysiology is line-frequency pickup (noise), often referred to as hum. Hum can occur not only at the line frequency but also at multiples of a particular line frequency. The GeneClamp has inherently low hum levels (less than 1 μV_{p-p}). To take advantage of these low levels, great care must be taken when integrating the GeneClamp into a complete recording system. The following procedures should be followed.

1) Only ground the preparation bath by directly connecting it to the yellow ground connector on the back of Headstage #1 or using the VG-2A x100 bath probe.

2) Place the GeneClamp in a position in the rack where transformers in adjacent equipment are unlikely to radiate into its electronics. The most sensitive part of the electronics is the right hand side looking from the front. A thick sheet of steel placed between the GeneClamp and the radiating equipment can effectively reduce the induced hum.

3) Initially make only one connection to the GeneClamp: to the oscilloscope from the SCALED OUTPUT. Ground the Electrode #1 headstage input through a 1 MΩ resistor to the yellow ground connector. After verifying that the hum levels are low, start increasing the complexity of the connections one lead at a time. Leads should not be draped near transformers which are inside other equipment. In desperate circumstances the continuity of the shield on an offending coaxial cable can be broken.

4) Try grounding auxiliary equipment from a ground distribution bus. This bus should be connected to the GeneClamp via the yellow 0.16 inch (4 mm) socket on the rear panel. This socket is connected to the GeneClamp's signal ground (*i.e.,* the outer conductors of all the BNC connectors). The signal ground in the GeneClamp is isolated from the chassis and power ground.

5) If more than one headstage is used, all the headstage cables should run from the GeneClamp to the preparation in a bundle. The bundle can be formed either by gently twisting the cables together or by loosely tying them together.

6) Experiment. While hum can be explained in theory (*e.g.*, direct pickup, ground loops), the ultimate theory is the practical result. Following the rules above is the best start. The final hum level can often be kept to less than 100 μV_{p-p} referred to V_m. One technique that **should not** be used to reduce the hum is the delicate placement of cables so that a number of competing hum sources cancel out. Such a procedure is prone to accidental alteration and irreproducibility.

Model Cell

The **MCO-2U** model cell (Figure 6) can be used to assist in setting up and testing the GeneClamp 500. The MCO-2U contains connections for Electrodes #1 - #3, as well as two inputs for the VG-2A bath clamp headstage. The cell membrane (R_m) is modeled by the parallel combination of either a 1 MΩ or 10 kΩ resistor (selectable via an external switch) and a 200 nF capacitor (C_m), all in series with a 1 kΩ resistor

(R_s). The micropipettes for TEVC (E #1 and E #2) are modeled as 1 MΩ resistors, while the bath agar bridges (R_{b1}, R_{b2}) are modeled as 2 kΩ resistors. The patch (E #3) is modeled as a 10 GΩ resistor.

NOTE: The predecessor **MCO-1U** model cell was designed with a "1 M" R_m position *only*, with no switch. Thus, the MCO-1U is identical to the MCO-2U when the latter is used in the 1 M R_m position. If your unit has been modified with the STEADY-STATE RESTORE function, then you will need the MCO-2U for testing purposes (see Functional Checkout chapter).

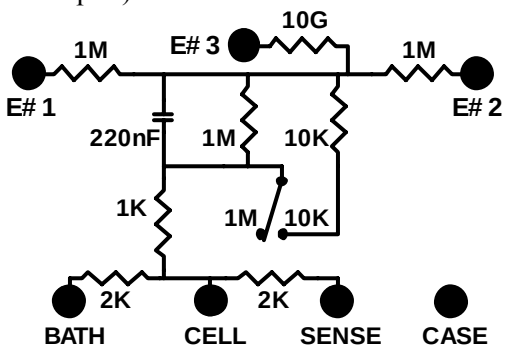


Figure 6. MCO-2U model cell schematic

Power-Supply Glitches

The GeneClamp has been designed to minimize the effects of power supply transients (glitches). Nevertheless, some power-supply glitches do get through. These can cause transients to appear on the voltage and current outputs which may corrupt high-sensitivity recordings (for example, during fluctuation analysis).

The only completely effective way to gain immunity from power supply glitches is to eliminate them at the source. Most glitches are due to the switching on and off of other equipment and lights on the same power-supply circuit. Precautions to be taken include:

1) Avoid switching equipment and lights on or off while recordings are being made.

2) Water baths, heaters, coolers etc. should operate from zero-crossing relays.

3) RFI (radiofrequency interference) filters should be installed in glitch-producing equipment.

In most circumstances occasional transients on the outputs are inconsequential and, therefore, no precautions have to be taken.

Ten-Turn Potentiometers

The ten-turn potentiometers used in the GeneClamp are high-quality wirewound types.

An inherent problem of wirewound potentiometers is that the wire elements tend to oxidize. When this happens, significant instrument noise becomes noticeable when the potentiometer is turned. This condition can be cured easily. If a potentiometer becomes noisy, the potentiometer manufacturer recommends rapidly spinning the knob 20-30 times between full clockwise and full counterclockwise. This clears the oxide off the element and restores noise-free operation.

Principles of Operation

The major topics in this section are arranged alphabetically.

Bath Error Potentials

In most experiments, the bathing solution is grounded by a solid grounding electrode (such as an agar/KCl bridge) and all measurements are made relative to ground (on the assumption that the bath is also at ground). This assumption may not be true in experiments in which the Cl^- concentration or temperature of the bathing solution is significantly changed, or where the membrane current is sufficiently large as to cause a significant voltage drop across the resistance of the grounding electrode. The latter circumstance would normally occur only when voltage clamping very large cells such as *Xenopus* oocytes, in which case the ionic current may be of the order of several microamperes or even several tens of microamperes.

Depending upon the method of grounding, the resistance of the bath grounding electrode (R_b) could be as much as 10 kΩ, although with care it is not difficult to achieve R_b values less than 1 kΩ.

In a simple TEVC setup, the voltage drop across R_b is indistinguishable from the membrane potential. That is, the potential recorded by the voltage-recording micropipette (V1) is the sum of the trans-membrane potential (V_m) and the bath potential (V_b). Problems arise if the product of the clamp current (I2) and R_b is significant. For example, for I2 = 5 μA and R_b = 2 kΩ, the voltage error is 10 mV. In some experiments, a worst-case error of this magnitude might be tolerable, but if the error were to be much greater, the position of I-V curves of channels with a steep voltage dependence and other responses would be seriously affected.

To faithfully record V_m, either V_b must be made equal to or nearly equal to zero, or the value of V_b must be independently measured and subtracted from the potential recorded by Electrode #1. The GeneClamp does not support the independent measurement and subtraction of V_b, since this technique does not yield a performance as good as either of the two techniques that follow. The two procedures to minimize the effect of errors introduced by R_b are listed in the preferred order of implementation. Please see the summary at the end of this section.

Minimize R_b

The best method is to minimize R_b in the first place. There are three main contributors to R_b:

1) The cell access resistance from the membrane surface to the bath;

2) The resistance of the grounding pellet; and

3) The resistance of the agar bridge (if used).

Typical values of the access resistance of a 1 mm diameter sphere in Ringers (such as an oocyte) are on the order of 150-200 Ω. No amount of manipulation can alter this for a given set of experimental conditions; fortunately it is relatively small. On the other hand, the resistance of the grounding pellet and agar bridge are larger, but one can take precautions to minimize them. A 1 mm diameter Ag/AgCl pellet in Ringer has a resistance of 300-600 Ω, depending on how much of the surface is in contact with the saline. The larger the surface area in contact with the saline, the smaller the resistance.

The resistance of an agar bridge depends on the length and diameter of the bridge, as well as what is inside (*i.e.,* Ringer's Solution *vs*. 3M KCl). For a 1 cm long bridge:

	1 mm diameter	2 mm diameter
Ringer's	10.2 kΩ	2.6 kΩ
3 M KCl	510 Ω	130 Ω

Therefore, to minimize R_b, it would be best to eliminate the agar bridge and ground the preparation directly with a Ag/AgCl pellet. The pellet should be as large as practical, and the area of it in contact with the solution should be maximized. However, if the bathing solution is changed during the experiment, the DC offset of the Ag/AgCl pellet will change with the chloride activity. In these cases, it is essential to use an agar bridge to prevent the DC offset of the bath from changing. Another advantage of an agar bridge is that it prevents metal ions from the grounding electrode from entering the bathing solution.

In order to minimize R_b when using an agar bridge, it is best to fill the bridge with 3 M KCl instead of Ringer's solution. When the agar bridge is filled with 3 M KCl, the sum of all components of R_b will be approximately 1-2 kΩ. If leakage of KCl from the agar bridge is a problem, it may be necessary to fill the agar bridge with Ringer. In this case, R_b will be several kilohms.

One can actually measure R_b in SETUP mode by placing both micropipettes in the bath. Put the micropipette connected to Headstage #1 on the far side of the micropipette connected to Headstage #2, away from the bath electrode. A 100 Hz, 10 V_{p-p} square wave command delivered to the external E #2 CURRENT COMMAND input produces a 1 μA_{p-p} current. The response measured with the micropipette connected to Headstage #1 is 1 mV/kΩ. If the product of the measured bath resistance and the magnitude of the expected current is such that significant voltage errors may result, then it will be necessary to clamp the bath potential to zero using a virtual ground circuit, as described below.

Clamp V_b Using a Bath Clamp or Virtual Ground

Another means to eliminate the effect of the voltage drop across R_b is to actively control the bath potential, measured near the outside surface of the cell. An independent clamping circuit clamps V_b to zero. This is achieved using a two-electrode virtual-ground circuit:

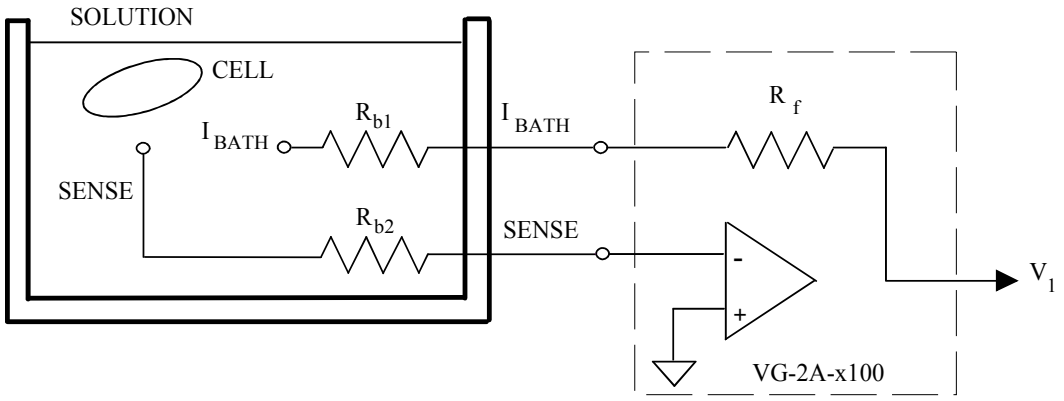


Figure 7. Bath clamp

One electrode (SENSE) is a voltage-sensing electrode. It is placed in the preparation bath near the cell surface. It is connected to the virtual-ground circuit by an agar bridge, or other means, of resistance R_{b2}.

Since there is no current flowing through this electrode, there is no voltage drop across R_{b2}. The other electrode (I_{BATH}), with resistance R_{b1}, is placed in the preparation bath. This electrode carries the ionic current. The feedback action of the operational amplifier ensures that the potential at the SENSE electrode is equal to the potential at the positive input, *i.e.,* 0 mV, irrespective of the voltage drop across R_{b1}.

Summary

As a first priority, we recommend that the value of R_b be minimized by the techniques described above. If it is still considered to be important to compensate for the IR voltage drop, a virtual-ground headstage (such as the VG-2A-x100 supplied with the GeneClamp) can be used to clamp the bath potential to zero.

Current and Voltage Conventions

The terminology used in this discussion applies to all amplifiers manufactured by Axon Instruments.

Positive Current

The flow of positive ions *out* of the headstage into the microelectrode and out of the microelectrode tip into the preparation is termed positive current.

Inward Current

Current that flows across the membrane, from the outside surface to the inside surface, is termed inward current.

Outward Current

Current that flows across the membrane, from the inside surface to the outside surface, is termed outward current.

Positive Potential

The term *positive potential* means a *positive* voltage at the headstage input with respect to ground.

Transmembrane Potential

The *transmembrane potential (V_m)* is the potential at the inside of the cell minus the potential at the outside. This term is applied equally to the whole-cell membrane and to membrane patches.

Depolarizing / Hyperpolarizing

The resting V_m value of most cells is negative. If a positive current flows into the cell, V_m initially becomes less negative. For example, V_m might shift from an initial resting value of -70 mV to a new value of -20 mV. Since the absolute magnitude of V_m is smaller, the current is said to *depolarize* the cell (*i.e.*, it reduces the "polarizing" voltage across the membrane). This convention is adhered to even if the current is so large that the absolute magnitude of V_m becomes larger. For example, a current that causes V_m to shift from -70 mV to +90 mV is still said to depolarize the cell. Stated simply, *depolarization* is a *positive* shift in V_m. Conversely, *hyperpolarization* is a *negative* shift in V_m.

Whole-Cell Voltage and Current Clamp

Depolarizing / Hyperpolarizing Commands

In whole-cell voltage clamping, a *positive* shift in the command voltage causes a positive shift in V_m and is said to be *depolarizing*. A *negative* shift in the command voltage causes a negative shift in V_m and is said to be *hyperpolarizing*.

Transmembrane Potential *vs.* Command Potential

In whole-cell voltage clamp, the command potential controls the voltage at the tip of the intracellular voltage-recording microelectrode. The transmembrane potential is thus equal to the command potential.

Inward / Outward Current

In a cell generating an action potential, depolarization is caused by a flow of positive sodium or calcium ions *into* the cell. That is, *depolarization* in this case is caused by an *inward* current.

During intracellular current clamping, a depolarizing current is a *positive* current out of the microelectrode tip into the interior of the cell. This current then passes through the membrane *out* of the cell into the bathing solution. Thus, in intracellular current clamping, a *depolarizing (positive)* current is an *outward* current.

An *inward* sodium current flows in some cells after a depolarizing voltage step. When the cell is voltage clamped, the sodium current is canceled by an equal and opposite current flowing into the headstage via the microelectrode. Thus it is a *negative* current. When two-electrode voltage clamping was first used in the early 1950's, the investigators chose to call the *negative* current that they measured a *depolarizing* current because it corresponded to the depolarizing sodium current. This choice, while based on sound logic, was unfortunate because it means that from the recording instrument's point of view, a negative current is *hyperpolarizing* in intracellular current-clamp experiments but *depolarizing* in voltage-clamp experiments.

To prevent confusion, Axon Instruments always uses current and voltage conventions based on the instrument's perspective. That is, the current is defined with respect to the direction of flow into or out of the headstage. Axon Instruments amplifiers do not have switches that the reverse the current or the voltage command polarities. This prevents forgetting to move the switch to the correct position. The data are recorded unambiguously and the correct polarity can be determined during subsequent data analysis.

Patch Clamp

By design, the patch-clamp command voltage is positive if it increases the potential inside the patch pipette. Whether it is hyperpolarizing or depolarizing depends upon whether the patch is "cell attached", "inside out" or "outside out". The patch-clamp pipette current is positive if it flows from the headstage through the tip of the patch pipette into the patch membrane.

Cell-Attached Patch

The membrane patch is attached to the cell. The patch pipette is connected to the outside surface of the membrane. A *positive* command voltage causes the transmembrane potential to become more negative, therefore it is *hyperpolarizing*. For example, if the intracellular potential is -70 mV with

respect to 0 mV outside, the potential across the patch is also -70 mV. If the potential inside the pipette is then increased from 0 mV to +20 mV, the transmembrane potential of the patch hyperpolarizes from -70 mV to -90 mV.

From the examples it can be seen that the transmembrane patch potential is inversely proportional to the command potential, and shifted by the resting membrane potential (RMP) of the cell. A positive current flows through the patch pipette, across the patch membrane into the cell. Therefore a *positive* current is *inward*.

Inside-Out Patch

The membrane patch is detached from the cell. The surface that was originally the inside surface is exposed to the bath solution. Now the potential on the inside surface is 0 mV (bath potential). The pipette is still connected to the outside surface of the membrane. A *positive* command voltage causes the transmembrane potential to become more negative, therefore it is *hyperpolarizing*. For example, to approximate resting membrane conditions of V_m = -70 mV, the potential inside the pipette must be adjusted to +70 mV. If the potential inside the patch pipette is increased from +70 mV to +90 mV, the transmembrane potential of the patch hyperpolarizes from -70 mV to -90 mV.

From the example it can be seen that the transmembrane patch potential is inversely proportional to the command potential. A positive current flows through the patch pipette, across the patch membrane from the outside surface to the inside surface. Therefore a *positive* current is *inward*.

Outside-Out Patch

The membrane patch is detached from the cell in such a way that the surface that was originally the outside surface remains exposed to the bath solution. The potential on the outside surface is 0 mV (bath potential). The patch pipette interior is connected to what was originally the inside surface of the membrane. A *positive* command voltage causes the transmembrane potential to become less negative, therefore it is *depolarizing*. For example, to approximate resting membrane conditions, assuming that V_m = -70 mV, the potential inside the patch pipette must be adjusted to -70 mV. If the potential inside the patch pipette is then increased from -70 mV to -50 mV, the transmembrane potential of the patch depolarizes from -70 mV to -50 mV.

The membrane potential is directly proportional to the command potential. A positive pipette current flows through the patch pipette, across the patch membrane from the inside surface to the outside surface. Therefore a *positive* current is *outward*.

Summary

1) *Positive* current corresponds to:

Cell-attached patch	patch inward current
Inside-out patch	patch inward current
Outside-out patch	patch outward current
Whole-cell voltage clamp	outward membrane current
Whole-cell current clamp	outward membrane current

2) A *positive* shift in the command potential is:

Cell-attached patch	hyperpolarizing
Inside-out patch	hyperpolarizing
Outside-out patch	depolarizing
Whole-cell voltage clamp	depolarizing

3) The correspondence between the command potential (V_{cmd}) and the transmembrane potential (V_m) is:

Cell-attached patch	$V_m = RMP - V_c$
Inside-out patch	$V_m = - V_c$
Outside-out patch	$V_m = V_c$
Whole-cell voltage clamp	$V_m = V_c$

Capacitance Neutralization and Input Capacitance

The Capacitance (C_{in}) at the input of the headstage amplifier is due to the capacitance of the amplifier input itself (C_{in1}) plus the capacitance to ground of the microelectrode and any connecting lead (C_{in2}). C_{in} combined with the micropipette resistance (R_e) acts as a low-pass filter for signals recorded at the tip of the micropipette. This leads to a slowing of the response of the voltage-measuring portion of the voltage-clamp circuit, and thus the speed of the clamp itself. The following two techniques may be used to increase the recording bandwidth:

Bootstrapping

One technique used to keep the contribution to C_{in} from the input amplifier as small as possible is to add the input signal voltage to the power-supply voltages used by the input stages. This technique, known as bootstrapping, fixes the voltage drop across C_{in1} to a constant value thereby preventing current flow through C_{in1}. The effective value of C_{in1} is thus reduced to well below its real value. This method is used only with Electrode #2.

Capacitance Neutralization

A commonly used technique, known as capacitance neutralization, is used to negate C_{in2} and the effective remnant of C_{in1}. The capacitance neutralization circuit attempts to inject into the headstage input a current which it anticipates will be required to charge and discharge C_{in} during signal changes. To adjust the capacitance neutralization, a current step is required and the voltage response to this current step must be displayed either using an oscilloscope or suitable A/D system. Pressing the TUNE button of the GeneClamp injects a hyperpolarizing line-frequency current into the micropipette connected to Headstage #1. The amplitude of the current is determined by the headstage current gain (*e.g.*, -1 nA for a x1L headstage). The capacitance neutralization control is advanced as far as possible without introducing overshoot in the step response. This setting is optimal for current passing and is also optimal for recording potentials at the tip of the micropipette.

It is important to recognize that the capacitance neutralization circuit is not more than 90% effective even for ideal micropipettes. This is because of the finite frequency responses of the headstage amplifiers and the capacitance neutralization circuit, and also because C_{in} does not behave ideally as a linear lumped capacitor. Consequently, the amount of C_{in} that the circuit must neutralize should be kept as small as possible. To this

end, **avoid using long lengths of shielded cable to connect the micropipette to the input. If possible, plug the micropipette holder directly into the input. Use shallow bathing solutions. Avoid having grounded objects near the pipette. Do not ground the headstage case.**

Headstages

In SETUP mode, an HS-2A series headstage functions as a ±12 V compliance unity gain buffer amplifier that has an input resistance many orders of magnitude greater than that of the micropipette and the input resistance of the cell. The output of this buffer amplifier follows the voltage at the tip of the micropipette, and since the amplifier draws no current, the current through the micropipette is clamped at the value of the current supplied at the input node. A high-quality current injection circuit is connected to the input node, and all of the injected current flows down the micropipette into the cell (Figure 8). The current source can be used to inject a pulse of current to stimulate or it can be used to apply a steady depolarizing or hyperpolarizing current to the cell. In addition, this current source is used by the TUNE function for capacitance neutralization of the micropipette connected to Headstage #1, and it is used during the measurement of the resistance of micropipettes connected to Headstage #1 or #2.

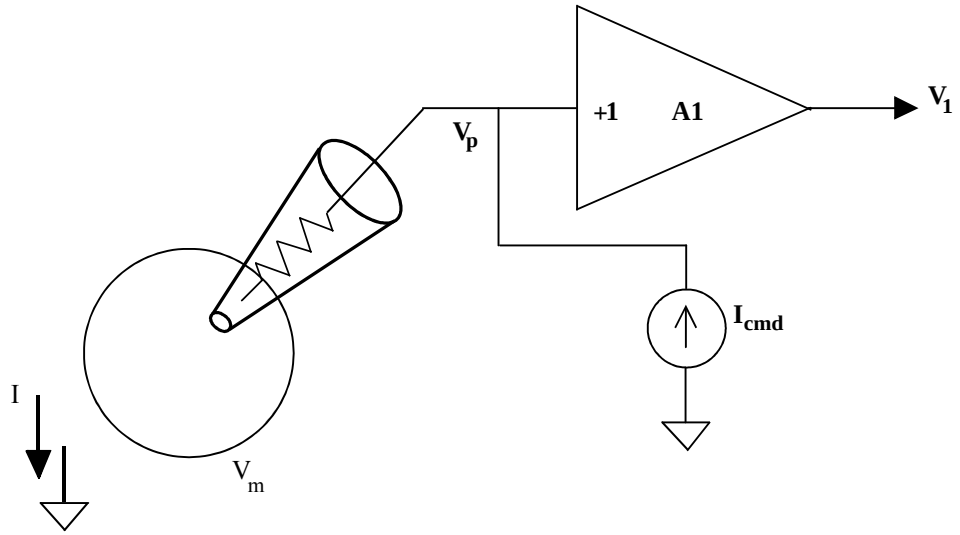


Figure 8. Current injection circuit

All of the HS-2A headstages have unity voltage gain but come in a variety of current-passing capabilities. The current-passing range and resolution are determined by the size of a single resistor (R_o) in the headstage. This resistor serves two functions: (1) it determines the size and resolution of the current that can be passed, and (2) it determines the sensitivity with which the current is measured. The lower the value of R_o, the more current can be passed; however, the resolution (in V/nA) with which the current can be measured is also lower. Current is measured by monitoring the voltage drop across R_o by means of a differential amplifier.

In TEVC mode, Headstage #1 still functions as in SETUP mode, but now Headstage #2 functions as part of a ±130 V compliance current-passing circuit.

Which HS-2A Headstage Should You Use?

One should use the headstage that is appropriate for the cell input resistance as well as the maximal currents one expects to encounter. Too little current-passing capacity will result in the voltage clamp being unable to control the cell's transmembrane potential, while too high a current gain means that the sensitivity with which the current is measured will be too low. Recommended headstage gain values for various cell input resistances can be found in the **SPECIFICATIONS**.

To clamp smaller cells in which the ionic current never exceeds 10 µA (and is usually much less) the signal-to-noise ratio of the current record might be improved by using an HS-2A-x1MGU as Headstage #2. If the voltage-recording electrode resistance is in excess of about 30 MΩ, the noise performance may be enhanced by an HS-2A-x0.1LU as Headstage #1.

Compatibility with HS-2 Headstages

All HS-2 headstages work in SETUP mode. However, in TEVC mode, HS-2 headstages used for Electrode #2 might latch up (*i.e.*, the output voltage might become stuck at plus or minus 130 V). This will not occur with the newer headstages of the HS-2A series. HS-2 headstages do not exhibit problems when used for Electrode #1.

With the HS-2A series headstages, coding resistors were added to the x10, x1.0 and x0.1 headstages. If uncoded, a x10 headstage will be recognized by the system as if it were a x0.01 headstage. To circumvent this, add a 100 kΩ resistor between pin 9 and pin 5 on the D connector.

A VG-2 headstage could work as the bath clamp; but for the fastest responses, the newer VG-2A headstage is required.

HS-4 -x1MGU Headstage

There is no capacitance neutralization control provided for in the Electrode #2 circuit. If a current-passing electrode of several tens of megohms is used, the voltage clamp speed of the GeneClamp may be slightly slower than that of an Axoclamp. The user has the option of employing an HS-4 series headstage. When connected to the GeneClamp, the current-measuring resistor (R_o) inside the HS-4 headstage is switched out of the circuit, requiring the use of a virtual ground headstage (VG-2) to measure the current.

There are two advantages to using the HS-4-x1MGU headstage in the two-electrode voltage clamp mode (TEVC). The first is that even in the linear operating region the time to establish a step voltage change is quicker, and the second is that larger step changes can be established without entering the nonlinear (*i.e.,* saturating) region. The disadvantage is that the HS-4 series headstages must be used in conjunction with a virtual-ground current-measurement headstage. This is because the normal built-in current monitors need R_o in order to operate.

It would appear that the HS-4 series headstage is superior to the HS-2 or HS-2A in TEVC. However, for reasons discussed below, the slight improvement attained probably does not warrant using an HS-4 instead of an HS-2A headstage.

The output voltage of the main unit during TEVC is ±130 V. This compliance is usually sufficient to drive the current through the current-passing microelectrode that is required to charge the membrane capacitance during a step voltage change. Thus, it is unlikely that even for large steps in cells with very large currents that the output would saturate and thereby increase the time required to establish the step change.

It is also true that the charging time constant is influenced, in part, by the microelectrode resistance and the current measuring resistor which are in series. Without a current-measuring resistor, as when using an HS-4 headstage during voltage clamp, the charging time constant is reduced. However, the charging time constant is also dependent on the closed loop gain. In fact, by increasing the gain, it is possible to decrease the charging time nearly as much as can be achieved by eliminating the current-measuring resistor.

A second apparent advantage of current not passing through a current-measuring resistor is that the full output voltage is applied to the microelectrode. Assuming that the resistance of the current-measuring resistor and the microelectrode are equal, the maximum voltage applied across the microelectrode would double without the current-measuring resistor. However, the time to reach the final step potential is not halved simply by doubling the output compliance. This is because the increased compliance is only useful during the saturated portion of the capacitance transient. The time constant during the exponentially decaying phase of the capacitance transient depends on the gain setting but not the output compliance.

NOTES

Do **not** touch the microelectrode input connector of the HS-4 series headstage in TEVC mode since it is directly connected to the high-voltage amplifier.

Do **not** ground the microelectrode input connector of the HS-4 series headstage directly in TEVC mode since its input is directly connected to the high-voltage amplifier output.

Headstage Connectors

The GeneClamp 500B is supplied with the U-type headstages. This type of headstage only connects with "U" (universal) type adapters, pipette holders and model cells. The U-type design offers several advantages and these are detailed in the section of the manual entitled *Holder*. The non-U-type headstages can still be used with the GeneClamp 500B, but these headstages offer less advantages than their U-type counterpart.

Because Axon Instruments sells replacement pipette holders, adapters and model cells in two varieties, the U-type and the non-U-type, please specify the complete name of the product.

Refer to Figure 9 for the following description of the U-type HS-2A headstage.

Microelectrode Input Connector (White)

The threaded white socket is the microelectrode input. The connection between the microelectrode and this socket should be kept as short as possible. Some excellent methods are:

1) Solder a silver/silver-chloride wire directly to one a 1 mm plug. Use the wire to connect to the microelectrode which can be supported on a separate mounting;

2) For greater mechanical stability, use an HL-U series microelectrode holder from Axon Instruments; or

3) Use a BNC-type microelectrode holder. This requires an HLB-U adapter from Axon Instruments.

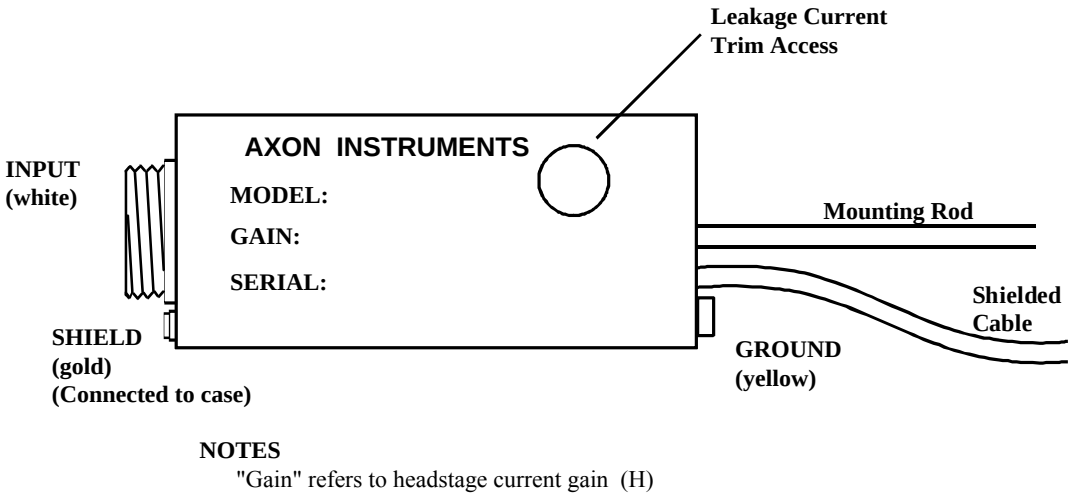


Figure 9. U-type HS headstage.

Shield Drive Connector (Gold)

The Shield drive is connected to the gold-plated guard socket and to the case of the headstages with gains of x1L, x0.1L, x0.01M and x0.0001M. This drive is protected against continuous short circuits, however for best frequency response **the case must not be grounded.** In general, this necessitates using an insulated mounting for the headstage (such as the polycarbonate rod or the acrylic plate provided).

The shield connection is provided primarily for driving the shield of microelectrodes prepared for deep immersion. It may also be used for driving metal objects near the input, or even the hutch in which the preparation is housed. It can be used for driving the shield of a coaxial cable used to connect the microelectrode to the input, although it is not recommended that the microelectrode be connected in this way. If not used, the shield socket is simply left unconnected.

There are two reasons why we do not recommend using shielded cable to connect the microelectrode to the headstage. First, the leakage resistance of shielded cable can degrade the input resistance when used with ion-sensitive and other high-impedance electrodes. If shielded cable is used it should have Teflon as the insulating material separating the shield and the inner conductor. Second, shielded cables add significant input capacitance. The shield drive circuit mostly removes the effect of this capacitance on microelectrode response speed. However, from a noise point of view the capacitance remains and causes an increase in high-frequency noise.

To optimize the response speed of low- and medium-resistance microelectrodes (up to approximately 300 MΩ) when a driven shield is used, the shield of headstages with H = x0.1 and larger is driven from the capacitance neutralization circuit. To optimize the headstage input resistance when a driven shield is used, the shield of headstages with H = x0.01 and smaller is driven from the output of the unity gain buffer inside the headstage.

If a shielded cable is being used and unusual microelectrode responses are observed, try disconnecting the shield.

No shield drive is provided on the headstages with the "G" designator (*e.g.,* x1MGU, x10MGU and the HS-4-x1MGU). On these headstages the case is grounded. This is because they are primarily

used for current passing in a two-electrode voltage clamp (TEVC). In TEVC, it is essential to minimize the amount of coupling capacitance between the voltage-recording headstage and the current-passing headstage. This coupling can be minimized most conveniently if the case of the current-passing headstage is grounded.

Ground Output Connector (Yellow)

The yellow ground socket of the ME1 headstage is the signal ground and is used for grounding the preparation. Using this connection as the preparation ground minimizes hum.

Input Leakage Current and How to Trim it to Zero

All DC-connected systems suffer from the problem of drift. With changes in temperature and the passage of time, the DC transfer functions of all semiconductor devices can drift by many millivolts away from their initial values. The major worry in a microelectrode system is that the cumulative effects of drift in various parts of the circuit may lead to the development of a DC offset across the resistor (R_o) used to set the headstage current gain. As a result, an undesired DC leakage current is injected into the micropipette.

Careful consideration to this problem has been applied throughout the design of the GeneClamp 500B. The overall DC offset has been made as insensitive as possible to the drift in the integrated circuits. Furthermore, special low-drift integrated circuits have been used in all critical positions. The magnitude of the DC leakage current increases with decreases in R_o. This normally does not increase the DC offset voltage error developed across the micropipette or the cell membrane because smaller R_o's are usually used with lower-resistance cells and micropipettes.

Before leaving the factory, the DC offset voltage of each HS-2A headstage is trimmed so that the input leakage current is no more than:

100 pA	for	R_o =	1 MΩ	(H = x10)
10 pA	for	R_o =	10 MΩ	(H = x1)
1 pA	for	R_o =	100 MΩ	(H = x0.1)

These input current levels are very low and cause negligible shifts in the cell membrane potential when the headstages are used with the recommended ranges of cell input resistances.

If you ever suspect that the input current has grown to a level where V_m is significantly affected, it can be re-adjusted by the following procedure:

1) Switch off all current commands and disconnect any external current commands.

2) Remove the plastic cap from the access hole in the headstage cover.

3) Ground the headstage input via a resistor equal to $R_o \div 10$ (where R_o is given above). Set an oscilloscope at 2 mV/div and observe the $10V_m$ output through the filter set to 100 Hz. Use the ZERO V1 (or ZERO V2) control to center the trace on the screen.

4) Ground the headstage input via a resistor equal to R_o. Observe the shift of the oscilloscope trace.

5) Repetitively swap from grounding via $R_o \div 10$ to grounding via R_o. Adjust the trim potentiometer inside the headstage until there is no shift.

Depending on the reason for a trim being necessary, the trim procedure may have to be repeated if the headstage is changed.

NOTE

If an external source is connected to the E #1 or E #2 CURRENT COMMAND input, any time the source is non-zero a proportional current will flow in the micropipette. Many external sources do not put out a true zero voltage when in the "off" state, thus there may be an unwanted current due to the fact that an external source is connected. To avoid this, use an external source in which you can adjust the off-state voltage.

DC Removal

The discussion in this section is only relevant for the HS-2 headstages with gains of x0.01 and x0.0001. Both headstages are compatible with the GeneClamp 500B.

One potential source of a small but variable input leakage current is due to DC current flow through the dielectric of the capacitor (C_n) used for capacitance neutralization. For example, the micropipette potential might be 200 mV (although the experimenter does not see this potential because of the offset compensation). To compensate several picofarads of input capacitance, the gain of the capacitance neutralization circuit might be 2. Thus 400 mV would be fed back to C_n, resulting in 200 mV across this capacitor. If the dielectric resistance of C_n were 10^{11} Ω (the guaranteed minimum of high-quality capacitors), there would be 2 pA flowing through the capacitor.

To eliminate this source of leakage current, a DC removal circuit removes the DC voltage from across C_n. The DC removal circuit operates with a 1 s or 10 s time constant. There may be a transient shift in the electrode voltage while the CAPACITANCE NEUTRALIZATION control is being adjusted. The DC voltage is also removed from the shield drive.

Input Resistance

The input resistance of the headstages is predominantly related to R_o. A circuit inside the GeneClamp 500B, called a constant current source (CCS), controls the voltage across R_o. Ideally, the voltage across R_o is independent of the micropipette voltage. The accuracy of the CCS in controlling the voltage across R_o is preset at the factory. Extremely stable components are used in the CCS so that the accuracy will not deteriorate with time. In general, the CCS is effective to one part in 10^4 so that the input resistance is 10^4 R_o.

Other possible factors that would decrease the input resistance are minimized. For example, the field effect transistor (FET) input of the headstage is referenced to the input voltage rather than to ground. Known as "bootstrapping", this technique causes the effective resistance of the input to be much greater than the already high resistance of the FET. Leakage current and resistive loading through the insulation of the input socket are minimized by using Teflon insulation and by driving the case with the DC input voltage.

Overloads in Voltage Clamp

Overloads in the voltage clamp circuit are not regarded as an error. In fact, for the fastest clamping, one would expect that the current transient will be saturated for several hundred microseconds or more. The electronic circuitry recovers almost instantly for the saturation condition. For this reason, an overload light is not provided because it may suggest to the inexperienced user that the overload condition is to be avoided.

Leak Subtraction

Typically the passive membrane response to a voltage step consists of a transient and a steady-state component. It is often helpful to subtract these from the output so that only active responses are observed. The transient component is eliminated by using the CAPACITANCE COMPENSATION controls discussed earlier. The steady-state component is eliminated by the LEAK SUBTRACTION control. When the leak subtraction option is selected, the GeneClamp applies a series of 10 mV hyperpolarizing pulses. Their pulse amplitude and polarity were chosen on the assumption that they would only activate passive currents. The microprocessor then determines the leak resistance automatically. Since both the CAPACITANCE COMPENSATION and LEAK SUBTRACTION controls are driven by the command voltage, the passive responses remain eliminated for all polarities and magnitudes of command.

Patch-Clamp Headstages

Patch-clamp headstages are current-to-voltage (I-V) converters. That is, the voltage output corresponds to the current input. In contrast, traditional microelectrode amplifier headstages are voltage followers, for which the voltage output corresponds to the voltage input.

Resistor Feedback

The essential parts of the headstage are shown in the following figure:

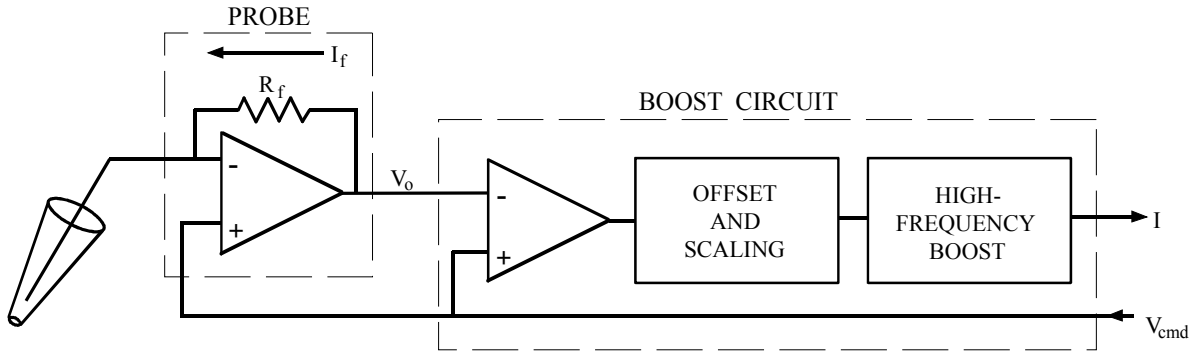


Figure 10. Resistive headstage

The patch pipette current is the same as the current through the feedback resistor R_f. Since the op amp in the probe acts to keep the voltage at its two inputs equal to each other, the potential at its negative input equals V_{cmd}. Thus the voltage across R_f is V_o - V_{cmd}, which is calculated by the differential amplifier in the boost box. Subsequent amplifiers are used to scale the gain and remove voltage offsets.

A fundamental problem of this circuit, when used for patch clamping, is that the output bandwidth of the probe is inherently low. To a first approximation, the bandwidth is set by the product of R_f and the stray capacitance across it. For example, if R_f is 50 GΩ and the stray capacitance is 0.1 pF, the bandwidth is about 30 Hz. To overcome this limitation, the probe output is passed through a high-frequency boost circuit. The function of the boost circuit is to recover the output signal bandwidth that has been lost because of the presence of the stray capacitance, thereby allowing a more realistic measurement of the wideband patch pipette current to be made. This circuit has a gain which is proportional to frequency.

If the Probe Output is Slow, How Can Voltage Clamping Be Fast?

After a casual consideration, you might conclude that if the output of the probe is slow, the voltage clamping of the patch pipette must also be slow because the high-frequency boost occurs after-the-fact and cannot influence the clamp speed. In fact, despite the slow current output of the I-V converter, the voltage clamp of the patch pipette is rapid. The patch pipette is connected to the negative input (summing junction) of the op amp. The command potential is connected to the positive input of the op amp. The operation of the op amp in this configuration is to force the potential at the summing junction to rapidly follow the potential at the positive input. If the command potential is a step, the potential at the summing junction, and hence the patch pipette, is also a step. The current required to achieve this step is passed through the feedback resistor (R_f) and the associated stray feedback capacitance (C_{Rf}) of the I-V converter. The output of the I-V converter settles to the final value with time constant $R_f C_{Rf}$. This relatively slow settling occurs despite the fact that the step at the summing junction is fast.

In this discussion, we have carefully referred to the fact that it is the "patch pipette" that is rapidly voltage clamped. The membrane potential is voltage clamped to its final value much more slowly. To a reasonable approximation, the time constant for voltage clamping the membrane is R_pC_m, where R_p is the patch pipette resistance and C_m is the membrane capacitance.

What is Clamped?

Voltage clamping is the intrinsic mode of operation of the CV-5 headstage. The series combination of the patch pipette and the patch of membrane is voltage clamped. Since there is no independent means of measuring the membrane potential, the inside of the patch pipette is actually clamped to the value of the command potential (V_{cmd}), which might lead one to assume that the patch itself is also clamped at V_{cmd}. However, this is only true if the current flowing through the circuit causes a negligible voltage drop across the patch pipette. For example, a current of 10 nA flowing across a 1 MΩ patch pipette resistance will lead to an error of 10 mV. However, even in the "worst" case (macropatch recording of channels expressed in *Xenopus* oocytes) the maximal currents encountered by the GeneClamp user will be on the order of 1 nA; so this source of error is negligible.

Which CV-5 Headstage Should You Use?

Four different CV-5 headstages are available. Their properties are described in the section on **SPECIFICATIONS**.

The boost circuit is part of the headstage itself so that the headstages are field interchangeable.

CV-5-100GU: This is the standard headstage for single-channel recording or continuous amperometry[3], suitable for currents of up to 100 pA.

CV-5-1GU: This headstage is suitable for macropatch recording. The maximum current that it can pass is on the order of 10 nA, which is more than sufficient for this purpose.

[3,4] Several biologically important analytes have molecule-specific oxidation-reduction potentials that can be detected with the electrochemical method, voltammetry. The oxidation-reduction potentials of these molecules is in the range of ± 1 V. The GeneClamp 500B has been designed to deliver the increased voltage range and to accept headstages adapted for voltammetric applications. The picoamp currents in DC amperometry and the nanoamp currents of cyclic voltammetry can be detected using the CV-5-100GU and CV-5-100MU headstages, respectively. Please see **RECORDING TECHNIQUES WITH CV-5 PROBES** for details.

CV-5B-100GU: This headstage is specialized for bilayer clamping. The FAST ELECTRODE CAPACITANCE COMPENSATION range is 100 pF — ten times larger than the range in the standard CV-5-100GU. The SLOW CAPACITANCE COMPENSATION range is 10 pF (compared with 1 pF in the standard headstage). The extended compensation range for this headstage can be used to rapidly charge the bilayer capacitance. This headstage should **not** be used for normal single-channel recording because the high-frequency noise is greater than in the CV-5-100GU.

CV-5-100MU: This headstage is designed for use in cyclic voltammetry[4] where the total current can be tens of nanoamperes.

Pipette Capacitance Compensation

We have previously discussed capacitance neutralization as a means of increasing the recording bandwidth of the voltage-measuring circuit in the TEVC mode. One source of this capacitance is the capacitance-to-ground of the microelectrode itself. In addition to the filtering effect that this capacitance can have, there is another unwanted effect that becomes apparent in the patch-clamp configuration. When the command voltage is stepped to a new potential, a large amount of current flows into the patch pipette capacitance during the transition from one potential to the next. In a patch-clamp amplifier, a variation of the capacitance neutralization technique described previously is used to reduce or even eliminate this charging transient. Compensating the patch pipette capacitance in the GeneClamp has two main purposes. First, many researchers want to remove the transient from the records for "cosmetic" reasons. Second, during the saturating portions of the transient, the potential at the top of the patch pipette is changing slowly while the patch pipette capacitance charges. By rapidly charging the patch pipette capacitance through the compensation circuitry, the potential at the top of the patch pipette is stepped more rapidly, thereby reducing the likelihood that rapid-onset ionic currents will be distorted.

Two pairs of patch pipette capacitance compensation controls are available in the GeneClamp patch section. With these controls, it is often possible to reduce the patch pipette transients to extremely low levels. The bulk of the transient is reduced by using the FAST MAGNITUDE and time constant (TAU) controls. The MAGNITUDE control compensates the net charge. The TAU control adjusts the time constant of the charge compensation to match the time constant of the command pathway and to compensate for small non-idealities in the frequency response of the patch pipette and electronics. The residual slow component seen in many patch pipettes is reduced by using the SLOW MAGNITUDE and TAU controls. A simplified circuit of the FAST and SLOW compensation circuitry is shown in Figure 10.

When the command potential (V_{cmd}) changes, current I_p flows into C_p to charge it to the new potential. If no compensation is used, I_p is supplied by the feedback element (R_f) resulting in a large transient signal on the output (I). By properly setting the FAST and SLOW MAGNITUDE and TAU controls, a current (I_{C1}) can be induced in capacitor C1 (connected to the headstage input) to exactly equal I_p. In this case no current needs to be supplied by R_f, and there is no transient on the output.

The FAST controls compensate for that part of the C_p that can be represented by a lumped capacitance at the headstage input (the major part of C_p). A small amount of C_p can only be represented as a capacitor with a series resistance component. This takes longer to charge to its final value and is compensated by the SLOW controls.

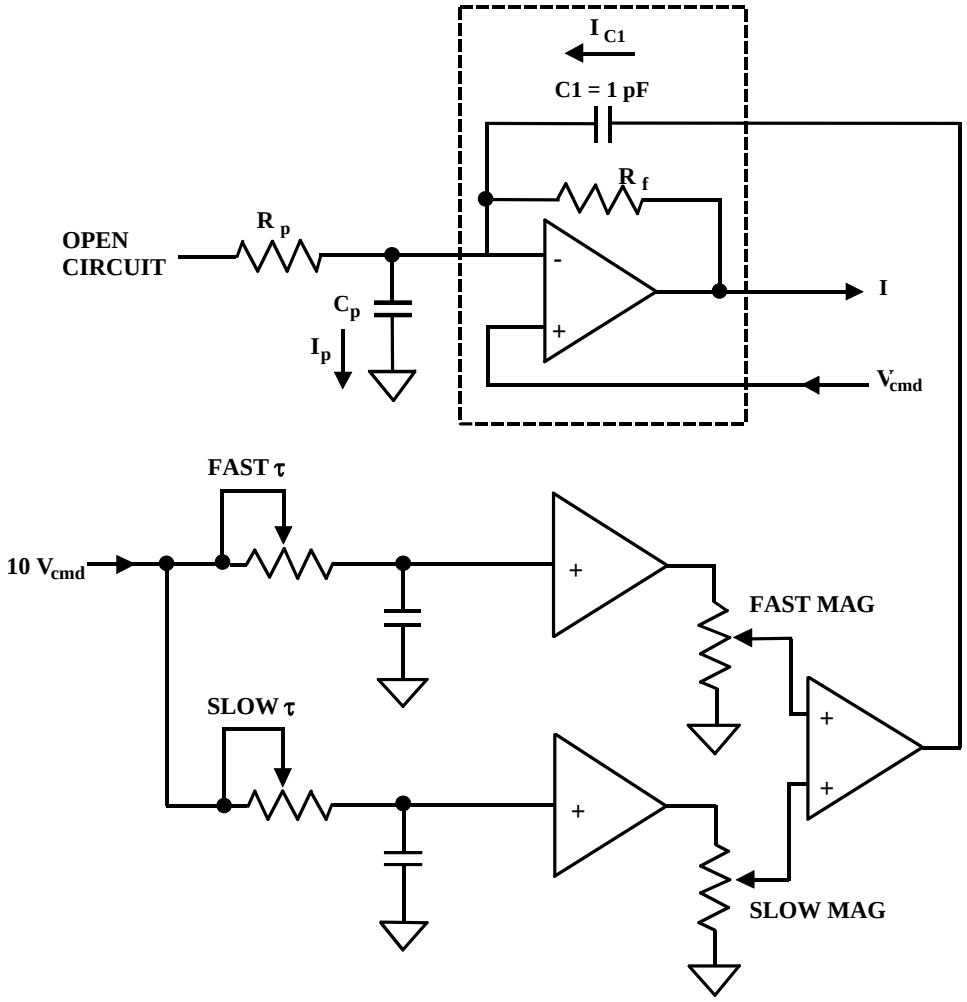


Figure 11. Patch pipette Capacitance Compensation Circuit

Two-Electrode Voltage Clamp (TEVC)

Gain

A voltage clamp circuit is designed to compare the measured membrane potential (V_m) to a desired command potential (V_{cmd}), and then inject a current proportional to the difference between the two inputs into the cell, thus driving V_m towards V_{cmd}. Therefore, in the absence of any errors, the negative feedback circuit will force V_m to be at V_{cmd}.

The steady-state membrane potential after a step change in the command voltage is given by:

$$V_m = V_{cmd} \frac{\mu K}{\mu K + 1}$$

where μ is the gain of the clamp amplifier and K is the attenuation of the clamp amplifier caused by the cell membrane resistance (R_m) and the resistance (R_{e2}) of the output micropipette connected to Headstage #2.

$$K = \frac{R_m}{R_m + R_{e2}}$$

As the product μK becomes very large, the difference between V_m and V_{cmd} becomes very small. Ideally, the error will be very low, just a fraction of one percent. The clamp gain μ is set by the front-panel GAIN control in the GeneClamp and ranges from 20 to 10,000. (NOTE: This GAIN is boosted throughout its range by 100 when the STEADY-STATE RESTORE feature is active. Thus, the maximum range would be 1 million when STEADY-STATE RESTORE is turned ON.)

From above, if K were unity, the error would vary from 5% down to 0.01%. However, if K is less than unity (which it always is), the error will be worse. If both the current-passing micropipette resistance and membrane resistance are 1 MΩ, K is 0.5 and the error will be twice as bad than if K were unity. As a general rule, it is desirable to use a current-passing micropipette whose resistance is as low as possible, ideally about the same size or smaller than the membrane resistance.

Step Response and Bandwidth

After a step command, the membrane potential relaxes exponentially towards its new value. For μK >> 1, the time constant for the relaxation is:

$$\tau = \frac{R_{e2}C_m}{\mu}$$

Increasing the clamp gain decreases the time constant for the step response.

In summary, in order to achieve both maximal fidelity and speed of response, a voltage clamp should operate at the highest gain possible. However, as we will see in the next section, non-idealities in the cell, as well as the recording configuration, can put a limit on the amount of gain at which a voltage clamp can operate.

Stability

A voltage clamp is a negative-feedback circuit that requires a 90° phase shift within the circuit. Ideally this phase shift is supplied by the capacitance of the membrane. In practice, however, other factors combine to make the clamp unstable at high clamp gains. The two main contributors to this instability are the coupling capacitance between the two microelectrodes and the non-ideal nature of the cell membrane.

The coupling capacitance between the micropipettes is extremely destabilizing. However, there are several easy ways to minimize the coupling capacitance. The two best ways are (1) have the two electrodes at as wide an angle as possible, preferably greater than 90°; and (2) place a grounded metal shield between the two micropipettes. The shield should be large enough to block all line-of-sight pathways between the two micropipettes and their holders.

Another destabilizing factor is the non-ideal nature of the preparation; *i.e.*, the cell membrane cannot be described as a simple parallel resistor and capacitor circuit. For real membranes, the phase shift at high frequencies is less than 90°. The voltage-clamp circuit can be adjusted with the STABILITY control to compensate for the non-ideal phase response of real membranes through the addition of phase lag to the circuit to build the total high-frequency phase shift up to 90°. Phase lag simultaneously **cuts** the high-frequency gain of the voltage-clamp circuit. This can be used to reduce the noise but at the same time it slows the response and introduces ringing. Therefore, the STABILITY control should only be advanced the minimum amount required to eliminate the oscillatory responses.

Inter-Electrode Coupling Capacitance and Shielding

When the voltage-recording microelectrode and current-passing microelectrode are applied to a cell there is considerable coupling capacitance (C_x) between them due to their proximity. This capacitive coupling introduces a low impedance feedback pathway at high frequencies that completely bypasses the cell membrane. The presence of this capacitance is the single most significant destabilizing influence on the voltage clamp, and for this reason it is essential that it be reduced as far as is practical. In point of fact, coupling capacitance as low as 0.01 pF can destabilize the response at high gain settings.

There are three ways to reduce C_x:

1) Introduce the two microelectrodes into the preparation at a wide angle, preferably greater than 90°. Keep the tips of the microelectrodes as far apart as possible. Generally it is better to have the tip of the current microelectrode nearer the center of the cell, so that field potentials do not affect the local membrane potential unevenly. (See *Eliminating the Voltage Error Due to Coupling Between Intracellular Microelectrodes* on page 58.)

2) Place a grounded metal shield between the two microelectrodes. If the shield cannot be placed in the middle, then place it nearer the current-passing microelectrode where added stray capacitance is not so critical. A shield near the voltage-recording microelectrode will increase the input capacitance and thus will increase noise levels. The shield should block all line-of-sight pathways between the two microelectrodes and their holders and should extend as close as possible to the tip of the current-passing microelectrode without coming into direct electrical contact with the bath solution. In extreme cases you can coat current-passing micropipette very close to the tip with conductive silver paint which can then be insulated by a coat of Sylgard. One effect of the shield is to vastly increase the output capacitance of the current-passing microelectrode which may affect the high-frequency measurement of I_2 and 0.1 x I_2.

3) **NOTE:** The use of a driven shield on voltage-recording microelectrode is not recommended as this will increase the noise; it is far better to place a grounded shield on the current-passing microelectrode.

Eliminating the Voltage Error Due to Coupling Between Intracellular Microelectrodes

Another potential source of voltage error in TEVC mode is introduced by the field potential that can develop around the tip of the current-passing microelectrode when current densities are high.

The equation for the access resistance, R_a, in a sphere is: $R_a = \rho/4\pi r$. The resistivity, ρ, is typically 100 ohm•cm for physiological saline and the radius, r, of the sphere is in cm.

If the potential picked up by the voltage microelectrode after the capacitance transient has decayed away is to be less than 5 mV and the ionic current is 10 µA, R_a must be less than 500 Ω. This requires a separation of 160 µm between the microelectrode tips, a significant distance even on the scale of amphibian oocyte dimensions. For a 100 nA current the separation need only be 1.6 µm. Clearly, as current becomes very large the voltage error due to the coupling of intracellular microelectrodes worsens. This may present a special problem when the concentration of heterologously expressed excitable proteins in amphibian oocytes is very large. If the current generated is larger than 10 µA, an alternative technique such as measuring the current using the macropatch technique might be considered (see *Macropatch* in **RECORDING TECHNIQUES WITH CV-5 HEADSTAGES**).

It is good practice to keep the tips of the two intracellular microelectrodes as far apart as possible inside the cell. Generally, it is better to put the tip of the current-passing microelectrode, ME2, near the middle of the cell so that the field potentials near its tip do not unevenly affect the local membrane potential.

Chapter 7

CV-5 Headstages

Recording Techniques

Planar Bilayers

The planar bilayer technique, in which ion channels are incorporated into an artificial, planar lipid bilayer by either fusion of channel-containing vesicles with the bilayer or by direct insertion of solubilized channels, provides a unique approach for the study of single ion channels. This approach enables experimental designs that are impractical or impossible using standard patch-clamp methods. For example, the effects of membrane composition on channel function can be rigorously studied; changes in the solution composition on both sides of the membrane can be made easily; and channels can be incorporated from membranes that are normally inaccessible to patch-clamp methods.

The utility of the planar bilayer method for studying single ion channels, however, is limited by a low recording bandwidth, primarily due to the high background current noise, and poor resolution of voltage step activation due to the large, slow capacitive current transient associated with a voltage step applied to the bilayer. These limitations may be problematic in investigations of the kinetics of channel-blocking drugs, or any voltage-gated channel.

Noise vs. Access Resistance

The CV-5B-100GU headstage is quite comfortable with loads less than 500 pF of pure capacitance (the maximum bandwidth decreasing to about 1 kHz with no overshoot). This can be used to great advantage when doing bilayer experiments: the lower the access resistance, the lower the noise. While there will be some lower limit on the value of the access resistance, it will not be set by the instrument's stability criteria. The CV-5B headstage is designed for complete stability even with extremely low access resistances.

In bilayer applications, one is usually working with bandwidths below 1 kHz. In this region, the e_nC_{in} noise has not yet become the major contributor to the overall noise (where e_n is the voltage noise of the probe input FETs[5] and C_{in} is the capacitance of the input of the headstage, which is primarily the bilayer membrane capacitance). However, a resistance in series with the bilayer membrane capacitance produces voltage noise just as though the headstage had high intrinsic noise. If this resistance is large enough, it becomes the major noise contributor.

5 FET -- Field Effect Transistor

Figure 11 shows peak-to-peak noise versus series resistance in a 1 kHz bandwidth for a given bilayer membrane capacitance.

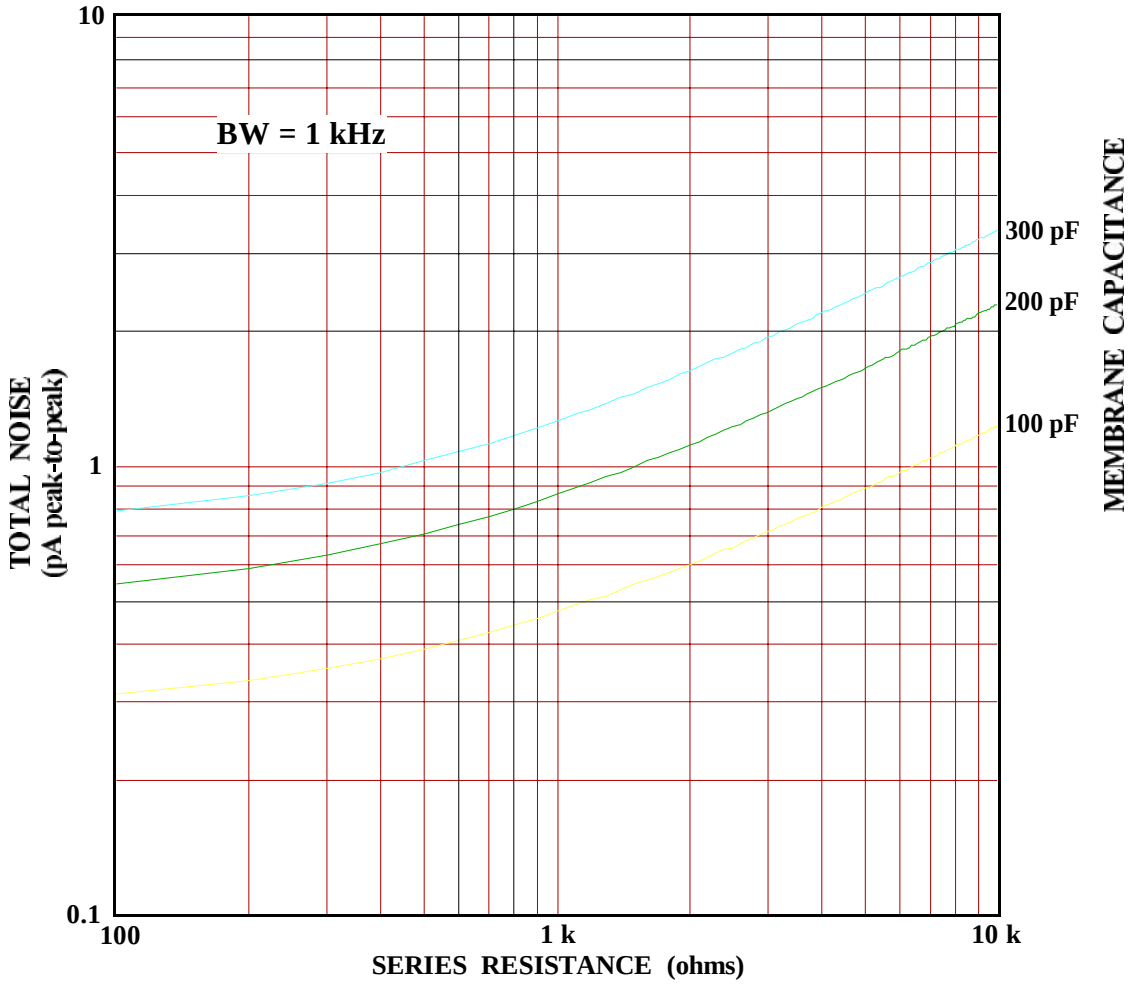


Figure 12. Typical Current Noise in Bilayer Experiments

Macropatch

The enormous capacitance of the *Xenopus* oocyte can limit the time resolution of rapidly activating or inactivating voltage-dependent channels studied with the two-electrode voltage clamp. Since the membrane voltage is not at the desired clamped potential until the capacitive transient is over, the early phases of activation may not be resolved. One way to be able to measure the kinetics of rapidly gating channels is to use a patch-clamp configuration and to record the currents from a large area of membrane (macropatch). In general, the density of channels expressed in the oocyte membrane is such that one can obtain up to several hundred channels in a macropatch, producing "macroscopic" currents suitable for standard kinetic analysis. Macroscopic patch currents can be recorded using standard patch-clamp instrumentation and a large patch pipette having a resistance of a few hundred kilohms. A gigaseal is formed using gentle suction, and then one can use either the cell-attached or excised patch mode. Since the capacitance of the system (membrane plus pipette) is relatively small, the membrane can be charged many times faster than that of a whole oocyte by a two-electrode voltage clamp.

There are a few problems associated with this method that need to be considered in the experimental design. First, the patch-clamp amplifier must be able to fully compensate for the capacitance of the larger patches; the 50 pF compensation range of the CV-5-1GU headstage is suitable for this. Second, when voltage pulses are applied to macropatches in the cell-attached configuration, current flowing in the patch will change the cell voltage; in the case of small cells, this can be significant. For example, a patch current of 100 pA and a cell input resistance of 100 MΩ can lead to a voltage change of 10 mV. This type of dynamic current-dependent error is difficult to correct and it may be impossible to use cell-attached macropatches to obtain accurate I-V curves, especially for rapidly activating currents. This problem can be avoided with the use of excised patches. Excised patches have the advantage that the transmembrane potential is known, rather than known relative to the resting potential (which is the case for cell-attached patches). In the case of oocytes, the input resistance of the oocyte is so low that this type of current-dependent voltage shift is negligible.

Single-Channel Patch Clamp[6]

The single-channel patch clamp is another special case of the voltage clamp. This technique permits the dynamic activity of individual membrane-bound channel proteins to be measured directly. A blunt patch pipette is sealed onto a patch of membrane. The current recorded is the current that flows through the membrane at the tip of the patch pipette. Since this membrane area is very small, there is a good chance that just one or a few ion channels are located in the patched membrane. Individual ion-channel currents can thus be recorded. In single-channel recording, the current through the series resistance of the patch pipette is negligible, perhaps only a few picoamperes through a series resistance of just 10 MΩ. The resulting error voltage is just a few tens of microvolts and is always ignored.

The Importance of a Good Seal

When a heat-polished patch pipette is pressed against a cell membrane, it may adhere tightly. The interior of the patch pipette is then isolated from the extracellular solution by the seal that is formed. If the resistance of this seal is infinite, no current can leak across it.

Current leakage though the seal resistance has a crucial bearing on the quality of the patch current recording. In the first place, depending on the size of the seal resistance, some fraction of the current through the patch of membrane will leak out through the seal and will not be measured. The lower the seal resistance, the larger the fraction of undetected current. In the second place, thermal movement of the charges in the conducting pathways of the seal constitute the major source of noise in the recording unless the seal resistance is very high (several gigohms or more). A high seal resistance is a prerequisite of low-noise recordings.

High-resistance seals, often called "gigaseals", require very clean patch pipettes used only once. If this requirement is met, gigaseals will routinely form after the application of gentle suction. When good gigaseals are formed, the noise due to the leakage current is virtually eliminated and other sources of noise remain as the dominant limitations in the resolution of the recording technique. These include the noise of the electronics, the pipette glass, the input capacitance and the feedback resistor. The noise of the electronics and the feedback element have been minimized in the GeneClamp. The section on glasses in **LOW NOISE RECORDING TECHNIQUES** describes the attributes of desirable glasses and the ways to fabricate low-noise patch pipettes. It also discusses the use of Sylgard coatings to minimize the patch pipette capacitance.

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6 Parts of this section have been reprinted, with permission, from Finkel, A.S., *Progress in Instrumentation Technology for Recording from Single Channels and Small Cells*, in **Cellular and Molecular Neurobiology: A Practical Approach**, Oxford University Press, 1991.

Methods

The four configurations for studying single channels are:

1) Cell-attached (on-cell) patch
2) Excised inside-out patch
3) Excised outside-out patch
4) Perforated vesicles

The cell-attached patch method of recording is simple and less disruptive to the cell than the other methods. The patch pipette is lowered to the cell surface and gentle suction is applied until a gigaseal is formed. Intracellular components necessary for the activity and modulation of the channels are retained within the cell.

The excised inside-out method requires that a gigaseal be made after which the patch pipette and the attached membrane are withdrawn from the cell. The cytoplasmic surface of the membrane is exposed allowing this surface to be exposed to ions and various modulatory substances from the bath.

The excised outside-out method also requires that a gigaseal be formed. The membrane is then ruptured by applying further suction. The patch pipette is then carefully and slowly withdrawn from the cell. As cell membrane is stretched, it resembles a tube. Eventually the membrane breaks and the portions attached to the patch pipette seal with the extracellular surface of the membrane facing the extracellular solution and the cytoplasmic surface of the membrane facing the patch pipette solution.

The perforated vesicle method involves the use of the ionophore nystatin. A patch pipette containing a buffered salt solution with nystatin is pressed against the cell and a portion of the membrane is sucked into the patch pipette. The patch clamp is then switched from V-clamp mode to I-clamp mode. As the patch pipette is withdrawn, the membrane breaks from the cell and seals with the outer surface of the membrane still facing the extracellular solution. Held within the tip of the patch pipette is a vesicle whose outer membrane is the same as the region of the cell from which it was taken. The vesicle contains a portion of the cell's cytoplasm. Once the vesicle has formed, the patch clamp is then switched to V-clamp mode. The ionophore in the patch pipette ensures that ionic current will flow across the membrane of the vesicle.

Voltammetry

The GeneClamp 500B is ideally suited for voltammetry, a recent extension of electrochemistry to the single cell level. The technique, pioneered nearly twenty years ago, combines electrochemistry and carbon-fiber electrodes. The general method has a wide bandwidth, lends itself to localization at the micrometer level, and can be applied to any analyte which has a reduction potential (E°) that permits detection in an aqueous solvent (±2 V), and for which a suitable sensing electrode has been developed. Two methods are used for *in vivo* work. *Scanning voltammetry* provides the highest degree of specificity, while *constant-voltage amperometry* provides the best temporal resolution. Both methods detect an analyte by measuring the current generated by its oxidation or reduction at the surface of a sensing electrode held at a given potential. In scanning voltammetry, the voltage is scanned through a range of from -1 to +1 V. The resulting I-V curve (voltammogram) is characteristic of the analyte. In constant-voltage amperometry, the sensing electrode is clamped at a constant voltage and the current flow induced by an electroactive substance is observed.

Amplifier and Headstage

The amplifier for constant-voltage amperometry must clamp the sensing electrode near the reduction potential of the analyte; provide the low-noise faradaic current, high-frequency bandwidth; and work well with a second amplifier that is used to clamp the cell potential. Held at a constant voltage, a carbon-fiber electrode has a very high resistance, and most recorded current is due to the diffusion of electroactive substances to the surface of the carbon-fiber electrode. With this recording method, it has been shown that cellular voltammetry correlates well with increases in cell capacitance as a measure of single-vesicle release in adrenal chromaffin cells.

Equipped with a **CV-5-100GU** headstage, the GeneClamp 500B is ideal for cellular constant-voltage amperometry. As with Axon Instruments Axopatches, the low-noise and high-frequency bandwidth of the GeneClamp 500B make it possible to record both individual quantal events and aggregate release. Because they do not use driven grounds, two Axon Instruments amplifiers can easily be used on the same preparation via a common Ag/AgCl reference electrode.

In scanning voltammetry, two currents in addition to faradaic currents must be supplied by the recording amplifier. During each cycle, the sensing electrode capacitance is charged and electroactive moieties on the surface of the electrode are oxidized and then reduced. This residual current can be as large as 50 nA, and produces a predominant background I-V curve that must be subtracted from the voltammogram. With the **CV-5-100MU** headstage, the GeneClamp will generate the required current. Data acquisition software can be used to generate and analyze a wide variety of voltammetry protocols to control data acquisition and perform data analysis.

Electrodes

Carbon fibers, variously treated and available in sizes as small as a few micrometers, have been used for a variety of analytes including epinephrine, norepinephrine, serotonin, dopamine and DOPAC. Carbon fiber electrodes have been used to detect somatostatin, and are proposed for the detection of any neuropeptide containing tyrosine, tryptophan or cysteine. Voltammetry can even record intracellular levels of neurotransmitters. Carbon fiber electrodes have been used to penetrate large invertebrate neurons and record intracellular dopamine levels.

For those substances not directly accessible to voltammetry, it may be possible to enzymatically produce an electroactive substance whose production depends on the concentration of the analyte of interest. The enzyme can be coupled to the derivatized surface of the electrode.

Carbon fibers are available from Amoco. The majority of researchers use P55S pitched-based fibers. The "S" designates sizing, which tends to keep the strands together in a bundle. It is prudent to clean the fibers before use with a brief wash in acetone, followed by air drying.

In the U.S. the general information number for Amoco carbon fibers is:
1-800-222-2448. Customers in Canada should call 803-299-9470.

NOTE: Fragments of carbon fibers that dislodge from the carbon strands are fine enough to enter electronic equipment and cause a short. Therefore, it is advisable to fabricate electrodes in a hood or at point distant from electronic equipment.

In some instances, platinum electrodes may be appropriate. For example, a platinum electrode and constant-voltage amperometry were used to record nitric oxide (NO) release from tissues *in vivo*. Also, electrolytically etched platinum electrodes can be used for recording intracellular levels of some neurotransmitters.

Tuning Procedure

The headstages are carefully calibrated at the factory. The following tuning procedure should be used if you have reason to believe that the offset, gain or frequency response has changed. Any headstage to be tested must be connected to the E #3 connector of the GeneClamp 500B. Allow a half hour warm-up before tuning.

Note: The headstage probe should be placed in a well shielded box throughout the test.

CV-5-100GU and CV-5B-100GU Headstages

Tuning should not be necessary more often than every 6-12 months.

Gain

1) Connect a digital voltmeter to Scaled Output on GeneClamp 500B.
2) Select a Gain of 0.1 pA/V on GeneClamp. Select I3 on SCALED OUTPUT.
3) Connect the Patch position of the MCO-2U model cell to the headstage.
4) Set DC Command to 100 mV on GeneClamp. Press ZERO I3. Press OUTPUT ZERO UPDATE.
5) Repetitively switch from + to -100 mV.
6) Trim GAIN for ± 1 V on the digital voltmeter.

Offset

1) Connect a digital voltmeter to TP1 inside the boost box.
2) Trim RT1 inside the probe box to minimum offset.
3) Connect a digital voltmeter on TP27 inside GeneClamp.
4) Trim OFFSET inside the boost box to minimum offset.

Tuning

1) Switch on the Speed Test and apply a 10 Hz triangular wave to the Speed Test BNC.
2) Attach scope probe to TP27 inside the GeneClamp.
3) Trim adjustments VL and L1 to achieve maximum square response.
4) Trim adjustment L2 to achieve response. You may need to go back to VL and L1.
5) Trim tuning adjustments M1 and M2 to eliminate the overshoot. You may need to go back to L2.
6) Increase the frequency of the triangular wave to 100 Hz.
7) Trim tuning adjustments M1 and M2 to achieve the flattest response.
8) Trim tuning adjustments H1 and H2 to obtain the fastest rise time with no overshoot and oscillation.
9) When adjusted correctly the rise time should be between 18 µs and 22 µs.
10) Check the noise level
    a) Connect the digital voltmeter to the Scaled Output of the GeneClamp.
    b) Switch the Speed Test to OFF.
    c) Set Filter to 10 kHz.
    d) The noise should be less than 0.25 pA rms.

Chapter 8

Troubleshooting

It has been our experience at Axon Instruments that the majority of troubles reported to us have been caused by faulty equipment connected to our instruments.

If you have a problem, please disconnect **all** instruments connected to the GeneClamp except for the headstages. Ideally, remove the GeneClamp from the rack. Work completely through the **FUNCTIONAL CHECKOUT**. This can often uncover a problem that is in your setup. If the problem persists, please call us for assistance.

Another common problem is caused when dirt or corrosion build up in the headstage connector socket, which can cause unstable current and voltage offsets. It is important to keep the holders and the headstage inputs clean.

Voltage Clamp Problems

TEVC

Symptom: The voltage clamp becomes unstable even at low gains. Oscillation is seen on the current trace during voltage steps.

Possible cause: The inter-electrode coupling capacitance may be too high.

Suggestion: Check that the shield between the microelectrodes is correctly placed and adequately grounded.

Symptom: The voltage clamp is slow to respond. There may also be a DC error.

Possible causes: The voltage-clamp gain is too low.

Suggestions: Use the STABILITY control (*i.e.*, phase lag) if necessary to enable the gain to be increased. If the problem is present even at maximum clamp gains then the current-passing microelectrode is probably blocked. This is most likely to occur when using high-resistance micropipettes. Withdraw the current-passing microelectrode, replace it, and try again. Microelectrodes filled with 4 M K-acetate or K-citrate tend to pass current better than KCl filled electrodes.

Symptom: The peaks of the current transients are clipped (have a flat top).

Possible cause: The current-passing side of the voltage clamp is saturating. If this is the case then variation in the gain around its maximum level will have little effect on the voltage rise time. Most likely the output

amplifier is saturating, but if you are using a virtual ground headstage to measure current then the saturation may be occurring there.

Suggestions: If the rise time is adequate and non-linear capacitive currents are acceptable, then there is no problem. If the saturation is not acceptable, then reducing the resistance of the current-passing microelectrode may help considerably. If you halve this resistance you halve the output voltage required to drive the same current. If the problem lies in the virtual-ground unit, use another one with higher range.

TEVC or Patch

Symptom: Unable to offset the voltage microelectrode voltage to zero.

Possible causes: There may be a break in the connection between the headstage input and ground, causing the input to float. The capacitance neutralization or pipette compensation circuits may be oscillating.

Suggestions: Check the electrical continuity and DC stability of the voltage recording and bath ground electrode holders. Check for bubbles in the microelectrodes. Check that the capacitance neutralization or electrode capacitance compensation is not in oscillation by observing the microelectrode voltage at a wide bandwidth.

Symptom: Persistent overshoot during a voltage step. High voltage and current noise. Cannot be "tuned" out.

Possible cause: If you are using an external voltage command, examine the command signal on an oscilloscope. The command signal itself may overshoot during a voltage step. Some D/A converters can also have high levels of digital switching noise.

Suggestions: Either get another D/A converter or low-pass filter the voltage command signal. Select the filter cut-off frequency such that the rise time of V_{cmd} is faster than the rise time of the voltage-measuring microelectrode voltage. The V_{cmd} signal should not be the limiting factor determining the clamp step response rise time. Never apply filtering to V_{cmd} as a way of compensating for performance in the voltage clamp setup — this is a false benefit.

Symptom: Extraneous noise in the V_m signal. V_m may drift several mV/min.

Possible cause: The Ag/AgCl pellet or Ag wire in an electrode holder may be defective.

Suggestions: Check the DC stability of the various voltage recording and bath ground electrode holders and replace where necessary.

Symptom: Time-course of the voltage step is faster than the current transient.

Possible causes: 1) A large series resistance or 2) the cell is not isopotential. The latter is common when you voltage clamp a neuronal soma with an axon and dendrites attached. The slower components in the current transient are due to charging of the distributed capacitance of these processes. In the absence of significant series resistance the voltage recorded is an accurate representation of the soma potential, but not that in the processes. Series resistance will exaggerate the difference in time course.

Suggestions: 1) Reduce the series resistance (see *Bath Error Potential* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION**) or 2) if your cell is not isopotential there is not much you can do short of cutting off or ligating the processes.

Noise

To realize optimal noise performance, the user must pay close attention to noise sources. All potentially contributing noise sources must be minimized. Specifically, the headstage, the pipette glass, the holder and, in the case of patch pipettes, the membrane seal contribute significant noise even under circumstances where extraneous noise pickup from the environment is negligible. It is absolutely crucial that the entire preparation be properly shielded, and that hum from power supply, mains and other sources be negligible.

Chapter 9

Software Control

This section is included to assist software developers. Knowledge of the information in this section is not required for normal use of this instrument.

Programmer's Guide

The following provides information for programmers who wish to add GeneClamp 500B control to any program. This guide assumes that the programmer is familiar with the features and basic operation of the GeneClamp 500B, as described in the Introduction and Functional Description chapters.

Libraries and Sample Code

The contents of the support disks distributed with the GeneClamp 500B are as follows:

1) The DOS command-line control program CMDGENE.EXE.

2) All of the source and information files necessary to compile CMDGENE.EXE.

3) Communications libraries written in both QuickBASIC and C.

These source files and libraries are not copyrighted. The libraries are for use by owners of the GeneClamp or by third-party vendors of data acquisition software. The source code for CMDGENE is made available as a guide to how applications can use the libraries.

The GeneClamp 500B Interface Library

The CGENELIB software interface library provides support routines that user-written programs can use to send commands to and receive status information from the GeneClamp 500B. The library contains support routines for programs written in either Microsoft C or BASIC. Other Microsoft languages and languages from other vendors may also be supported with minor modifications to the source code of the library routines.

The following description of the library interface uses a non-language-specific calling convention that describes the calling interface to the support routines. All procedure arguments are passed by value in C

(unless otherwise noted) and by reference in BASIC. This convention uses the following types for procedure arguments and return values:

Convention	C type	BASIC type
integer	int	integer%
float	float	single or single!
string	char [ ]	string$
ref integer	int *	N/A
ref float	float *	N/A
ref string	char *	N/A

The procedure naming convention is also non-specific to any language. The following naming convention and the corresponding language specific names are used:

Naming Convention	C Name	BASIC Name
int Initialize ( )	int CGENE_Initialize (void)	function CGENE.Initialize% ( )
define COM1	#define CGENE_COM1	const CGENE.COM1

Note that names that are fully capitalized represent constants that are defined in the header and include files.

Implementation Notes

All procedures that are available in the library return an integer value which is zero (defined by the constant SUCCESS in the header file or include file for the corresponding language) if the call was successful or non-zero if unsuccessful (error return codes are also defined in the header/include files). This return value can be used in an IF statement to perform any necessary error action. The most recently generated return value is always available by calling the routine **GetLastError**.

Most of the procedures that send simple commands to the GeneClamp 500B do not send the commands directly to the unit but buffer them until either a call is made to the **FlushCommands** routine or until a command is to be sent that would require a response. The routine **FlushCommands** will do nothing if there are no buffered commands to be sent. Therefore a call to **FlushCommands** should be coded in the program prior to exiting. Library procedures that buffer the generated GeneClamp commands are indicated by the **<buffered>** annotation in their definition.

RS-232 "AT" Command Protocols

The "AT" command set consists of ASCII commands and rules governing the commands that are sent by the host computer to instruments on the Axon expandable RS-232 bus.

1) Each command from the host must commence with the letters "AT". Upper case is required for the letters "AT" but case is irrelevant for subsequent characters in the command string. This prefix is crucially important because it is used by the instrument to determine the baud rate and the parity.

   An incidental benefit of prefixing all command strings with "AT" is that it ensures that the instrument will not respond to spurious characters on the serial port.

2) The third character in the command string is usually a number from 0 to 9. This is the address of the device for which the command is intended. This manual uses "d" for "device" number when referring to this address.

   If no device number is specified, all devices on the bus will respond. (There is a potential conflict if more than one device is connected.) This mode of operation is useful for test and development purposes. However, application programs should always specify a device number.

3) All characters after the "ATd" prefix represent the command.

4) Certain commands are reserved as being applicable to all instruments that reside on the Axon expandable RS-232 bus. At the time this manual was printed, only one such command was defined. This is "ATdS", where "S" stands for status.

   On receipt of an "ATdS" command, the instrument numbered "d" must respond with a description of its model number and ROM version number. By sequentially issuing the ATdS command for all values of d from 0 to 9, the host computer can determine what instruments are connected to the bus.

   There is a verbose form of this command, called "ATdS+". The instrument numbered "d" must respond with a description of its model number, serial number, ROM version number and the internal settings, in a prescribed format.

5) All command strings from the host must be terminated by a carriage return. All responses from the instrument are terminated by a right arrow, ">".

6) If a command is not recognized, or if there is an error executing the command, the instrument responds with a question mark (?) followed by a carriage return and the right arrow.

7) Multiple commands can be concatenated into a single command string, prefixed by "ATd" and terminated by a carriage return. Spaces can be inserted between commands to improve clarity. Spaces can also be inserted between commands and data. There is a 64 character limit to the command string length.

8) Leading zeros are always ignored and do not have to be supplied.

RS-232 Commands

A wide variety of RS-232 commands allow the host computer to control or report the state of the GeneClamp 500B. The "Command" string is specific to the GeneClamp 500B, however, the "S" and "L" commands are the same as CyberAmp commands. Commands supported are:

System Commands

S	Return model number
S+	Return complete system status
L	Load factory defaults
V	Verify system hardware is OK
V3	Verify ME3 EEPROM is OK
V3RAssssnnnn	Reads ME3 probe EEPROM from location ssss for nnnn bytes in ASCII
V3RHssssnnnn	Reads ME3 probe EEPROM from location ssss for nnnn bytes in HEX
V3WAsssstext	Write ASCII "text" to ME3 probe EEPROM from location ssss
V3WHssssHEX	Write HEX DATA to ME3 probe EEPROM from location ssss

Electrode Commands

E1 variants

E1Z> ZERO E1 electrode
E1Z- Remove zero
E1Z? Report current zero

E1T+ Turn on TUNE oscillator
E1T- Turn off TUNE oscillator
E1T? Report state of TUNE oscillator

E1DP DC COMMAND potentiometer to positive
E1DN DC COMMAND potentiometer to negative
E1D- DC COMMAND potentiometer to zero
E1D? Telegraph DC COMMAND potentiometer voltage

E1? Report probe type

E2 variants

E2Z> ZERO E2 electrode
E2Z- Remove zero
E2Z? Report current zero

E2TH Turn on FAST TEST oscillator
E2TL Turn on SLOW TEST oscillator
E2T- Turn off TEST oscillator

E2T? Report state of TEST oscillator

E2DP DC HOLDING POTENTIAL potentiometer to positive
E2DN DC HOLDING POTENTIAL potentiometer to negative
E2D- DC HOLDING POTENTIAL potentiometer to zero
E2D? Telegraph DC HOLDING POTENTIAL potentiometer voltage

E2? Report probe type

E3 variants

E3Z> ZERO E3 electrode current
E3Z- Remove zero
E3Z? Report current zero

E3TH Turn on 200 mV SEAL TEST oscillator
E3TL Turn on 20 mV SEAL TEST oscillator
E3T- Turn off SEAL TEST oscillator
E3T? Report state of SEAL TEST oscillator

E3DP DC COMMAND POTENTIAL potentiometer to positive
E3DN DC COMMAND POTENTIAL potentiometer to negative
E3D- DC COMMAND POTENTIAL potentiometer to zero
E3D? Telegraph DC COMMAND POTENTIAL potentiometer voltage

E3? Report probe type

E4 bath probe variants

E4? Report probe type

Clamp Control

C+ Clamp Mode
C- SETUP Mode
C? Return clamp status

Meters

MVn+ Set voltmeter to electrode "n"
MVn- Turn off voltmeter on electrode "n"
MVn? Telegraph last voltmeter "n" reading

MIn+ Set ammeter to electrode "n"
MIn- Turn off ammeter on electrode "n"
MIn? Telegraph last ammeter "n" reading

MRn+ Set resistance mode on electrode "n".
MRn- Turn off resistance mode electrode "n".
MRn? Telegraph last resistance of electrode "n".

Note Since resistance takes at least 2/3 second to stabilize (up to 5 seconds for ME3), the ATRn? command is illegal unless it has been previously selected using the ATRn+ command. The user must not issue the ATRn? command until the reading has stabilized, otherwise a transitory value will be returned.

Scaled Output

O? Report SCALED OUTPUT selection

OVn+ Turn on SCALED OUTPUT voltage for electrode "n"
OVn- Turn off SCALED OUTPUT voltage for electrode "n"

Special case: If n = 4, then OV4? reports bath clamp voltage.

OIn+ Turn on SCALED OUTPUT current for electrode "n"
OIn- Turn off SCALED OUTPUT current for electrode "n"

OFf Set SCALED O/P filter to f Hz. f=10,20,50, _ 20000,50000
OF? Report current filter setting

OG? Report current gain setting
OGg Set SCALED OUTPUT gain to "g" g=1,2,3,4

Note Since there is a complex relationship between gains and probes as well as the type of output selected. Gains are specified by indices, *e.g.* 1=first, 2=second ...etc. The actual gain units will be returned to the host using the OG? command.

OZ> Zero scaled output
OZ+ Enable output zero
OZ- Remove zero
OZ? Report zero

OL> Recalculate leak subtract parameters
OL+ Enable leak subtract operation
OL- Inhibit leak subtract operation
OL? Return leakage resistance

CMDGENE — Control Software

The GeneClamp is supplied with a DOS command-line program called CMDGENE (for Command GeneClamp) that can be used to control all of the functions and capabilities of the instrument. The program can be used in command-line mode, or it can accept commands interactively or from a command file on disk.

Installation

The CMDGENE program and other software supplied on the diskette can be installed on a hard disk in a directory called \CMDGENE. With the diskette in Drive A:, issue the following DOS commands.

```
MD \CMDGENE
CD \CMDGENE
COPY A: \*.*
```

The CMDGENE.EXE program can be renamed to G.EXE to make it easier to type the name.

General

The main purpose of CMDGENE is for factory testing. It is described in this manual and supplied in source code form on disk because it is a useful example for applications software developers.

The first time that CMDGENE is run it will ask which COM port is being used to communicate with the GeneClamp. The COM port number can be entered or highlighted, followed by the <Enter> key. A selected COM port will be remembered during subsequent executions of the program. The COM port can be changed by using the CMDGENE "COM" command.

Similarly, the first time that CMDGENE is run it will use a COM port speed of 9600 bits/second by default. The default speed can be changed by using the CMDGENE "BAUD" command. Once changed, the speed will be remembered between runs of the program.

Note that most CMDGENE commands have minimum abbreviations that can be used interchangeably with the full commands. For example, the minimum abbreviation for the "STATUS" command is "S". Likewise, most command modifiers also have minimum abbreviations. For example, the command "STATUS DEFAULTS" could be entered as "S DEF". The minimum abbreviation for all commands and modifiers is documented in the command reference section of this chapter.

Most 80286 and 80386 class PC computers should be able to communicate with the GeneClamp at 9600 or 19200 bps. The correct communication speed can be found with the "FINDSPEED" command that will exercise the communications line starting at the maximal speed until it finds a speed at which communications is reliable and error free.

CMDGENE Version 1.0 Help

Summary of Commands

The GeneClamp commands are divided into two groups that correspond to commonly used commands (Basic commands) and commands that may not be used frequently or may only be used to test the unit (Advanced commands).

Basic Commands		Advanced Commands	
BAUD	INITIALIZE	CONTINUOUS	RESTART
CLAMP	LEAK	DELAY	BEEP
COM	METER	FILE OR @	VERIFY
DC	OUTPUT	INTERACTIVE	WRITEPROBE
DEVICE	PROBE	RAW	SERIAL
FILTER	QUIET	READPROBE	SMARTSTEP
FINDSPEED	STATUS	REPEAT	LARGEOUTPUT
GAIN	TEST	STEP	SMALLOUTPUT
HELP OR ?	VERBOSE	ECHO	
	ZERO		

Details of Basic Commands

The following basic commands and modifiers are supported by the CMDGENE program. The minimum abbreviation of the commands and modifiers is indicated by the large capitalized text portion of each token.

1) **B**AUD <baud rate setting>

   or

   BAUD **DEF**AULT

   This command is used to specify what speed will be used for communication with the GeneClamp. The default speed that is assumed by the CMDGENE program when it is first executed is 9600 bps. The current speed is remembered between executions of the program. The <baud rate setting> may be selected from the following list: 19200, 9600, 4800, 2400, 1200, 600 or 300.

   The COM port is always set to 1 Stop bit, 8 data bits and non-parity bit. If you wish to set the communications speed outside the CMDGENE program or want to use a COM port setup other than the program defaults then use the BAUD DEFAULT command which will prevent the program from setting up the COM port. In this case it is assumed that a DOS MODE command will have been issued for the COM port that is to be used. See also the FINDSPEED command for determining the optimum communications speed.

2) **C**LAMP **ON**

   or

   CLAMP **OF**F

   or

   CLAMP REPORT

   This command sets or reports the mode of the GeneClamp. CLAMP ON sets the GeneClamp in CLAMP mode, CLAMP OFF sets the GeneClamp in SETUP mode.

3) **COM** <COM port number>

This command is used to specify which COM port will be used for communication with the GeneClamp. If you have not specified a COM port when a command must be sent to the GeneClamp then the program will prompt you for a COM port to use. The <COM port number> must be in the range 1-4 and the port selected must be valid for your computer configuration. Once a COM port is selected it is remembered between executions of the CMDGENE program.

4) **D**C <electrode> **P**OS

or

DC <electrode> **N**EG

or

DC <electrode> **Z**ERO

or

DC <electrode> REPORT

This command sets or reports the DC potential for the specified electrode.

5) **DEV**ICE <device number>

or

DEVICE **OF**F

This command allows you to specify Axon expandable RS232 bus device numbers. By default the program does not use device numbers and hence assumes that the GeneClamp is the only device on the RS232 bus on that COM port. If you are using multiple units on the RS232 bus then each unit will need to be explicitly addressed by its device number. Once a device number is used then it is remembered by the CMDGENE program until device numbers are disabled (DEVICE OFF) or until another device number is specified.

The <device number> may be in the range 0-9. The current device number may be reviewed with the STATUS DEFAULTS command.

6) **F**ILTER <filter value>

This command sets the low-pass filter for the selected channels. The permissible values for <filter value> are as follows:

10, 100, 1000, 10000
20, 200, 2000, 20000
50, 500, 5000, 50000

There is a permissible shorthand for the whole multiples of 1000 using the letter "k" as a multiplier. For example, 10k is equivalent to 10000.

7) **FIND**SPEED

This command can be used to determine the optimum speed that can be used for communications with the GeneClamp. The program attempts to communicate with the GeneClamp at all speeds starting with 19200 bps until it can find a speed that is reliable and error-free. If a speed is selected that is different than the current speed setting then you will be prompted to confirm whether the selected speed should be used as the default in future executions of the program.

8) **G**AIN <gain setting>

This command sets the overall gain to the preset value specified by the setting. The permissible values for <gain setting> are as follows:

1, 2, 3, 4.

9) **H**ELP

or

?

This command displays a help file in full-screen mode. You can navigate around the help file by use of the <PageUp> and <PageDn> keys.

10) **I**NITIALIZE

This command is used to return the GeneClamp settings for all channels to the factory defaults. The factory default settings are as follows:

Positive input	=	DC
Negative input	=	Ground
Pre-filter gain	=	x1
Output gain	=	x1
Low-pass filter	=	10 kHz
Offset	=	0
Notch filter	=	bypassed

11) **L**EAK **C**ALCULATE

or

LEAK **ON**

or

LEAK **OF**F

or

LEAK REPORT

This command is used to calculate the leak subtract value, and enable or disable leak subtract, as well as report the current leak value.

12) **M**ETER REPORT

or

METER <electrode> **V**OLT | **A**MP | **O**HM **ON** | **OF**F | REPORT

or

METER RMS **ON** | **OF**F | REPORT

This command is used to set the meters or report the current meter settings.

13) **O**UTPUT REPORT

or

OUTPUT <electrode> **V**OLT | **A**MP **ON** | **OF**F | REPORT

or

OUTPUT **Z**ERO **N**OW | **ON** | **OF**F | REPORT

This command is used to set the scaled output, report the current output settings, or zero the output.

14) **P**ROBE <electrode>

This command reports the specified probe type.

15) **Q**UIET

This command indicates that all subsequent command responses should be minimal. In minimal mode no confirmation is given for simple commands such as changes to gain, filter settings, etc.

16) **S**TATUS

or

STATUS **DEF**AULTS

or

STATUS **ID**

The STATUS command display the GeneClamp unit type, serial number and firmware revision number.

The STATUS DEFAULTS command displays information about the COM port being used for communications at the moment and the RS232 bus device number being used if any.

The STATUS ID command displays information about the unit itself.

17) **T**EST **TU**NE **ON** | **OF**F | REPORT

or

TEST **FA**ST | **SL**OW

or

TEST **OS**CILLATOR **OF**F | REPORT

or

TEST **HI** | **LO**

or

TEST **SE**AL **OF**F | REPORT

The TEST TUNE command turns on or off the tune oscillator, or reports the status of the tune oscillator.

The TEST FAST|SLOW command turns on the fast or slow test oscillator.

The TEST OSCILLATOR OFF|REPORT command turns the FAST|SLOW test oscillator off or reports its status.

The TEST HI|LO command turns on the seal test oscillator.

The TEST SEAL OFF|REPORT command turns the seal test oscillator off or reports its status.

18) **VERB**OSE

This command indicates that subsequent command responses should be detailed. All commands provide some confirming response in this mode.

19) **Z**ERO <electrode> **N**OW

or

ZERO <electrode> **D**ISABLE

or

ZERO <electrode> REPORT

This command requests that the GeneClamp attempt to zero the specified electrode, disable the zero, or report the current zero.

Command Details of Advanced Commands

The following advanced commands and modifiers are supported by the CMDGENE program. The minimum abbreviation of the commands and modifiers is indicated by the large capitalized text portion of each token.

22) **CONT**INUOUS

This command sets the program into continuous command-file mode. If the program is currently in command-file mode then the current command file will be processed continuously.

21) **DEL**AY <seconds delay>

This command delays the processing of subsequent commands by the number of seconds specified. When used in conjunction with the REPEAT command the program can be made to repeat a command sequence at regular intervals. For example, the following command displays the status of the GeneClamp every 2.5 seconds:

```
C:\> CMDGENE REPEAT STATUS DELAY 2.5
```

22) **FILE** or **@**

This command specifies the name of a command file that is to be loaded in command-file mode. If there was a previous CONTINUOUS command then the command file is loaded and is processed line by line until the end of the file, until a STEP command is processed or until the user interrupts processing with the <Esc> key. Only the filename itself is allowed to follow the FILE token.

23) **INT**ERACTIVE

This command places the program into interactive command mode. This command is only allowed on the command line.

24) **RAW**

The RAW command allows the user to pass an arbitrary command the to GeneClamp. The remainder of the command line is passed unedited to the GeneClamp. The CMDGENE program will append the necessary "AT" command prefix and device number if applicable. If there is any text response from the GeneClamp then it is displayed to the user.

25) **R**EADPROBE <probe field>

This command allows the reading of information that is stored in the EEPROM of a probe that is attached to channel 3 of the GeneClamp. The <probe field> can be one of the keywords:

ALL, **MO**DEL, **SE**RIAL, **NA**ME, **MA**NUFACTUREDATE,
CALIBRATEDATE, **CE**CAP, **CM**CAP, **UN**ITS,
SCALEFACTOR or **ZE**ROVALUE

26) **REP**EAT <repeat count>

This command causes the CMDGENE program to repeat the following commands the number of times specified. If the <repeat count> is omitted then the following commands are repeated indefinitely until the <Esc> key is hit. This command is most useful when used in conjunction with the DELAY command. The following command monitors the overload status of all the channels by continuously polling the GeneClamp:

C:\> CMDGENE REPEAT OVERLOAD

27) **STEP**

This command sets the program into step by step command-file mode.

28) **ECHO ON**/**OF**F

This command controls whether non-executable lines from the input command file are echoed to the output screen. The default is ECHO ON.

29) **REST**ART

The RESTART command repositions the current command input file to the beginning.

30) **BEEP**

The BEEP command generates an audible tone.

31) **V**ERIFY INSTRUMENTS

or

VERIFY **P**ROBE <probe number>

This command causes the GeneClamp to run a verification test on the main system unit or on the EEPROM in an attached probe. If the main system unit is function normally then the program will display a message of the form:

RAM OK
EEPROM OK
E3 EEPROM OK

32) **W**RITEPROBE <probe number> <probe field> <text>

This command allows you to change or update the standard information that is stored in the EEPROM of a probe attached to a channel of the GeneClamp. The <probe number> is any allowed channel number for this GeneClamp, or the word "CHANNEL", in which case the current channel number is used. The <probe field> can be one of the keywords:

MODEL, **SE**RIAL, **NA**ME, **MA**NUFACTUREDATE, **CA**LIBRATEDATE, **CE**CAP, **CM**CAP, **UN**ITS, **SC**ALEFACTOR or **ZE**ROVALUE

The <text> can be any string of alpha or numeric characters. If the string contains a mix-ture of alpha, numeric or special characters, then the whole string should be surrounded by double quotation marks ("). The MANUFACTUREDATE and CALIBRATEDATE keywords may use the special <text> DATE which substitutes the current date for the string DATE.

33) **SE**RIAL <serial number>

The SERIAL command should only be used during final checkout of the GeneClamp by Axon test technicians. It is used to set the EEPROM based serial number of the GeneClamp unit. The user is prompted with the current serial number and is asked to confirm that the serial number should be changed.

34) **SMART**STEP **ON**

or

SMARTSTEP **OF**F (default)

The SMARTSTEP command is used to enable and disable intelligent command cursor positioning. Turned ON, the command cursor steps over comment lines and is positioned on the next active command line after a command is executed. Turned OFF, the cursor will be positioned at the next line in sequence after each command is executed. In the context of SMARTSTEP, an active command line includes all non-comment lines except blank lines, and comment line that include a check-box.

In the following script, the cursor will be positioned on the lines indicated by a preceding asterisk:

```
*  1:    'This is a comment
*  2:
*  3:    'Use <Enter> to move from line to line to ensure
*  4:    'proper execution of all instructions.
*  5:
*  6:    STEP
*  7:
*  8:    DEVICE OFF INIT STAT*  9:    ' []
         GeneClamp status should have been displayed
* 10:
* 11:    SMARTSTEP ON  12:
* 13:    TEST TUNE ON
* 14:    ' []Should stop here,
  15:    ' but not here.
  16:
* 17:    INIT
* 18:    ' [tick here]
```

35) **LARGE**OUTPUT

The LARGEOUTPUT command resizes the height of the output window in file-command mode to approximately 60% of screen height.

36) **SMALL**OUTPUT

The SMALLOUTPUT command resizes the height of the output window in file-command mode to three lines.

Chapter 10

Specifications

Unless otherwise noted T_A = 20°C, 1 hour warm up time

CURRENT CLAMP

Electrode #1: Voltage recording (Setup and Voltage Clamp modes) (HS-2A-x1LU Standard).

Electrode #2: Voltage recording (Setup) or current passing (Voltage Clamp mode) (HS-2A-x10MGU Standard).

Suitable HS-2A headstages:

Gain	Maximum current for Electrode #1*	Maximum current for Electrode #2*	Notes
x0.1LU	0.13 µA	1.3 µA	Driven case. Low capacitance neutralization range.
x1LU	1.3 µA	13 µA	Driven case. Low capacitance neutralization range.
x1MGU	1.3 µA	13 µA	Grounded case. Medium capacitance neutralization range.
x10MGU	13 µA	130 µA	Grounded case. Medium capacitance neutralization range.

* Maximum current specifications assume that electrode resistance is negligible.

Recommended combinations for two electrode voltage clamp:

Cell input resistance *	Electrode #1 recommendation	Electrode #2 recommendation	Notes
<300 kΩ to 3 MΩ	HS-2A-x1LU	HS-2A-x10MGU	Standard combination
3 MΩ to 30 MΩ	HS-2A-x1LU	HS-2A-x1MGU	N/A

* Considerable overlap in the cell input ranges can be tolerated.

Hum (line frequency pickup): Less than 1 μV_{p-p} grounded input.

Total noise: Using the HS-2A-x1LU or HS-2A-x10MGU probes with grounded inputs, the total noise is 15 μV_{p-p} for Electrode #1 and 50 μV_{p-p} for Electrode #2.

Using the HS-2-x1LU probe and a 2 MΩ electrode resistance, the input noise for Electrode #1 or Electrode #2 is 100 μV_{p-p}. Using the HS-2A-x10MGU probe and a 2 MΩ electrode resistance, the input noise for Electrode #1 or Electrode #2 is 150 μV_{p-p}.

Working input voltage range: ±10V for transients and steady state.

Input resistance: 10^{12} Ω, headstage gain = x0.1

10^{11} Ω, headstage gain = x1

10^{10} Ω, headstage gain = x10

Input leakage current: Adjustable to zero.

Input leakage current *vs.* temperature:

100 fA/°C, headstage gain = x0.01, x0.1

1 pA/°C, headstage gain = x1

10 pA/°C, headstage gain = x10

Capacitance neutralization: Single-turn knob for Electrode #1 only.

Range: HS-2A "LU" version: -1 to 7 pF

HS-2A "MU" version: -2 to 20 pF

HS-2A "MGU" version: -4 to 18 pF

Tune: Injects (x Gain) nA hyperpolarizing line-frequency current into Electrode #1. (*e.g.*, -1 nA for Gain = x1L). This can be used to tune capacitance neutralization.

Zero (V1 & V2): Push button to zero the electrode offset (±300 mV). Voltages are first filtered at 15 Hz.

DC current command: Ten-turn potentiometer delivers depolarizing or hyperpolarizing currents selected with push buttons.

External current commands (Electrodes #1 & #2): 10 x headstage gain nA/V.

VOLTAGE CLAMP

Mode: Selected with Voltage Clamp push button.

Rise Time: 10%-90% rise time is 60 µs for a 10 mV step command when clamping a 220 nF // 1 MΩ model cell via 1 MΩ electrodes.

Noise: Voltage noise (V1) = 30 µV rms (10 kHz) (150 μV_{p-p}) with MCO-2U model cell.

Current noise (I2) = 140 nA rms (10 kHz) (1 μA_{p-p}) for Gain = 10,000 when clamping the MCO-2U model cell (1 MΩ Rm).

Gain: Range is 20 - 10,000 on a logarithmic scale. When *STEADY-STATE RESTORE* is active, this scale is boosted x 100, for a maximum of 1,000,000 gain.

Output compliance: ±130 V (Internal supply voltages are ±150 V.)

Stability: Phase lag range is 3 µs to 2 ms.

Holding potential: Ten-turn potentiometer with ±1 V range.

External voltage-clamp command: 20 mV/V.

Test: 10 mV_{p-p} pulse, hyperpolarizing from rest.

Fast: Line-frequency square wave.

Slow: One-tenth line frequency.

BATH POTENTIAL COMPENSATION

Bath potential is clamped to zero by the bath headstage (VG-2A x100).

PATCH CLAMP

Pipette capacitance compensation:

Fast τ: 0.1-4 μs, single-turn dial.

Fast magnitude: Ten-turn dial.
Ranges specified in CV-5 table.

Slow τ: 0.1-10 ms.

Slow magnitude: Ten-turn dial.
Ranges specified in CV-5 table.

Seal test: 100 μV_{p-p} or 10 mV_{p-p}, line-frequency square wave.

DC command potential: Ten-turn potentiometer with dial.
Range is ±1 V. Push-button polarity control.

Patch-clamp step command: Two inputs; 20 mV/V and 200 mV/V.

Speed test: Requires user-supplied triangular wave input.
Controlled by an on/off switch.

Zero I3: Push button to zero the electrode offset (±300 mV).
Voltages are first filtered at 15 Hz.

CV-5 headstages: All CV-5 headstages are resistor-feedback current-to-voltage converters.

An EEPROM holds coded information about the headstage type. CV-5 headstages are very stable with capacitive loads. Electrode capacitances of many hundreds of pF can be tolerated, but at large electrode capacitances, the bandwidth is reduced.

CV-5 type	**100GU**	**B-100GU**	**1GU**	**100MU**
Transfer resistance	100 GΩ	100 GΩ	1 GΩ	100 MΩ
Feedback resistor	50 GΩ	50 GΩ	1 GΩ	100 MΩ
C_e injection capacitor	2 pF	20 pF	10 pF	50 pF
C_e Fast Mag. comp range	10 pF	100 pF	50 pF	250 pF
C_e Slow Mag. comp range	1 pF	10 pF	5 pF	25 pF
Bandwidth 20	kHz 5	kHz 40	kHz 70	kHz
Guaranteed stable with C_e=	<50 pF	<500 pF	<200 pF	<1000 pF

Maximum noise: Measured with minimal external noise sources (*e.g.*, radiated line-frequency noise, mechanical vibration), 8-pole Bessel filter.

		CV-5-100GU	CV-5-1GU
Without holder:	DC-1 kHz	0.04 pA rms	0.15 pA rms
	DC-2 kHz	0.06 pA rms	0.20 pA rms
	DC-10 kHz	0.25 pA rms	0.80 pA rms
With holder:	DC-10 kHz	0.27 pA rms	0.80 pA rms

DC METERS

Current, voltage or electrode resistance is displayed on either of two digital meters; rms noise measurement bandwidth is 20 Hz to 5 kHz. Upper -3 dB frequency is set by 4-pole Butterworth filter. In all cases magnitudes are displayed with the correct units. Values exceeding the range are displayed as EEE.

PUSH-BUTTON CONTROLS

Key press causes a click tone when pressed and LED illuminates. By pressing specific keys simul-taneously, tone and/or LED can be disabled. Tone re-enabled by pressing specific key combination and LEDs re-instated at power up. There are two error tones; medium duration tone indicates opera-tional error (*e.g.,* headstage is not connected) and longer duration tone indicates data are out of range.

SCALED OUTPUT

Push-button selection: V1, V2, V3, I1, 2 or I3. Only one signal can be processed at a time.

Gain and frequency: Can be set independently for each signal. Settings for the six signals are retained in memory.

Gain: Three gains are available for all selections (four on I2). Display gains and units are dynamically displayed appropriately for the probes used.

Filter: 4-pole low-pass Bessel filter with twelve settings (Hz): 10, 20, 50, 100, 200, 500, 1k, 2k, 5k, 10k, 20k, 50k.

Leak subtract: Causes a signal linearly proportional to the command to be subtracted from current record. Signals are first filtered at 15 Hz.

Output zero: Subtracts a signal equal to that entering the circuit, so output is zero. Averaging with 1 ms time constant. Signals are first filtered at 150 Hz.

OUTPUT BNCs

Two identical Scaled Outputs.

10 x V1, 0.1 x V2, 10 x V2, and 5 x V3.

I1 (10/Gain V/µA), 0.1 x I2 (1/Gain V/µA), and I3 (R V/nA).

I_{BATH} (10/Gain V/µA).

COMPUTER CONTROL

Host computer requirements: RS-232 interface.

Interface format: Conforms to the Axon Instruments Expandable RS-232 bus specification for connecting several instruments to one RS-232 bus.

Auto baud rate: Responds at baud rate initiated by host computer.

Allowable baud rates: 1200, 2400, 4800, 9600 and 19200

Configuration: Null Modem (DTE mode)

Expansion cable length: Up to ten compatible instruments can be "daisy chained" onto one RS-232 interface. Only one host is allowed. A rate of 19200 baud can be sustained if the total cable length is less that 6 m (20 feet). Longer cable lengths are permissible if the baud rate is reduced.

Device address: Range 0-9. Device must have a unique address.

TELEGRAPH OUTPUTS

Control settings sent to computer via RS-232 output:

Scaled Output Filter	Electrode Offsets
Scaled Output Gain	Electrode Resistances
Scaled Output Input Signal Selection	Holding Potentials
Mode	Leak Subtraction Impedance

GROUNDING

Signal ground is isolated from chassis and power ground and is available on rear panel.

CABINET DIMENSIONS

Cabinet is 3.5" (89 mm) high, 19" (483 mm) wide, 12.5" (317 mm) deep. Mounts in standard 19" rack. Handles included. Net weight 10.0 lbs (4.5 kg).

MCO-2U Model Cell

Intracellular electrodes:	Two, 1 MΩ each.
Membrane (switchable):	10 kΩ or 1 MΩ // 220 nF in series with 1 kΩ.
Patch:	10 GΩ
Bath current electrode:	2 kΩ
Bath sense electrode:	2 kΩ

HEADSTAGE DIMENSIONS

HS-2A case is 2.25 x 1.14 x .0.87" (57.2 x 29.0 x 22.1 mm). CV-5 case is 2.25 x 1.37 x 1.13" (57.2 x 34.8 x 28.6 mm). Mounting rod is 4" (102 mm) long. Available mounting rod diameters are 1/4, 5/16 (standard) or 3/8" (6.3, 7.0 or 9.5 mm). Specify non-standard mounting rod diameter with order. Input sockets for the microelectrode shield and ground are 0.08" (2 mm) diameter. Cable length is 10 feet (3 m). Acrylic mounting plate is provided.

PIPETTE HOLDERS

HL-U holders mate to threaded Teflon input connectors of the HS headstages and optional CV-headstages. Post for suction tubing is 1 mm O.D. on both types of holders. **HL-U holder** accepts glass 1.0-1.7 mm OD. Supplied with silver wire. Optional **HLR-U** right-angle adapter and **HLB-U** BNC adapter are available.

SUPPLY REQUIREMENTS

Line voltage:	100 - 240 V~ universal voltage input.
Line frequency:	50-60 Hz
Power:	30 W
Fuse:	2.0 A slow. 5x 20 mm.

ACCESSORIES PROVIDED

Theory and Operation Manual

HS-2A-x1LU headstage

HS-2A-x10MGU headstage

Other HS-2A headstages may be substituted on request with order.

VG-2A-x100 bath-clamp headstage

MCO-2U model cell

RS232-01 cable

Two HL-U pipette holders

Spare fuse

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Warranty

Standard Warranty

Axon Instruments warrants its non-consumable hardware products to be free from defects in materials and workmanship for 12 months from date of invoice. The warranty covers the cost of parts and labor to repair the product. Products returned to our factory for repair must be properly packaged with transportation charges prepaid and the shipment fully insured. We will pay for the return shipping of the product to the customer. If the shipment is to a location outside the United States, the customer will be responsible for paying all duties, taxes and freight clearance charges if applicable.

The warranty is valid when the product is used for its intended purpose and does not cover products which have been modified without approval from Axon Instruments, or which have been damaged by abuse, accident or connection to incompatible equipment.

To obtain warranty service, follow the procedure described in the Repair Service section. Failure to do so will cause long delays and additional expense to the customer.

This warranty is in lieu of all other warranties, expressed or implied.

Repair Service

The company reserves the right to cease providing repair maintenance, parts and technical support for its non-consumable hardware products five years after a product is discontinued. Technical support for old versions of software products will cease 12 months after they are upgraded or discontinued.

If you purchased your instrument from a Distributor or OEM Supplier, contact them for repair service.

If you purchased your instrument from Axon Instruments, contact our Technical Support Department. If it is determined your instrument must return to the factory for repair, the Technical Support Representative will issue a Return Merchandise Authorization (RMA) number. Our RMA Coordinator will contact you with specific instructions.

Out-of-Warranty Repair Service

Out-of-warranty repair service is available. Contact the supplier for current rates.

Circuit Diagrams Request Form

All the information that you require for operation of the GeneClamp 500B is included in the operator's manual. In the normal course of events, the GeneClamp 500B does not require any routine maintenance.

Should you need the circuit diagrams for the GeneClamp 500B, Axon Instruments will be pleased to supply them to you. However, we caution you that the GeneClamp 500B is a sophisticated instrument and that service should only be undertaken by talented electronics experts.

To request a copy of the circuit diagrams and the parts lists, please complete the form at the bottom of this page and mail it to:

Customer Service
Axon Instruments, Inc.
3280 Whipple Road
Union City, CA 94587 USA

This form must be completed in full and signed. Telephone orders will not be accepted.

Name of registered owner: _____

Department: _____

University/Institute: _____

Street address: _____

City: _____State: _____Zip Code: _____Country: _____

Telephone: _____ Fax: _____

Model: GeneClamp 500B Serial number: _____

Declaration
Please send me the circuit diagrams and parts lists for the GeneClamp 500B. I agree that I will only use the circuit diagrams and parts lists for service of the GeneClamp 500B. I will not use them to create equivalent or competing products. If I transfer the circuit diagrams or copies thereof to someone who is assisting in the service of the GeneClamp 500B, I will ask them to make the same undertaking that I am declaring herein.

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